Underground networks of arbuscular mycorrhizal fungi – development and functioning of the external mycelium of *Glomus mosseae* and *G. intraradices* in soil substrate and plant residues

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Abbreviations

AM	Arbuscular mycorrhiza
DAP	Days after planting
DAS	Days after sowing
DC	Donor root compartment
DS	Dry (soil) substrate
DW	Dry weight
ERM	Extra-radical mycelium
FC/ FT	Fungal compartment/ Fungal tube
IRM	Intra-radical mycelium
LC	Labelled root compartment
RC	Receiver root compartment
v/v	Volume per volume
w/w	Weight per weight

Chapter 1

General introduction

The development of symbiotic associations between different organisms is a strategy that has evolved over millennia to allow species to cope with the large variety of environmental conditions existing on earth. Plants have developed different strategies for adaption and diversification, one of which is the co-evolution with microorganisms to acquire nutritional elements from the soil. Special importance can be attached to the symbiotic interaction of plants with soil-borne fungi, mycorrhizal fungi. Fossil finds of plants, dated to the Ordovician / Devonian period (Redecker *et al.* 2002), have revealed mycorrhizal colonisation of plant roots as early as 400 million years ago, indicating that this inter-specific connection has existed since plants first inhabited terrestrial ecosystems. It is therefore not astonishing that mycorrhizal symbioses can be found in the vast majority of land plant species occupying all different terrestrial ecosystems, thus making them one of the most widespread plant-microbial associations. From the point of view of the plant, one main benefit within this relationship is the fungal derived nutrient supply to the plant. This chapter will provide some information on the mycorrhizal symbiosis and its significance for plant nutrition.

1.1 Mycorrhiza

The earliest experimental studies on mycorrhiza, describing the symbiotic connection between fungi and plant roots, have been published in the late 19th century. Frank (1885) was the first to coin the term mycorrhiza which comes from the Greek words: mykes (fungus) and rhiza (root). It has been shown that the fungus is supplied with carbon by the host plant and in return provides mineral nutrients to the plant. The type of symbiosis mainly reported for mycorrhizal associations is a mutualistic form of symbiosis, where in most cases the plant benefits from the

fungal colonisation (Smith and Read 2008). From the nutritional point of view, when characterising mycorrhiza, it should be considered that this symbiosis (similar to other symbiotic forms) is the result of a cost-benefit ratio between plant and fungal nutrient contribution and consumption. In an optimal, balanced case, both partners would benefit from each other. Previous studies, quantifying costs and benefits of mycorrhizal symbioses, have indicated that not all mycorrhizal associations are mutualistic but rather shift into a one-sided benefit within the relationship. This underlines the complexity of this symbiosis and makes it important to understand processes that influence the outcomes of the mycorrhizal symbiosis.

Mycorrhiza is formed by fungi belonging to the phylum *Glomeromycota* (Redecker and Raab 2006) and includes both aseptate fungi, belonging to *Glomeromycetes,* and septate fungi, belonging to *Ascomycetes* and *Basidiomycetes*. Referring to their specific morphology and characteristics of the association between the fungal mycelium and plant roots, the mycorrhiza fungi are subdivided into several different types: ectomycorrhiza, ectendomycorrhiza, endomycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza and orchid mycorrhiza (Smith and Read 2008). The most abundant groups of these mycorrhiza are the ectomycorrhiza and the endomycorrhiza.

Ectomycorrhizal fungi form thickened mycelium capsules around host plant roots, termed the 'Hartig net', and mycelium growth is limited to the intercellular space between root cortical cells (Massicotte *et al.* 1989; Finlay 2008). The common plant types hosting ectomycorrhiza are woody perennial species (Smith and Read 2008). In contrast to ectomycorrhiza, the growth of endomycorrhizal fungi occurs in both, inter- and intracellular spaces of cortical cells. Endomycorrhiza forms a complex intra-radical mycelium (IRM) within the root cortex which is differentiated into hyphae, arbuscules and vesicles. One important member of this group is the arbuscular mycorrhiza, the main subject of this thesis.

1.2 Arbuscular mycorrhizal symbiosis – general characteristics

To complete their life-cycle, arbuscular mycorrhizal fungi depend on the carbohydrate supplied by their host plant and are therefore classified as obligate biotrophs (Parniske 2008). They colonise the outstanding majority of known land plant species, belonging to all land plant phyla, and are established in very diverse terrestrial ecosystems. To date, about 200 morphospecies have traditionally been described, distinguished by features of the spore wall (Smith and Read 2008). The way the spore is formed on the hyphae is used to circumscribe genera and families, and the layered structure of spore walls is used to distinguish species

(Morton and Benny 1990; Blaszkowski *et al.* 2010). The following families were distinguished within the class *Glomeromycetes*: *Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Diversisporaceae, Entrophosporaceae, Geosiphonaceae, Gigasporaceae, Glomeraceae, Pacisporaceae* and *Paraglomeraceae* (Redecker and Raab 2006).

AM fungi are multinucleate with several hundreds to thousands of nuclei within a single spore (Becard and Pfeffer 1993; Marleau *et al.* 2011). Nuclei migrate through hyphae and aggregate in developing spores and are formed by mitosis (Marleau *et al.* 2011). Since the nuclear population within a spore or hyphae fragment is hetero-karyotic, genetic variation within individual AM fungi is high. It has been shown that *glomalean* spores originated from single-spore cultures hold different genetic fingerprints (Zeze *et al.* 1997; Koch *et al.* 2004). It can be assumed that the multi-genomic character of these fungi is necessary, since they have to face a huge variability of micro-environmental conditions, differentiating inside plant roots and proliferating extra-radically into the soil. At the same time they are challenged by macro-environmental abiotic and biotic factors.

AM fungal species can colonise a wide spectrum of plant species and are known to be mainly host unspecific. One factor that influences the outcome of the symbiosis is thought to be the host plant dependence on the AM symbiosis for nutrient uptake and growth, varying from almost independent to highly dependent. The AM symbiosis seems to be particularly beneficial when plants possess a relatively low capacity for nutrient uptake via their own root system or when nutrient availability in soils is limited by abiotic factors (Mosse 1977; Saif 1987; Smith and Read 2008). When quantifying net benefit derived from AM colonised compared with uncolonised host plants, research has brought variable results in terms of nutrient uptake and plant growth. A better understanding of the processes that influence the outcome of AM symbiosis in terms of plant nutrition may contribute to improve management strategies for plant production in sustainable agriculture.

1.3 AM fungal morphology and development

This section describes the structures and growth processes of AM fungi involved in the AM life cycle, starting with the resting propagule, followed by the colonisation of a plant root, the formation of extra-radical structures up to the ending of the life cycle.

1.3.1 AM fungal presymbiotic growth and plant root colonisation

Root colonisation by AM fungi can be initiated in different ways: i) Asymbiotic infection originated from spores, mycelium fragments, or from AM fungal colonised plant roots; ii) Symbiotic infection originated from neighbouring roots of the same or different plants and plant species. In terms of asymbiotic infection and establishment of new colonies, spores are important inoculum sources and therefore are studied in the present work. Depending on the AM fungal species, spore diameters range between 15 and 800 µm (Sieverding 1991). Spores contain cytoplasm and storage lipids, their energy source, and can maintain their germinability for several years in the soil despite being exposed to harsh and changing environmental conditions. By these means spores are the main generative organs for AM fungi which is in contrast to excised mycelium fragments that can only maintain their viability for a relatively short period. Spores and mycelium fragments differ in life-span depending on the fungal species and their relevance as propagules to establish new colonisation varies between fungal families. For example, members of the family *Glomeraceae* are able to infect effectively from spores and mycelium fragments while representatives of *Gigasporaceae* infect only from spores (Klironomos and Hart 2002).

The life cycle of AM fungi usually starts with the germination of a propagule, either a resting spore or mycelium fragment located within the bulk soil or within a former AM colonised root fragment. The germination process happens in absence of the host plant during presymbiotic growth and is characterised by the germ tube development and elongation which is usually interrupted after a few millimetres when no potential plant root is present, so that stored resources are used economically (Koske 1981). In this state the propagules exist in an asymbiotic way and are not influenced by the presence of a host plant but merely by abiotic factors, predominantly soil moisture, soil pH and temperature (Daniels and Trappe 1980; Siqueira *et al.* 1982; Clark 1997). In the case that propagules germinate in the presence of a plant, germination is triggered by signal molecules such as strigolactones, flavonoids and phytoestrogenes contained in plant root exudates (Akiyama *et al.* 2005; Steinkellner *et al.* 2007). These compounds of root exudates may be detected by the fungus as chemotropic guidance to accelerate host root location and therefore reduce energy loss during presymbiotic growth (Sbrana and Giovannetti 2005). Prior to the contact with the root, AM fungi produce the so called MYC-factor stimulating formation of AM symbiosis as well as root branching in host plants (Smith and Read 2008).

As soon as the hypha comes in contact with a root it differentiates to form hyphal swellings on the root surface, termed appressoria (Hause and Fester 2005). Natural plant defence responses are increased at this early stage of association, but are suppressed to low levels very soon thereafter (Kapulnik *et al.* 1996). The fungal entry into the host plant root cortex is accompanied by various changes in the root cell, including cell wall loosening (Balestrini *et al.* 2005), reorganisation of cortical cell organelles and finally the formation of a pre-penetration apparatus that finally forms a 'hollow tube' in the plant cell facilitating the fungal hyphae growth through the root epidermis (Genre *et al.* 2005). At this stage the hyphae proliferates intensively longitudinally between parenchyma cortex cells and develops manifold side branches that form characteristic intra-radical structures including arbuscules and, depending on the fungal species, also vesicles. It is recognised that two different types of AM fungi can be distinguished in terms of the structures they form in cortical cells: *Arum* type which is characterised by arbuscules and *Paris* type, that forms hyphal coils (Smith and Read 2008). There is evidence that a given AM fungus can develop either arbuscules or hyphal coils depending on the host plant (Dickson 2004). The present description refers to the *Arum* type which was observed in the host-AM interactions examined in the experiments of this study.

Until arbuscules are formed, the fungus relies on its propagule resources for development. The arbuscule formation starts with hyphal penetration into a cortical cell which subsequently branches dichotomously into a tree-shaped structure, the arbuscule (Hause and Fester 2005). The pronounced branching allows for increased surface contact between the interfaces of both symbiotic partners and this is assumed to be the location where carbohydrates are exchanged for nutrients (Harrison 1999). Arbuscule formation thus marks the beginning of the symbiotic phase. Arbuscules, like other intra-radical structures, remain in the apoplast and are always separated from plant cell cytoplasm. The separation consists of a thin matrix including the fungal cell wall, the plant-derived apoplast and the periarbuscular membrane which originates from the plant cell plasma membrane (Dexheimer and Pargney 1991; Harrison 1999; Parniske 2008). Subsequent to their formation, arbuscules remain active for about seven days (Alexander *et al.* 1988; Hause and Fester 2005) before they senesce and degrade. After arbuscule development, many AM fungal species aggregate their resources within hyphal swellings, the vesicles containing high levels of cytoplasm as well as storage lipids and functioning as propagules within root fragments (Smith and Read 2008).

1.3.2 AM fungal extra-radical growth

During intra-radical colonisation the fungus is supplied with carbohydrate by the host. The extra-radical mycelium (ERM) development occurs by spreading intensively out of the root and into the substrate beyond the rhizosphere. Hyphae that develop extra-radical and spread into bulk soil differ in diameter between 1 and 20 µm (Sieverding 1991). Fine hyphae with diameters between 1 and 5 µm are assumed to be responsible for nutrient uptake, since they form branched absorbing structures (BAS) with increased surfaces, similar to arbuscules (Bago *et al.* 1998). Coarse hyphae (5-20 µm) can be observed to run longitudinally along the root surface (runner hyphae) and appear to serve mainly for extension and fast spread of the fungal colony (Friese and Allen 1991). By re-colonisation of roots the fungus connects not only neighbouring roots of the same plant but also connects root systems belonging to different host plants. Depending on the host carbohydrate supply, the extra-radical mycelium (ERM) proliferates into the surrounding substrate about 15 cm distant from the host root surface (Jansa *et al.* 2003) and therefore can acquire nutrients far beyond the rhizosphere. Proliferation strategies, in terms of spread intensity into the root surrounding substrate, differ between AM fungal species (Mikkelsen *et al.* 2008). Once colonisation is well advanced (between 3 weeks and 6 months post initiation of root colonisation), depending on the fungal species, asexual spores can form on the ERM (Sieverding 1991). The importance of spores as infective units varies between fungal species, the local abundance of the fungus, and the environmental conditions. Spores are the most stable and effective propagules to establish infection compared with other inoculum sources such as colonised roots or excised hyphae (Bellgard 1993).

1.4 Photosynthate costs in the AM symbiosis

For their proliferation and maintenance, AM fungi depend on the carbohydrate supplied by their host. Substantial amounts of mycelium biomass can be present within roots and mycorrhizal roots can receive 4-20% more photosynthates than non-inoculated roots (Douds *et al.* 1988; Jakobsen and Rosendahl 1990). By pulse labelling of extensively colonised plants with stable isotopes, Jakobsen and Rosendahl (1990) calculated that about 20% of the total plant fixed carbon (C) can be attributed to AM fungal use. It is possible that AM fungal colonisation can cause plant growth depressions, due to the C drain to the fungus especially under conditions were C reserves of young plants fail to meet AM fungal carbohydrate demand (Mortimer *et al.* 2005). Nevertheless, due to the nutritional benefits provided by the fungus, the

plant is usually able to compensate the C costs of the fungus by the increase of photosynthesis per unit leaf area (Mortimer *et al.* 2008).

The photosynthetically fixed C is translocated to the plant sink organs, predominantly in the form of sucrose which is lost from the plant cell along a concentration gradient and then released into the apoplastic interface. Sucrose first has to be cleaved by a cytosolic sucrose synthase or by invertases before being absorbed by the fungus as hexose, mainly in form of glucose and also fructose. The hexoses are rapidly incorporated into trehalose and glycogen which are supposed to buffer excess glucose accumulation in the cell (Smith and Read 2008). It is assumed, that hexose absorption is conducted via the plasma membrane of intra-radical organs including hyphae, arbuscules and hyphal coils (Smith and Read 2008).

1.5 Host plant benefits by AM fungal colonisation

1.5.1 Indirect benefits

The most important benefit of AM symbiosis for the host plant is the AM fungal function with respect to nutrient transfer to the plant partner and therewith the involvement in nutrient cycling processes. Indirect benefits for host plants mediated by AM fungal colonisation include the following:

i) Alleviation of the adverse effects of drought (reviewed by Augé 2001), salt stress (reviewed by Evelin *et al.* 2009), and high concentrations of heavy metals in AM fungal colonised host plants. AM fungi may function as an effective sink for heavy metal surpluses and passively adsorb heavy metal ions by binding them to the fungal cell wall (Joner *et al.* 2000) and to glycoproteins secreted by the fungi (Gonzalez-Chavez *et al.* 2004).

ii) The formation of beneficial relationships between AM fungi and other rhizosphere microorganisms, such as nitrogen fixing and plant growth-promoting bacteria which can physically attach to the fungal surface (Gerdemann and Trappe 1974; Ho 1988; Bianciotto *et al.* 2001; reviewed by Artursson *et al.* 2006).

iii) Improvement of soil structure due to the formation of water stable aggregates as a result of the secretion of glycoproteins by AM fungi (Rillig *et al.* 2002).

iv) Increased host plant resistance to soil-borne pathogens and nematodes, thought to be induced by stimulation of defence responses (Volpin *et al.* 1994; Morandi 1996; Li *et al.* 2006) or by competition with pathogens for root infection sites (Muchovej *et al.* 1991).

It is fairly widely acknowledged that soil-borne bacteria, present in the myco-rhizosphere are closely associated with AM fungi. Some such bacteria are capable of producing plant available mineral nutrients by decomposing soil organic matter and consequently play a crucial role in nutrient cycling. Therefore, when examining AM fungal contribution to plant nutrient supply, the impact of soil-borne bacteria should not be neglected. Bacterial species known to be beneficial for plant growth, due to their nitrogen fixation, P-solubilising or bio-degradative properties, can be attached to hyphae and spore surfaces (Toljander *et al.* 2006). Several mechanisms have been proposed to be involved in this inter-specific interaction: the accommodation of bacteria by fungal secretion of soil aggregate stabilising polysaccharides (Bianciotto *et al.* 2001), the improved fungal growth and establishment in presence of certain bacteria (Xavier and Germida 2003), or alternatively the organisms could also be in competition for nutrients (Ravnskov *et al.* 1999b). By spreading into soil, the large surface of the AM fungal extra-radical mycelium may not only directly take up nutrients available in the bulk soil distant from the host rhizosphere, but could also function as a means of transport for bacteria. Soil bacteria occurring together with AM fungi increase the nutrient availability from organic sources (Hodge *et al.* 2001) and thereby enhance AM fungal ability to promote plant growth. A possible function of external hyphae as a pathway for soil solutes other than mineral nutrients was recently demonstrated by Barto *et al.* (2011), who observed a transfer of hydrophilic and lipophilic substances between two colonised root systems of two plants interconnected by a common AM fungal hyphae network. Allowing for solute movement (either on hyphal surfaces or in the interior of hyphae), AM fungal mycelia may also serve as a 'highway' for substances such as signalling molecules that enable chemical communication between plants.

In this thesis the emphasis will be placed on the function of AM fungi in nutrient transfer, the fungi's most direct contribution to plant growth. Even though the production of external mycelium varies considerably between AM fungal species (Abbott and Robson 1985; Jakobsen *et al.* 1992; Smith *et al.* 2004), all develop extensively branched, absorptive structures (Bago *et al.* 1998) that enable them to access the soil solution captured within fine soil pores, otherwise unavailable to plant roots. Furthermore, hyphae spread can explore about 12 cm^3 of soil volume per centimetre of colonised root length, compared with a soil volume of about 1-2 cm^3 for a similar length of an uncolonised root (Sieverding 1991). Therefore during the symbiotic association, AM fungi may forage for nutrients far beyond the soil volume of the rhizosphere by bridging narrow depletion zones, especially those of relatively immobile nutrients such as

phosphorus (P), zinc (Zn), copper (Cu) and ammonium (NH_4^+). AM fungal colonisation has been shown to increase plant uptake of the elements mentioned above and also that of sulphur (S) and potassium (K) (Smith and Read 2008). The following subsections give a brief overview of plant P and N nutrition as affected by the arbuscular mycorrhizal symbiosis.

1.5.2 AM fungal contribution to plant P nutrition

For plant nutrition phosphorus (P) is one of the all-important macro-elements, required by the plant in relatively large quantities. Being a structural component of macromolecules, P is most prominent in nucleic acids, the phospholipids of bio-membranes and in the energy-rich intermediates and coenzymes involved in biosynthesis and degradation processes (Marschner 1995). In the plant tissue, P is very mobile and is transported within the phloem during plant development, depending on the demand of the respective organ (Biddulph *et al.* 1958; Rausch and Bucher 2002).

Soil P is contained in organic as well as mineral P pools (Sharpley and Smith 1985). Inorganic P is considered to be the most important form of P taken up by plant roots either as $H_2PO_4^-$ or HPO_4^{2-}. P-ions in soils are easily bound to Ca, resulting into the formation of hardly soluble Ca-phosphates mainly in high pH soils. P can also be bound to Fe or Al, forming hardly soluble complexes mainly in low pH soils (Scheffer and Schachtschabel 2009). The strong affinity of soils for P-ions may result in P immobilisation and in low concentrations of plant available P in the soil solution, especially under alkaline or acid soil conditions and in soils with a high Ca content (Koide 1991). As a result of these reactions, P depletion zones may develop rapidly around plant roots (Marschner 1995). To some extent, microbial mineralisation of P from soil organic matter can increase P concentrations and mobility in the soil solution (Seeling and Zasoski 1993). Thus, although the total content of P in the soil may be high, it is often present in unavailable forms. More than 80% of the soil P sources can become immobile because of adsorption, precipitation, or microbial conversion into immobile organic forms (Scheffer and Schachtschabel 2009), and this insufficient P availability has often been observed to limit plant growth in natural soils (Bucher 2007). Under such conditions, the value of the AM symbiosis for sustainable agriculture and re-vegetation practices may be great, since AM fungi are usually beneficial for plants in terms of improved P acquisition. Accordingly, AM fungal contribution to plant P uptake is most significant under conditions of low P availability in the soil solution (Marschner and Dell 1994).

To forage for P located in the immediate vicinity of the root rhizosphere, terrestrial plants have developed efficient phenotypic and physiological adaptations. The most important physical adaptations are maximisation of the absorptive surface area by increasing root length densities in bulk soil and root hair development per unit root length (Lynch 1995; Barber 1995). Furthermore, plants actively induce acidification of the soil solution by proton release from root tips (Kraus *et al.* 1987; Tang et al. 2004). Depending on the plant species, a complex of root exudates are produced, which can include organic acids to solubilise inorganic P fractions or acid phosphatase to catalyse the mineralisation of organic P fractions (Li et al. 1997). In addition to these P acquisition methods, plants can also be supplied with P by the mycorrhizal pathway. The extra-radical mycelium (ERM) of AM fungi spreads into the bulk soil beyond the depletion zone of plant roots, thus creating a larger P absorbing surface.

In terms of plant P acquisition, the increase of availability of P is the main advantage of the association with AM fungi. The fungal hyphae can enter soil pores with very small diameters that are inaccessible to roots (Drew *et al.* 2003). Moreover, it has been reported that the fungi have a great aptitude for mining P from the soil solution. They have the ability to excrete enzymes, namely phosphatases, which enable the mobilisation of P from organic matter (Joner and Johansen 2000). When interacting synergistically with P-solubilising microorganisms, AM fungi are thought to contribute also to the solubilisation of P from rock phosphate sources (Antunes *et al.* 2007). Moreover, AM fungal colonisation can reduce the severity of water stress to plants (Nelsen and Safir 1982; Neumann *et al.* 2009), an effect that has been attributed to an increased P nutrition through the mycorrhizal pathway under dry soil conditions (Neumann *et al.* 2009).

When in symbiosis with AM fungi, plants usually respond to improved P nutrition by the development of lower root, but higher shoot growth, compared with non-mycorrhizal plants. This is noticeable in the higher shoot-root dry weight ratio typically observed in mycorrhizal plants (Marschner 1995). Under conditions of pronounced P deficiency, root P uptake may not satisfy the plant's P requirement. In such cases, the benefit of mycorrhizal P delivery becomes increasingly important for plant growth, so that the resultant plant biomass accumulation is enhanced compared to that of non-mycorrhizal plants (Sieverding 1991). The transport of P from the AM fungi to plants has been studied using compartmented pot systems where labelled phosphorus isotopes were supplied to the fungus (Jakobsen *et al.* 1992; Pearson and Jakobsen 1993; Smith *et al.* 2003; Smith *et al.* 2004). These studies revealed that fungal-derived P ranges

from a small percentage to almost all of the P acquired by the plant, and huge variations exist depending on the plant/fungus combination (Pearson and Jakobsen 1993; Smith *et al.* 2003; Smith *et al.* 2004). Although P is delivered through the mycorrhizal pathway, plants may not necessarily respond to mycorrhizal colonisation with increased biomass production or increased net P uptake when compared with non-mycorrhizal plants (Smith *et al.* 2003; Smith et al. 2011). This effect has been explained by a down-regulation of the plant high-affinity Pi transporters (PiTs; usually expressed in actively P absorbing root tissue) in the root epidermis of AM colonised plants (Smith et al. 2011). Therefore the reduced direct pathway might be compensated by the independent AM fungal pathway resulting into similar quantities of total P uptake in mycorrhizal compared to non-mycorrhizal plants (Smith et al. 2011).

The concentration of P in soil solution is usually lower than in plant roots and fungal cytoplasm, and to counteract the concentration gradient, P uptake by the extra-radical mycelium requires energy. Therefore, inorganic P (P_i) is actively absorbed by the ERM and enters the fungal cytoplasm driven by H^+/P_i symporters, whilst the required proton gradient is produced by plasma membrane H^+-ATPases. After being taken up, P_i is incorporated into polyphosphates, which are translocated within the mycelium (Bücking and Shachar-Hill 2005). When fungal P uptake is higher than demand, surplus of P accumulates in vacuoles where it is stored for later use. When required, P transport through the interfacial apoplast is assumed to be regulated by the intracellular P_i concentration within the hyphae (Bücking and Shachar-Hill 2005). The vacuolar P pool contains mainly polyphosphates which probably play important roles in fungal derived P supply to the plant (Ezawa *et al.* 2002).
The intra-radical mycelium (IRM) of AM fungi is likely supplied with P derived from vacuolar components (Ezawa *et al.* 2002), and the transport may occur along a motile tubular vacuole system (Olsson *et al.* 2002; Uetake *et al.* 2002). The exact mechanism of P breakdown in the IRM is still not well understood, but it is assumed that the polyphosphate molecules are reduced in size by hydrolysation in the intra-radical hyphae (Ohtomo and Saito 2005), and then released to the host as P_i. The main site of nutrient exchange between the two symbionts is proposed to be the interface between the fungal arbuscular membrane and the plant periarbuscular membrane (Cox and Tinker 1976). P_i supposedly exits through the fungal plasma membrane into the interfacial apoplast where it is actively transported into plant cells (Ezawa *et al.* 2002).

1.5.3 AM fungal contribution to plant N nutrition

Nitrogen (N) is a macro-nutrient required in the highest quantities by the plant. N plays a central role in the synthesis of plant macro-molecules and is a component of structural proteins, enzymes and amino- and nucleic acids (Marschner 1995). Therefore, plant growth is first of all determined by the availability of N in the soil. Nitrogen becomes available as a result of the continuous cycling of inorganic and organic compounds crucially affected by the activity of soil-borne micro-organisms.

The soil N pool consists predominantly (about 90%) of organic forms, such as amino acids, amino sugars and N-containing heterocyclic compounds. Organic N molecules can be rapidly decomposed by heterotrophic microbes that mobilise N from organic sources by the conversion into ammonium (NH_4^+) which then underlies the nitrification process by microbial transformation into nitrate (NO_3^-). A considerable contribution to the soil N input is mediated by *Rhizobia* bacterial N fixation of gaseous nitrogen, and also soil organic matter is an important N pool releasing plant available N subsequent to microbial degradation. Following the mineralisation process, a relatively small proportion of the soil N pool (about 5%) is plant available in the form of inorganic N. Steadily produced ammonium is unlikely accumulated in most soils, since the conversion to nitrate occurs faster than ammonification (Scheffer and Schachtschabel 2009). In well aerated soils, mineral N is predominantly present as nitrate, a relatively mobile component susceptible to be lost to deeper soil layers by leaching. The concentration of mineral N (N_{min}) in agricultural field soils varies greatly; e.g. 30- 160 kg/ha N_{min} in dry soil from the top layer after crop harvest (Haberle et al. 2004; Sadej and Przekwas 2008). Low status of available N in field soils are usually compensated by fertiliser application, since N demand of crop plants is relatively high. The tissue N concentration of well-nourished crop plants ranges between 2 and 5% depending on the plant species, the developmental status and the considered organ (Marschner 1995).

The major forms of inorganic N taken up by plant roots are NH_4^+ and NO_3^-. Depending on the plant N demand and species specific preferences, NO_3^- is assimilated in the root plastids and in the shoot chloroplasts. NO_3^- is readily mobile in the xylem tissue and in case of surplus it is stored in the cell vacuoles of different plant organs. In contrast, NH_4^+ has to be assimilated immediately by the plant into amino acids at the site of uptake to prevent toxic effects of this compound. Plants incorporate the major part of the absorbed NO_3^- into essential organic compounds. Therefore, NO_3^- has to be transformed by enzymatic reduction to NH_4^+. Finally,

NH_4^+ derived from either NO_3^- reduction or root uptake, serves as a basis to build-up essential amino acids and other organic compounds relevant for plant development (Taiz and Zeiger 1999).

It has been shown that N is taken up by AM fungi and transported to host plants, thus the actual significance of AM fungal N acquisition for overall plant nutrition remains unclear. The capacity of AM fungi to improve N availability to colonised host plants can be explained by its intense hyphal proliferation in soil enabling better spatial exploration of N. Utilising ^{15}N labelled N, it has been reported that considerable amounts of N are taken up by AM fungi, transported through the ERM network and supplied to the host plants (Johansen *et al.* 1992; Frey and Schüepp 1993; Subramanian and Charest 1999; Tanaka and Yano 2005). When high amounts of N were supplied only to fungal compartments, thus separated from plant roots, the percentage of plant total N attributed to hyphal uptake were up to 20-30% (Ames *et al.* 1983; Frey and Schüepp 1993). In contrast, AM fungal plant-to-plant N transfer has been shown to not increase plant N uptake when compared with uncolonised plants (Johansen and Jensen 1996). The uptake by the hyphae occurs in the form of NH_4^+ and NO_3^- (Johansen *et al.* 1992; Subramanian and Charest 1999) and also amino acids (Hawkins *et al.* 2000). When taken up either as NH_4^+ or NO_3^-, both forms are likely assimilated into arginine as the main transport form within hyphae (Govindarajulu *et al.* 2005), and thereafter, N is probably transferred in the form of NH_4^+ to the plant (Govindarajulu *et al.* 2005; Tanaka and Yano 2005).

Results of previous studies have successfully highlighted the potential for AM fungal mediated N transfer to the host but failed to provide a clear evidence for a considerable contribution to plant N nutrition. The experimental conditions used in former reports have been largely based on artificial substrates and have used high quantities of inorganic N (offered only to the fungus). Not only did the results of these experiments differ dramatically between the individual trials, they probably also did not adequately simulate natural field site conditions. In nature, however, it seems likely that AM fungal contribution to plant N uptake could become important under circumstances where plant N demand exceeds N availability, for example under conditions of immobilised N sources or during drought (Tobar *et al.* 1994; Subramanian and Charest 1999). Soil organic matter is a possible nutrient source for AM fungi, and only little information is available on the quantities of N taken up and transferred from decomposing roots or litter (e.g. Johansen and Jensen 1996; Hodge *et al.* 2001; Hodge and Fitter 2010).

Therefore, more studies in soil are needed in order to understand the contribution of AM fungal N supply under field conditions, especially when N is taken from plant residues.

1.5.4 The plant nutritional status and the outcome of the AM symbiosis

Plant species differ in their requirements for AM symbiosis, mainly due to root morphological or physiological features and their demand for P. Plant species with coarse, poorly branched root systems and small surface areas (Hetrick 1991), and/or a low ability to excrete P-mobilising root exudates, benefit the most from an AM symbiosis (Marschner 1995). More than that, progress and eventual outcomes of the plant /AM fungal association depend greatly on the plant nutritional status, in particular the plant P status. A high plant availability of soil P reduces AM fungal root colonisation (Son and Smith 1988; Amijee *et al.* 1993; Vierheilig 2004), arbuscule development and also decreases the spread of the external mycelium in soil (Smith and Read 2008). In general, relatively pronounced beneficial effects of the AM symbiosis are observed when plant available soil P is low (Marschner and Dell 1994), or when plants have a high P demand, but root P uptake capacity is restricted by some means. Therefore, seedlings are highly responsive to AM fungal colonisation (Fisher and Jayachandran 2002; Guissou 2009). When highly receptive to AM symbiosis and grown in low P soils, mycorrhizal plants may take up three to four times more P than non-mycorrhizal plants (Smith and Read 2008). In contrast, under conditions where plant available P enables optimal P uptake by the roots, the extent of fungal colonisation declines. The mechanisms behind are still not fully understood (Smith and Read 2008). It has been suggested that this suppression of mycorrhiza development may result from a reduced carbohydrate allocation from roots to the fungus by the plant in response to high P status (Graham *et al.* 1997; Olsson 2002). For their development, AM fungi rely on the C contained in sugars synthesized and delivered by their host. Thus, any factors (such as irradiation, available nutrients or drought) that restrict photosynthate production or C distribution in the plant may also affect AM fungal colonisation.

1.6 AM fungal inoculum production and the request for adequate inoculum formulations

The volume of AM fungal inoculum traded worldwide increased considerably within the past twenty years (more than a 5-fold gain between 1999 and 2003; Grotkass *et al.* 2005), and regions with the most predominant demand have been Germany and North-America (Feldmann 2008). A realisation of the benefits of AM fungi for supplying nutrients under unsuitable abiotic soil conditions and their ability to act synergistically with other soil-borne micro-

organisms (see Section 1.5) have contributed to the success of AM inoculum products. They are being considered more and more in agriculture, horticulture as well as for re-cultivation activities. The most promising applications of AM fungal inoculum for plant production are:

i) To sustain or establish functional AM fungal populations in low-input (agro-) ecosystems (Sieverding 1991; Douds *et al.* 2005; Plenchette *et al.* 2005).
ii) To improve plant establishment for re-cultivation processes of degraded or polluted sites which have been disturbed by anthropogenic means (Menge 1983; Cuenca *et al.* 1998; Joner and Leyval 2003).
iii) To improve the development of cuttings (Douds *et al.* 1995; Druege *et al.* 2006) and micro-propagated plants after transplanting into non-sterile substrates, inoculated with AM fungi (Branzanti *et al.* 1992; Vestberg *et al.* 2004; Carretero *et al.* 2009).

The obligate biotrophic nature of AM fungi means that fungal propagation must take place in the presence of a host plant. This fact complicates and hinders cost-efficient mass propagation of AM fungal inoculum, and as a consequence, a commercial production is still in its infancy. However, in response to the growing demand for AM fungal inoculum in the last decades, producers and scientists are working specifically towards the development of large-scale production (Ijdo *et al.* 2011). At present, inoculum is produced for commercial purposes using several simple, and some more complicated techniques. The most important of these include (in ascending order of technical standard and cost expenses):

i) Production on inoculated plants within open field or nursery beds using soil (Sieverding 1991).
ii) Production in containers or raised beds, where plants are inoculated and grown under greenhouse conditions within different substrates. As starting inoculum individual AM fungal strains can be used, e.g. obtained from sterile cultures provided by gene banks (Ijdo *et al.* 2011; Feldmann and Schneider 2008).
iii) Production on pre-inoculated plants in hydroponic or aeroponic systems (Hung and Sylvia 1988; Hawkins and George 1997; Mohammad *et al.* 2000).
iv) Axenic production of pure AM fungal strains *in vitro* on transformed roots or autotrophic plants (Becard and Fortin 1988; Declerck *et al.* 1996; Voets *et al.* 2009). This technique produces a carrier-free inoculum, suitable for many applications. Disadvantages may include the relatively complicated and cost-intensive technological setup. Furthermore, not all AM fungal species can be propagated successfully on sterile media (Gianinazzi and Vosatka 2004).

In general, the procedure for formulating AM fungal inoculum involves placing fungal propagules (colonised root fragments, spores and hyphae fragments) into a given carrier material (e.g. sand, calcined clay, vermiculite, peat, etc.). Inoculum from substrate-based production therefore contains not only AM fungi but also associated microorganisms, and the producer has to ensure that those are not harmful to plants (Feldmann 2008). The final configuration of the formulation is determined by the target inoculum application method (mixing or surface incorporation by hand or machine, inoculation of bare roots, container substrate, seeds, culture substrates, field soils, etc.), and it is possible to adapt the carrier material to the demand of the user (Feldmann 1998). For certain application methods, solid carrier material may function as a protective unit, for example for the amendment on roof tops expanded clay can prevent spore damage during high-pressure application processes (Feldmann 2008). However, in many cases solid carrier material is undesirable, since the additional weight and volume increases the effort required for transport and application, and ultimately leads to higher costs for the user. Developing cheap and carrier-free inocula, easy to apply, would certainly increase the acceptance of AM inoculum among potential costumers and may allow the expansion into new fields of application. Accordingly, an increasing effort into research has been made over the past few years (Gianinazzi and Vosatka 2004; Ijdo *et al.* 2011). Still more studies are requested by inoculum producers (Feldmann 2008; C. Schneider, 2011, personal communication) therefore the present work should contribute to that.

1.7 Agricultural practices that affect AM fungal symbiosis

Since the middle of the last century the use of fossil fuels for input production has allowed agriculture to become intensified in temperate regions, and more recently, also in tropical areas (Craswell and Karjalainen 1990). As a result of this, a wide range of different cropping systems have been established, of which the most intensive forms have achieved great increases in yields. The pronounced rise in the use of agrochemicals for crop or energy plant production inevitably increases not only field operations and input costs, but also the costs for the environment. Associated with inappropriate agricultural management methods, consequences may include surface water pollution from leaching of fertilisers and pesticides (Flury 1996; Olarewaju *et al.* 2009), loss of soil C stocks due to insufficient organic fertilisation (Guo and Gifford 2002) and erosion due to fallow periods and due to soil compaction by passing over with machinery (reviewed by Hamza and Anderson 2005).

AM fungi have coexisted and coevolved with plants for millions of years (Remy *et al.* 1994). These fungi are hence commonly found in natural and agricultural soils and worldwide where they are symbiotically associated with both wild- and cultivated plant species (Sieverding 1991). It is thought, however, that intensive forms of cropping can be detrimental to soil-borne microbial symbionts, such as AM fungi. By altering the biotic and abiotic soil conditions, inapropriate agricultural practices can impact the development of AM fungi in the following ways:

i) High levels of P fertilisation can reduce AM fungal colonisation of host plants (Hayman *et al.* 1975; Braunberger *et al.* 1991; Vierheilig 2004), and also decrease the subsequent plant growth response to mycorrhizal colonisation (Schubert and Hayman 1986; Smith and Read 2008). In intensive conventional plant production systems where P is applied regularly, the contribution to plant nutrition by AM fungi is negligible. Sufficient P supply by fertilisation inevitably leads to a decreased dependency on the symbiotic fungal partner which may account for a reduced AM fungal abundance in such sites. When natural ecosystems are transformed into agricultural fields, over time this effect may lead to reduced genetic variability in AM fungal species populations (Schenck *et al.* 1989; Oehl *et al.* 2003). When compared with permanent grassland a high-input field site might select for fast developing AM fungal species, so called 'generalists' (Oehl *et al.* 2003).

ii) The infective potential of AM fungal propagules in soil may be altered by several agricultural practices. Crop rotations that include a considerable proportion of non-mycorrhizal plant species (e.g. sugar beet, rapeseed) and/or fallow periods can reduce dramatically AM fungal infectivity for the following growth season (Harinikumar and Bagyaraj 1988; Douds *et al.* 1997; Kabir *et al.* 1999). The absence of mycorrhizal plants during the vegetative period of the fungi may cause the most extensive harm to the survival of AM fungal species (Kabir *et al.* 1999; Plenchette *et al.* 2005).

iii) Soil disturbance (ploughing) has been shown to decrease AM fungal development and contribution to plant P uptake (Evans and Miller 1988; Fairchild and Miller 1988), and caused a reduction in AM fungal species richness (Brito *et al.* 2012). However, AM fungal species obviously differ in their susceptibility to disturbance (Hart and Reader 2004; Brito *et al.* 2012). Within the soil depth profile of a natural grassland, AM fungal spores are mainly present in the top 20 cm of soil, and only a small portion of AM fungal spores are also located in deeper layers of 60-100 cm depth (Abbott and Robson 1991; Oehl *et al.* 2005). Mouldboard ploughing may lead to spore relocation to deeper soil layers, or to a reduced density of propagules by diluting top soil (harbouring higher spore densities) with deeper soil layers. Accordingly, it has

been shown that AM fungal colonisation and early P uptake were higher in maize plants grown in no-till or ridge-tillage management compared to mouldboard ploughed plots (McGonigle and Miller 1993). Moreover, as a consequence of mechanical soil disruption the infection potential of an AM fungal network might be reduced. Olsen *et al.* 1999 suggested that the establishment and colonisation by fragments of a disrupted extra-radical mycelium might need more C expenditure from the subsequent plant, compared with an intact mycelium. Especially moderate forms of mechanical soil treatment (such as applied in reduced tillage systems) are not precisely studied with respect to their effects on the AM symbiosis and the present study gives more information about that.

In order to take advantage of the AM symbiosis in agriculture, conditions must be met that support AM fungal development. Most importantly this includes the use of sustainable management systems with reduced tillage, avoidance of non-mycotrophic plants in the rotation and the prevention of P accumulation in soils by fertilisation. In cases where the former AM fungal populations could not be maintained due to management practices, a targeted application of selected AM fungal inoculum might be recommendable. Inoculation in the field with efficient AM fungal isolates can be an effective means of re-establishing AM symbioses and improving plant yield and quality after transition from conventional to organic farming. It might also be an opportunity for farmers in regions were mineral P fertilisers are too costly. Any AM fungal strains directly selected from the farm itself are likely better adapted to the present soil conditions. To attain large quantities of the desired strains, inoculum could easily be propagated on-farm with low operating costs (Sieverding 1991).

1.8 Objectives of the study

This study aims at improving our understanding on some morphological and physiological aspects of the AM symbiosis. It focuses on investigating the N uptake from decomposing plant roots and delivery to the host plant, especially considering growth and development of the AM fungal extra-radical mycelium (ERM). It also addresses the question of inoculum potential of the ERM with respect to its developmental stage and spatial distribution in soil. Furthermore, the ERM as an infective unit for host plant colonisation was studied with respect to its susceptibility to mechanical disruption typically for many agricultural soil management practices. The study also gives more information about AM fungal spore production, as spores are the most important propagules in soil. This study also analysed quantities and patterns of

fungal spore production occurring within dead plant roots which are a ubiquitous in vegetated soils.

In view of these objectives the following hypotheses were formulated:

1. Nitrogen is absorbed by the extra-radical mycelium of the AM fungi from a dying donor plant root and delivered to a living receiver plant. Thereby fungal transfer of N to the receiver plant will be higher from AM colonised donor roots compared to uncolonised roots.
2. AM fungal N transfer to a colonised host plant will be reduced when soil, containing established mycorrhizal networks, is mechanically disturbed.
3. When fungal colonisation of plants is established exclusively by the ERM, AM fungal isolates with a higher extent of ERM proliferation in the soil volume prior to mycelium excision will have a higher inoculum potential and growth promoting effect on the subsequent plant.
4. The mechanical fragmentation of detached ERM, induced by soil disturbance, reduces AM fungal inoculum potential and consequently reduces fungal contribution to P uptake and growth of the next plant.
5. Spore development within dead plant roots will not depend on whether the root originated from a host or a non-host plant species, but rather will increase with root diameter.

The outcomes of the present study aim to contribute to our knowledge on the ecology of AM fungi and their potential to improve plant nutrition. Findings may also assist the development of suitable management practices to improve the use of AM fungi in agricultural systems for a more sustainable plant production.

Chapter 2

General materials and methods

Materials and methods routinely used in the experiments are described in this chapter. Applications and modifications related to specific experiments are described in the relevant sections.

2.1 Description and preparation of experimental plant growth substrate

Subsoil obtained from the C-horizon of a Luvisol from Weihenstephan, Southern Germany (48°25'N, 11°50'E) was used as growth (soil-) substrate. The substrate was classified as loamy sand (45.2% sand, 42.0% silt, 13% clay) and it contained (mg kg^{-1}): 5.2 and 3.4 $CaCl_2$ (0.0125 M)-extractable NH_4^+ and NO_3^-, respectively. The organic matter content was 0.3% in DS, with a substrate pH ($CaCl_2$) of 7.7 and a $CaCO_3$-equivalent of 23%. After heat sterilisation, the substrate contained (mg kg^{-1} DS) 6.5 acetate lactate-extractable P (CAL, Schüller, 1969); 65.7 CAL-extractable K; and 1.9 (Fe), 15.0 (Mn), 0.3 (Zn), 0.9 (Cu), 0.09 (B) and 0.04 (Mo) CAT-extractable micronutrients (Alt and Peters 1993). Substrate characteristics and plant available nutrients were analysed by LUFA Rostock according to VDLUFA, 2007. Prior to experimental use the substrate was sieved through a 5 mm sieve to homogenise and to exclude larger stones and other particles. It was then heat sterilised in a drying oven at 85°C for 48 h to eliminate all fungal propagules. Before use the substrate was fertilised with 200 mg K (K_2SO_4), 200 mg N (NH_4NO_3), 100 mg Mg ($MgSO_4$), 50 mg P (KH_2PO_4), 10 mg Fe (Fe-EDTA), 10 mg Cu ($CuSO_4$), 10 mg Zn ($ZnSO_4$) kg^{-1} dry substrate. All nutrients were dissolved in deionised water and then mixed homogeneously into the dry substrate. The planting pots were filled with the fertilised substrate at a bulk density of 1.3 g cm^{-3}.

2.2 Preparation of fungal compartments

Fungal compartments for the insertion into the growth substrate were constructed from 60 ml plastic tubes (height 6 cm, Ø 3 cm) with a latticed wall. The walls of the tubes and the two open ends were covered with a 30 µm mesh membrane (Sefar Nitex, Sefar AG, Switzerland) that allowed hyphae, but not roots, to grow into the compartments. The membrane was fixed to the walls of the tubes using a fungicide-free silicone sealant (Probau, Bauhaus AG, Germany).

2.3 Preparation of fungal compartment substrate

The substrate preparation and the extraction of the extra-radical mycelium (ERM) were done by a modified method of Neumann and George (2005b). The substrate consisted of a 1:1 mixture of wet sieved subsoil (particle size < 40 µm) and glass beads (Ø 1.7 – 2.1 mm; Carl Roth GmbH Karlsruhe, Germany), and with 20% w/w water. This mixture allows for the extraction of almost intact fungal ERM after harvest. The substrate used for the sieving was similar with that used for the planting pot substrate. To prepare the compartment substrate the subsoil was thoroughly mixed with water in a bucket by stirring. The soil suspension was allowed to stand for a few seconds to allow larger particles to settle to the bottom. It was then poured over a 40 µm sieve. The supernatant was decanted repeatedly and the remaining sludge was dried at 65°C in a drying oven for 48 h. The temperature was then increased to 85°C for 48 h to eliminate fungal propagules. This material was then mixed with glass beads and deionised water containing dissolved nutrients. The rate of fertilisation was similar to that of the planting pot substrate.

2.4 Extraction of the extra-radical mycelium from fungal compartments and estimation of hyphae length and spore number

To extract the ERM, the content of the fungal compartments was mixed with deionised water in a bowl. After descent of the glass beads to the bottom the water including the fungal ERM and substrate particles were poured through a 40 µm sieve. Tap water was used to wash remaining substrate particles through the sieve, leaving only the ERM. The ERM was subsequently freeze-dried at -30°C for four days. After the dry weight (DW) of the ERM had been determined, subsamples of approximately 0.5 mg were transferred to 2.5 ml Eppendorf tubes and stained overnight at room temperature with 0.05% trypan blue in lactic acid. Stained samples were transferred to a laboratory blender (Waring Blender 7009G, Waring, USA) with

300 ml tap water, and blended at low speed for 40 s. Aliquots of 90 ml of the suspension were filtered onto a gridded (3 x 3 mm) 0.5 µm nitrocellulose membrane (Micronsep; GE Water & Process Technologies, USA) following the modified membrane filter method of (Hanssen *et al.* 1974). The membrane filter was mounted onto a microscopic glass slide. Hyphae length was estimated by a modified gridline intersection method (Newman 1966) under the microscope at 200 x magnification. The number of spores was assessed by counting AM spores visible on a defined area with 50 x magnification.

2.5 AM fungal isolates

The following table presents a list of all AM fungal isolates used in this study:

Table 2.1: Identity and sources of the AM fungal isolates used in this study.

AM fungal isolate	Source
Glomus mosseae [a] (Gm IFP S/08) *Glomus intraradices* [b] (Glintra IFP S/08)	Commercially available single-strain inoculum; Carrier material: quartz sand (INOQ GmbH Schnega, Germany)
Glomus mosseae BEG 12 *Glomus intraradices* BEG 110	Self propagated on maize in C-Loess (substrate treatment and fertilisation similar as in the experiments)
Field soil with indigenous AM fungi	Soil sample from the top 10 cm of a loamy sand soil, collected from a field site near Banda Aceh, Indonesia

[a,b] The phylum *Glomeromycota* has recently been re-named, accordingly *Glomus mosseae* Gerd. & Trappe (1974) is now *Funneliformis mosseae* and *Glomus intraradices* N.C. Schenck & G.S. Sm. (1982) is now *Rhizophagus intraradices* (www.amf-phylogeny.com). Thus, similar to other recent publications also in the present work the former names were kept on using to facilitate comparison with other works studying the same fungi.

2.6 Establishment of non-inoculated control plants

To compare mycorrhizal [+AM] with non-inoculated [-AM] treatments, it is necessary to ensure a similar distribution of nutrients and microorganisms other than AM fungi. Therefore, [-AM] treatments received the same amount of sterilised (heated at 85°C for 48 h) AM fungal inoculum as in [+AM] treatments, plus a filtrate from living inoculum. The filtrate was obtained by mixing fresh inoculum with deionised water (100 ml water per 50 g dry inoculum) and then filtering through a Blue Ribbon filter paper (Schleicher and Schüll, Germany).

2.7 Estimation of the AM fungal colonised root length

Plant roots were washed free from substrate and a representative sample of the fresh roots (approximately one g) taken and stained with 0.05% trypan blue in lactic acid according to

Koske and Gemma (1989). The extent of AM fungal root colonisation was determined according to a modified gridline intersection method using a stereo microscope with transmitted illumination and 50 x magnification (Kormanik and McGraw 1982). Between 250 and 300 intersections were counted per sample.

2.8 Nutrient analysis in plant tissue

Phosphorus

The plant material was dried for 48 h in a drying oven at 65°C and the DW was estimated. Subsamples (200 mg) of ground plant material (particle size 0.25 mm) were dry-ashed at 550°C for 4 h, oxidised with 5 ml 21% HNO_3, and taken up into 25 ml 2% HCl. After staining with ammonium-molybdate-vanadate solution, the P concentration in the samples was estimated colorimetrically with a spectrophotometer (EPOS analyser, Eppendorf, Germany) at a wavelength of 436 nm (Gericke and Kurmies 1952).

Total nitrogen and atom% $^{15}N_{excess}$

For quantification of nitrogen and ^{15}N concentrations in plant material, 10 mg of dried, ground shoot and root samples were analysed in an elemental analyser (Elementar Vario EL, Elementar, Germany) following the DUMAS method. After total N measurement, the N fraction of the combustion gas was automatically transferred to a coupled emission spectrometer (NOI 7; Fischer Analysen Instrumente, Leipzig, Germany) where the atom% $^{15}N_{excess}$ was determined, meaning the percentage ^{15}N atoms of all N atoms above the natural abundance.

2.9 Experimental location

The experiments were conducted in a controlled climate glasshouse located at the Institute of Vegetable and Ornamental Crops (IGZ) in Grossbeeren, Germany (52°22 N, 13°20 E). Plants were grown in a single-glazed 'Venlo' glasshouse cabin (effective area 60 m^2; Width 6.4 m; Ridge height 4 m; Ridge aeration double sided; Light transmission factor 0.7).

Chapter 3

The symbiotic recapture of nitrogen from dead mycorrhizal and non-mycorrhizal roots of tomato plants[1]

3.1 Abstract

The aim was to quantify the nitrogen (N) transferred via the extra-radical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* from both a dead host and a dead non-host donor root to a receiver tomato plant. The effect of a physical disruption of the soil containing donor plant roots and fungal mycelium on the effectiveness of N transfer was also examined.

The root systems of the donor (wild-type tomato plants or the mycorrhiza-defective *rmc* mutant tomato) and the receiver plants were separated by a 30 µm mesh, penetrable by hyphae but not by the roots. Both donor genotypes produced a similar quantity of biomass and had a similar nutrient status. Two weeks after the supply of ^{15}N to a split-root part of donor plants, the shoots were removed to kill the plants. The quantity of N transferred from the dead roots into the receiver plants was measured after a further two weeks.

Up to 10.6% of donor-root ^{15}N was recovered in the receiver plants when inoculated with the arbuscular mycorrhizal (AM) fungus. The quantity of ^{15}N derived from the mycorrhizal wild-type roots clearly exceeded that from the only weakly surface-colonised *rmc* roots. Hyphal length in the donor *rmc* root compartments was only about half that in the wild-type compartments. The disruption of the soil led to a significantly increased fungal-mediated transfer of N to the receiver plants.

The transfer of N from dead roots can be enhanced by AM fungi especially when the donor roots have been formerly colonised by AM fungi. The transfer can be further increased with higher hyphae length densities, and the present data also suggest that a direct link between receiver mycelium and internal fungal structures in dead roots may in addition facilitate N transfer. A mechanical disruption of soil containing dead roots may increase the subsequent availability of nutrients, thus promoting mycorrhizal N uptake. When associated with a living plant, the external mycelium of *G. intraradices* is readily able to re-establish itself in the soil following disruption and functions as a transfer vessel.

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3.2 Introduction

In terrestrial ecosystems, root turnover is a key component of below-ground nutrient cycling, and so provides an important source of nutrients for plant growth. The quantity of nutrient released from dead roots can be substantial, although it differs from plant species to plant species. Aerts *et al.* (1992) estimated the volume of organic nitrogen (N) turnover in soil associated with root decay to be 1.7 g N m^{-2} yr^{-1} in *Deschampsia* and 19.7 g N m^{-2} yr^{-1} in *Molinia* grasslands. Detached *Holcus* grass roots lose up to 87% of their initial N within 42 days and approximately 40% of it is taken up by other plants (van der Krift *et al.* 2001). The activity of arbuscular mycorrhizal (AM) fungi enhances the ability of plants to recycle nutrients from decaying roots (Grime et al. 1987). AM fungal networks may also be associated with different mycorrhizal plant species and so provide access to N derived from the roots of distant plants. Interconnected mycorrhizal plants may be more competitive than non-mycorrhizal species or those which are less responsive to mycorrhiza (Hartnett et al. 1993). The use of isotope-labelled phosphorus has shown that AM fungal mycelia can transfer nutrients over a distance of as much as 50 cm (Walter et al. 1996). The application of ^{15}N-enrichment technology in AM fungal compartments (accessible to AM fungi but not to roots) has enabled the quantification of soil-to-plant N transfer via the AM fungal extra-radical mycelium (ERM) from inorganic as well as organic N sources (Ames et al. 1983; Frey and Schüepp 1993; Johansen et al. 1992; Johansen et al. 1994; Hawkins et al. 2000; Mäder et al. 2000; Hawkins and George 2001; Hodge et al. 2001; Cheng et al. 2008), For example, about 30% of receiver plant N content derived from AM fungal N transfer (Ames et al. 1983; Frey and Schüepp 1993; Mäder et al. 2000) suggesting that AM fungi may have a large potential to improve N nutrition of host plants.

Only a few studies have investigated N transfer between live mycorrhizal plants where roots have been separated by an AM fungal accessible barrier (Haystead et al. 1988; Bethlenfalvay et al. 1991; Hamel et al. 1991; Ikram et al. 1994; Johansen and Jensen 1996; Jalonen et al. 2009; Li et al. 2009). A possible undesirable side-effect of AM fungal colonisation is increased root biomass (which produces a larger nutrient pool) occurring especially in legume species (Haystead et al. 1988; Li et al. 2009) and the resulting substantial level of N transfer becomes difficult to interpret. The extent of AM fungal mediated N transfer is only minor from the live root, while killing the root by removal of the shoot clearly rises the level (Johansen and Jensen 1996). The implication is that decaying roots are a much more effective source of transferrable

N than are the root exudates from living plants. However, the relative contributions of live roots, dead roots and rhizodeposition remain as yet to be clarified. The direct uptake of N from the inner cortex of live roots by hyphae is unlikely, as it would contradict the accepted idea about a two-sided mycelium functioning, i.e. the site of N uptake and anabolic assimilation into the fungal tissue is thought to be the ERM, while N is catabolised within the intra-radical mycelium (IRM) and before being released to the host plant via the arbuscules (Govindarajulu et al. 2005; Tian et al. 2010). What occurs subsequent to the dieback of colonised donor plant roots is unclear. It appears possible, however, that the AM symbiosis can facilitate the efficient (re-) absorption of root N, so that this root N is transferred directly to the receiver host plant, rather than to the rhizosphere soil, soil-borne microorganisms or non-host plants.

The initial objective of the present study was to quantify the extent of mycorrhizal N transfer from the dead roots of a donor plant to a receiver plant. The working hypothesis was that a greater quantity of N is transferred from dead mycorrhizal roots than from dead non-mycorrhizal ones. To test this, a comparison was made between a wild-type [WT] tomato (*Solanum lycopersicum* L. cv. RioGrande 76R) and a mycorrhiza-defective [*rmc*] mutant tomato. The latter cannot support intra-radical colonisation by *Glomus intraradices* (Barker et al. 1998) but its above and below ground biomass production is similar to that of the WT (Bago et al. 2006; Cavagnaro et al. 2006).
The second aim was to asses the ability of the ERM to absorb and subsequently transfer N following physical damage to the AM fungal network caused by tillage which has been repeatedly been shown to reduce the infectivity of a mycelium (McGonigle et al. 1990; Jasper et al. 1991). Furthermore, the re-establishment of the network and fungal mediated N transport can be clearly reduced following the severe disruption of the ERM (Frey and Schüepp 1993). Nevertheless, various AM fungal isolates can differ considerably from one another in terms of their sensitivity to mechanical disruption (Duan et al. 2011).

3.3 Materials and methods

3.3.1 Pre-cultivation of plant material

Seeds of the mycorrhiza-defective [*rmc*] mutant tomato (Barker *et al.* 1998) and the wild-type [WT] progenitor *Solanum lycopersicum* (L.) cv. RioGrande 76R were germinated in the dark between two layers of paper soaked with saturated $CaSO_4$ solution. To obtain seedlings with a root system suitable to split between two pots, plants were pre-cultivated in nutrient solution.

Therefore, at height of 5-6 cm, germinated seedlings were transferred to an aerated nutrient solution (pH 6.8) composed of the following: 5 mM N (half $Ca(NO_3)_2$, half NH_4NO_3); 0.7 mM P (KH_2PO_4); 4 mM K (KH_2PO_4 and K_2SO_4); 2.5 mM Ca ($Ca(NO_3)_2$ and $CaSO_4$); 1 mM Mg ($MgCl_2$); 4 mM S ($CaSO_4$ and K_2SO_4); 10 µM Fe (Fe-EDTA); 10 µM B (H_3BO_4), 5 µM Mn ($MnSO_4$); 1 µM Zn ($ZnSO_4$); 0.7 µM Cu ($CuSO_4$); 0.5 µM Mo ($(NH_4)_6Mo_7O_{24}$). Fourteen days after transfer to nutrient solution, the main root of each tomato plant was cut off one cm above the tip to break apical dominance. The plants were grown another two weeks before transplantation to the experimental planting units.

3.3.2 Preparation of growth substrate and planting units

Tripartite planting units were constructed consisting of three square plastic pots (Teku-Tainer, Pöppelmann, Germany), placed in a row and fastened together with adhesive tape. One of the outer pots (compartments) with a volume of 0.5 L, served as the ^{15}N labelling compartment (LC). The other two compartments, with a volume of 1.2 L, served as donor (DC) and receiver (RC) root compartment, respectively (see Fig 3.1b). To allow for the growth of AM fungal mycelia but not of roots between the two larger compartments, a fungal window (height = 7 cm; width = 6 cm) comprising of a 30 µm mesh membrane (Sefar Nitex; Sefar AG, Switzerland) was cut into the two adjoining walls. The window was covered by a 30 µm mesh membrane (Sefar Nitex; Sefar AG, Switzerland), that allowed fungal hyphae, but not plant roots to grow through. Each 1.2 L and 0.5 L compartment was filled with 1.4 kg and 0.6 kg dry substrate, respectively. The preparation and fertilisation of the substrate is described in Chapter 2.1.

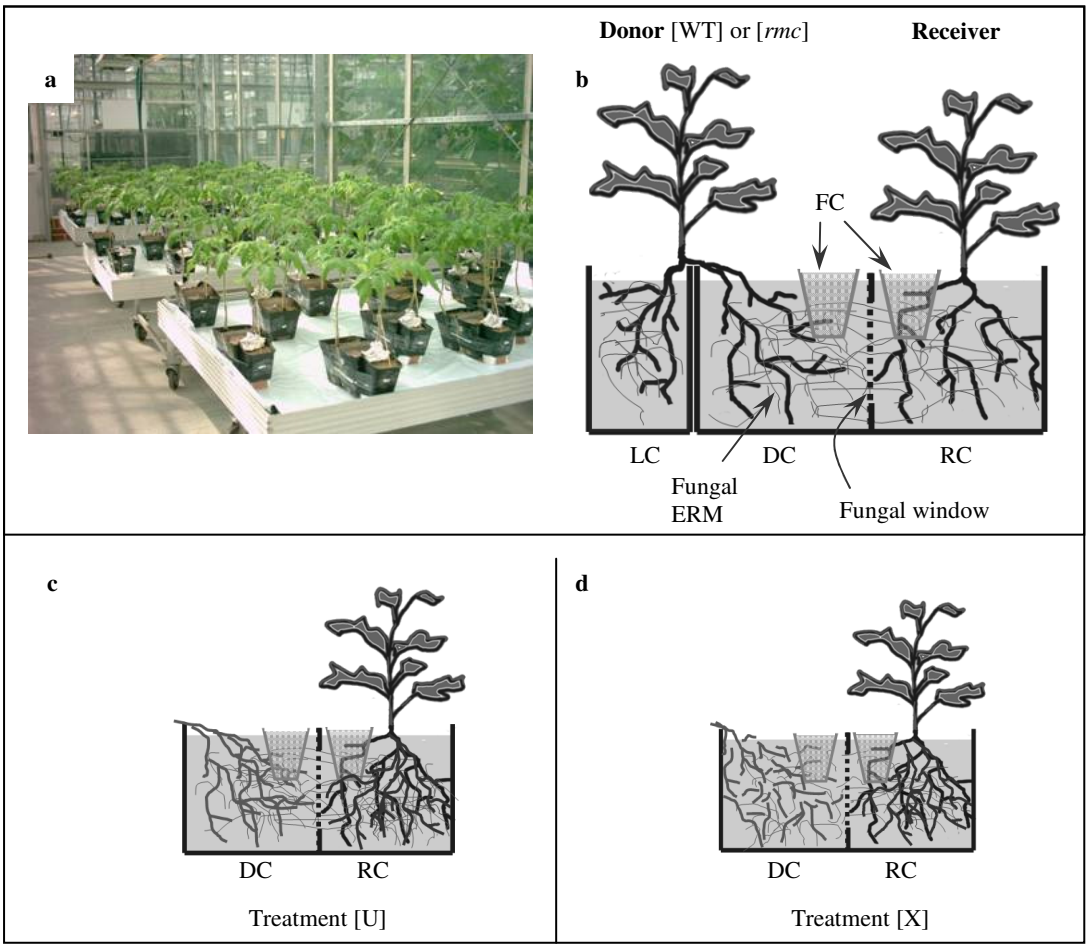

Fig. 3.1: a. Photograph of the tomato plants used in this experiment, four weeks after planting. **b.** The roots of a donor plant (either wild-type (WT) or a mycorrhiza-defective (*rmc*) mutant) were split between the donor root compartment (DC) and the ^{15}N-labelling compartment (LC). The receiver root compartment (RC) contained a WT tomato plant in each case. The RC and DC root compartments were separated from another by a 30 µm mesh membrane penetrable by AM fungal hyphae but not by roots. Both root compartments contained one fungal compartment (FC) each. Subsequent to a two–week labelling period, the LC and the donor shoots were removed and the substrate in DC was either **(c.)** left undisturbed (treatment [U]) or **(d.)** was mechanically disrupted (treatment [X]).

3.3.3 Arbuscular mycorrhizal inoculation and installation of fungal compartments

Inoculum of the AM fungus *Glomus intraradices* was used (Glintra IFP S/08; provided by INOQ GmbH; Schnega; Germany). It consisted of a mixture of AM fungal colonised roots with adhering growth substrate (quartz sand) and extra-radical mycelium with spores. To prepare [+AM] treatments, inoculum was mixed with the experimental growth substrate at a rate of 7% (w/w). [-AM] treatments were prepared as described in Chapter 2.6.

Fungal compartments (FC) were constructed from 60 ml plastic tubes (see Chapter 2.2) and filled with FC substrate prepared as described in Chapter 2.3. One fungal compartment was vertically inserted into the DC and RC of each planting unit. They were located in opposite corners, near the fungal window (see Fig 3.1b-d).

3.3.4 Plant cultivation, ^{15}N application and set-up of the donor plant treatments

At the age of 28 days, one wild-type tomato [WT] 'receiver' plant was planted into the centre of the receiver compartment, RC. At that time also one 'donor' plant, either [WT] or [*rmc*], was transferred into the labelling compartment (LC) and donor compartment (DC) with its root system split (see Fig 3.1 b). The main root of each split-root donor plant was directed into the DC and four to five upper lateral roots with a length of 5-8 cm were directed into the LC. In total, 32 pots were established.

Thirty days after planting, the substrate in the LC was supplied once with additionally 240 mg N kg^{-1} DS as $Ca(NO_3)_2$ that contained 10 atom% ^{15}N isotope (Chemotrade GmbH, Leipzig, Germany). Fourteen days after ^{15}N application, all LCs together with the split-root parts contained therein, were completely removed from the donor plants and the planting units. At that time all donor plant shoots were harvested one cm above the soil surface The growth substrate in the DC of harvested plants was either left undisturbed [U] or was disrupted [X; Fig. 3.1 c, d; Table 3.1]. To create disruption, the substrate inside the DC was cut vertically into columns of approximately one centimetre size and vertically mixed by hand using a spatula. Fungal compartments were removed from the DC during this process and were re-installed afterwards. The experimental plants were grown for 72 days in a glasshouse between September and November. The average day and night temperatures in the glasshouse were 22°C and 17°C, respectively, and the relative air humidity averaged 71%. For the last 42 days the plants received additional light for 8 h at a rate of 380 µmol m^{-2} s^{-1} at plant height provided by 400 W lamps (SON-T Agro; Philips, Germany). Daily water loss from the planting units was estimated gravimetrically and replaced with deionised water. The irrigation water was distributed among the three compartments of each planting unit, in order to maintain average water content in the substrate of each compartment at approximately 18% (w/w).

Table 3.1: Overview of the experimental treatments. The donor substrate treatment was set up after the ^{15}N-labelling period. Each treatment was replicated four times.

Donor substrate treatment	Donor genotype	Receiver genotype	Mycorrhiza inoculation of donor and receiver plant
[U]	[WT]	[WT]	[+AM]
			[-AM]
	[*rmc*]	[WT]	[+AM]
			[-AM]
[X]	[WT]	[WT]	[+AM]
			[-AM]
	[*rmc*]	[WT]	[+AM]
			[-AM]

[U] substrate in donor compartment undisturbed
[X] substrate in donor compartments disturbed
[WT] wild-type tomato plant
[*rmc*] mycorrhiza-defective tomato plant
[+ AM] inoculated with *G. intraradices*
[−AM] non-inoculated treatment

3.3.5 Harvest and analysis of plant and AM fungal material

Receiver plants and the roots in the donor compartments (DC) were harvested another 14 days after termination of the 14-days-^{15}N labelling period and the cutting off of the donor shoots (see Section 3.3.4). All roots were washed from substrate and stained to estimate the extent of AM fungal root colonisation as described in Chapter 2.7. As intra-radical AM fungal structures were absent from *rmc* roots, values for these plants represent root surface colonisation by appressoria and attached hyphae only. The ERM in the fungal compartments was extracted and freeze-dried and the spore number and hyphae length were assessed as described in Chapter 2.4.

The harvested plant material (shoot or root) was dried for 48 h at 65°C before DW was estimated. Biomass analyses for the donor split-root-parts LC and DC were conducted separately.

3.3.6 Nutrient analysis and statistics

Dried plant material (shoot or root) was finely ground and P concentration, N concentration and atom% $^{15}N_{excess}$ were analysed as described in Chapter 2.8. P and N analyses for the donor split-root-parts LC and DC were conducted separately.

The results on ^{15}N concentration measured with the method as described in Chapter 2.8 were used to calculate total ^{15}N uptake into the donor and receiver plant tissue. Assuming that ^{14}N and ^{15}N are both taken up and transferred in equal quantities, the relative amount of N transferred from the donor to receiver plant ($\%N_{transfer}$) was estimated from the ratio between ^{15}N content in the receiver plant and the sum of ^{15}N contents in both the receiver and donor plant. The $\%N_{transfer}$ was calculated using the donor plant total ^{15}N content comprising the labelled N contents in shoot and both split-root parts from LC and DC.

$$\%N_{transfer} = {}^{15}N\ content_{Receiver} \times 100 / ({}^{15}N\ content_{Donor} + {}^{15}N\ content_{Receiver}) \quad (1)$$

where

$$^{15}N\ content_{plant} = atom\%\ ^{15}N\ excess_{plant} \times total\ N\ content_{plant} / 100 \quad (2)$$

Since donor shoots and the LC were removed 14 days after labelling and 14 days before the harvest of the receiver plants, it may also be meaningful to estimate the N transfer percentage by taking into account only the N content in donor roots from the DC. Accordingly, the percentage N transferred to receiver plants from donor roots ($\%Root\ N_{transfer}$) was calculated as (according to Johansen and Jensen (1996)):

$$\%Root\ N_{transfer} = {}^{15}N\ content_{Receiver} \times 100 / ({}^{15}N\ content_{Donor\ root\ DC} + {}^{15}N\ content_{Receiver}) \quad (3)$$

The amount of N (mg per plant) transferred from the donor root ($Root\ N_{transfer}$) was estimated with the following equation:

$$Root\ N_{transfer} = \%Root\ N_{transfer} \times N\ content_{Donor\ root\ DC} / (100 - \%Root\ N_{transfer}) \quad (4)$$

The % of total N recovered in the receiver, derived from transfer ($\%N_{dfT}$), was calculated as:

$$\%\ N_{dfT} = Root\ N_{transfer} \times 100 / N\ content_{Receiver} \quad (5)$$

Four replicates per treatment were used. Provided that results passed the test for normal distribution (Kolmogorov-Smirnov test; $p > 0.05$) and homogeneity of variance (Levene test; $p > 0.05$), data were subjected to three-way ANOVA. Data for ^{15}N contents in receiver plant tissue were normalised by square root transformation prior to statistical analysis. In cases where the ANOVA indicated a significant effect of any factor, the multiple comparison Tukey-test was used to estimate differences between means of all treatments. *P* values below 0.05 obtained in both tests were interpreted as indicating significant effects. Statistic calculations were conducted using SPSS software, version 15.0 (SPSS Inc., USA). Results in tables and figures are presented as treatment means ± standard deviation.

3.4 Results

3.4.1 Dry weight and nutrient status of the donor plants

3.4.1.1 Donor plant dry weight and phosphorus uptake

Across all treatments the dry weight of the respective donor plant parts averaged 10.5 ± 0.7 g (shoot), 1.5 ± 0.3 g (root in donor root compartment DC) and 0.8 ± 0.2 g (root in ^{15}N-labelling compartment LC) per plant. The plant parts alone or the total plant dry weight were not affected by genotype and AM fungal inoculation. Donor shoot phosphorus (P) concentration was not affected by any of the treatments and averaged 1.4 ± 0.2 mg g^{-1} DW. The labelling compartment (LC) was removed from the growth unit after the labelling period, and the values measured for the nutritional status of roots from the LC in all cases reflected the results shown for the split-root part from the DC. Therefore no further results for root parts from the LC are shown. AM fungal inoculation lead to significantly higher root P concentrations in WT donor roots compared to non-inoculated controls. In contrast, *rmc* mutant plants showed no significant response to the presence of mycorrhiza (Table 3.2). The total plant P content was not affected by AM fungal inoculation or genotype (Tables 3.3). As a result of disruption of roots and mycelium in [X] treatments, P concentration and P content in donor roots were reduced by about one third compared to the undisturbed [U] treatment (Table 3.2).

Table 3.2: P concentration and P content in roots from the donor root compartment (DC). Shown are the mean values ± *SD* for wild-type [WT] or mycorrhiza-defective [*rmc*] mutant tomato plants inoculated [+AM] or non-inoculated [-AM] with *Glomus intraradices*. The donor shoots were cut off by the end of the labelling period and the substrate in the donor root compartment was either undisturbed [U], or was manually disrupted [X]. Means followed by different letters are significantly different from each other according to a multiple comparison Tukey-test ($p < 0.05$).

		Root P concentration (mg g^{-1} DW)		Root P content (mg per plant)	
Donor substrate treatment	Donor genotype	+AM	-AM	+AM	-AM
[U]	[WT]	3.12 d ± *0.22*	2.33 c ± *0.29*	4.45 b ± *1.12*	4.01 b ± *1.35*
	[*rmc*]	2.45 c ± *0.29*	2.54 c ± *0.25*	3.57 b ± *0.73*	3.69 b ± *0.46*
[X]	[WT]	2.12 c ± *0.13*	1.56 a ± *0.14*	2.89 ab ± *0.32*	1.76 a ± *0.18*
	[*rmc*]	1.84 b ± *0.09*	1.52 a ± *0.04*	2.37 ab ± *0.41*	2.19 ab ± *0.50*

Table 3.3: Three-way-ANOVA results of donor root P concentration and content and total plant P content. A significant effect of the donor genotype (G), AM fungal inoculation (M) or donor substrate treatment (T) is indicated with a black dot (n.s. = not significant).

				Interaction			
	G	M	T	G x M	G x T	M x T	G x M x T
Root P concentration in DC	●	●	●	●	n. s.	n. s.	n. s.
Root P content in DC	n.s.	n. s.	●	n. s.	n. s.	n. s.	n. s.
Plant P content	n. s.	n. s.	●	n. s.	n. s.	n. s.	n. s.

3.4.1.2 Donor plant total nitrogen and ^{15}N

Across all treatments the average shoot N concentration of donor plants averaged 18.2 ± 1.8 mg g^{-1} DW and was not affected by the genotype or AM fungal inoculation treatments. The root N concentration in inoculated treatments was by trend higher compared to non-inoculated plants, significant in the case of the [*rmc* / X] treatment. Root N content (Table 3.4) and total plant N content (Table 3.6) were not significantly different due to any of the treatments. Substrate disturbance did not significantly affect root N concentration or content.

Table 3.4: Total N concentration and N content in roots from the donor root compartment (DC). For abbreviations and statistics see Table 3.2.

		Donor root N concentration (mg g^{-1} DW)		Donor root N content (mg per plant)	
Donor substrate treatment	Donor genotype	+AM	-AM	+AM	-AM
[U]	[WT]	15.9 b ± *1.1*	13.3 a ± *1.7*	22.6 a ± *5.0*	22.7 a ± *6.3*
	[*rmc*]	14.5 ab ± *0.4*	13.9 ab ± *1.1*	21.1 a ± *3.1*	20.3 a ± *2.8*
[X]	[WT]	14.6 ab ± *1.1*	13.1 a ± *0.8*	20.0 a ± *3.0*	15.1 a ± *2.9*
	[*rmc*]	15.4 b ± *0.3*	13.3 a ± *1.1*	19.9 a ± *3.2*	18.5 a ± *3.0*

At harvest, the average atom% $^{15}N_{excess}$ in shoots was 5.3 ± 0.2% and it was not affected by the genotype or AM fungal inoculation. Atom% $^{15}N_{excess,}$ as well as ^{15}N content in roots from the DC were similar irrespective of any of the experimental factors (Table 3.5), although the ANOVA detected a significant effect on the ^{15}N status due to the AM fungal inoculation but not due to the donor genotype or substrate treatment (Table 3.6). Independent of the treatments,

the average quantity of ^{15}N recovered in the whole donor plant was 65 ± 11% of the amount applied to the labelling compartment of donor plants (about 16 mg ^{15}N was applied per plant; data not shown).

Table 3.5: Atom% $^{15}N_{excess}$ and total ^{15}N content in roots from the donor root compartment (DC) at harvest. For abbreviations and statistics see Table 3.2.

		Atom% $^{15}N_{excess}$ in donor root		^{15}N content in donor root (mg per plant)	
Donor substrate treatment	Donor genotype	+AM	-AM	+AM	-AM
[U]	[WT]	3.3 a ± *0.2*	2.9 a ± *0.4*	0.8 a ± *0.2*	0.7 a ± *0.2*
	[*rmc*]	3.8 a ± *0.6*	2.8 a ± *0.7*	0.9 a ± *0.3*	0.6 a ± *0.2*
[X]	[WT]	3.4 a ± *0.5*	3.3 a ± *0.4*	0.7 a ± *0.2*	0.5 a ± *0.1*
	[*rmc*]	3.6 a ± *0.6*	2.9 a ± *0.3*	0.8 a ± *0.2*	0.6 a ± *0.1*

Table 3.6: Three-way ANOVA results for uptake of total nitrogen and of ^{15}N into donor plant tissue. For abbreviations and statistics see Table 3.3.

				Interaction			
	G	M	T	G x M	G x T	M x T	G x M x T
Root N concentration in DC	n. s.	●	n. s.	n. s.	n. s.	n.s.	n. s.
Root N content in DC	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
Plant total N content	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
Root atom% $^{15}N_{excess}$ in DC	n. s.	●	n. s.	n. s.	n. s.	n. s.	n. s.
Root ^{15}N content in DC	n. s.	●	n. s.	n. s.	n. s.	n. s.	n. s.
Plant total ^{15}N content	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

3.4.2 Intra- and extra-radical AM fungal development

The AM fungal colonised root length of all AM fungal inoculated WT donor roots was 50 - 60% (Table 3.7), including appressoria on the root surface with attached extra-radical hyphae, spores, as well as intra-radical fungal structures. Donor roots of *rmc* mutant plants showed a colonisation rate between 12% and 16% (Table 3.7). These plants showed surface colonisation consisting only of appressoria and attached extra-radical hyphae and spores. No intra-radical fungal structures were found inside of decomposing *rmc* mutant roots, with the exception of a

few instances where intra-radical AM fungal spores were present. These spore clusters colonised a root length of not more than 1.2 ± 0.9%. Receiver root colonisation rates (WT only) ranged between 60% and 70% and were unaffected by the substrate treatments in the DC (Tables 3.7 and 3.8). No AM fungal colonisation was observed in non-inoculated treatments.

Table 3.7: Percentage of AM fungal colonised root length of roots from either the donor (DC) or the receiver (RC) compartment. For abbreviations and statistics see Table 3.2.

		AM fungal colonised root length (%)	
Donor substrate treatment	Donor genotype	DC	RC
[U]	[WT]	61.8 b ± *6.7*	67.3 a ± *20.3*
	[*rmc*]*	12.3 a ± *5.3*	60.5 a ± *6.2*
[X]	[WT]	48.0 b ± *8.7*	67.8 a ± *4.0*
	[*rmc*]*	16.2 a ± *7.9*	70.3 a ± *7.8*

*= surface colonisation

At the end of the experiment, in all AM fungal inoculated treatments the average dry weights of the ERM from the donor fungal compartments was 0.3 ± 0.1 mg cm^{-3} across all treatments (data not shown). No fungal material was found in [–AM] compartments. When the donor root was left untreated [U], the external mycelium in WT donor compartments developed approximately four times higher hyphae length and spore amounts per volume substrate compared to the ERM of the *rmc* donor fungal compartments (Fig. 3.2 a and b). Also, differences of external mycelium architecture were observed between the genotypes: Specific hyphae length and spore number per unit dry weight of mycelium in WT compartments were significantly higher than those found in *rmc* compartments (Fig. 3.2 c and d). The disruption treatment [X] did not significantly affect the hyphae length and spore density (Fig. 3.2 a,b). In contrast, the development and architecture of the ERM obtained from fungal compartments of the receiver root compartments (RC) were not significantly affected by genotype or disruption of the neighbouring donor plant root (see Fig. 3.2 and Table 3.8).

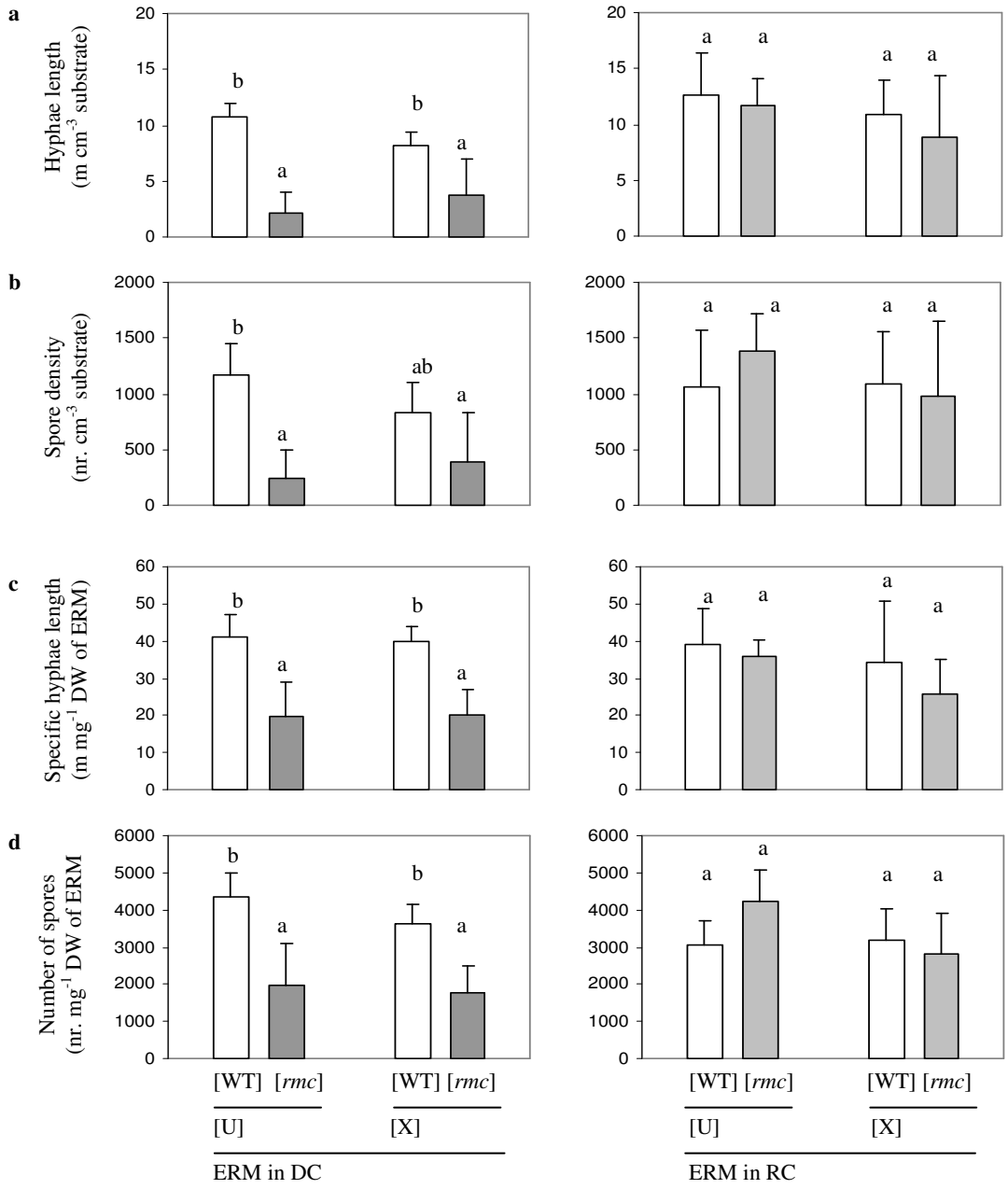

Fig. 3.2: Development of the extra-radical mycelium (ERM) obtained from fungal compartments, harvested at the end of the experiment. Shown are the results for **a.** Hyphae length density in substrate and **b.** Spore density in substrate; **c.** Specific hyphae length per mg DW and **d.** Number of spores per mg DW of ERM obtained from either donor (DC; figures left) or receiver (RC; figures right) root compartments. Different letters indicate significantly different mean values (multiple comparison Tukey-test; $p < 0.05$) due to the donor genotype [WT vs. *rmc*] or donor substrate treatment [U vs. X].

Table 3.8: Two-way-ANOVA results for the percentage of the AM fungal colonised root length of donor and receiver plants and ERM development in fungal compartments (for data see Table 3.7 and Fig. 3.2). The plant roots and the ERM were obtained from either the donor compartment (DC) or the receiver compartment (RC). A significant ($p < 0.05$) effect of the donor genotype (G), or donor substrate treatment (T) is indicated by a black dot, n.s. = not significant.

				Interaction
		G	T	G x T
Roots from DC	AM fungal colonised root length	●	n.s.	n.s.
Roots from RC	AM fungal colonised root length	n.s.	n.s.	n.s.
ERM from DC	Hyphae length density (m cm^{-3} substrate)	●	n.s.	n.s.
	Spore density (number cm^{-3} substrate)	●	n.s.	n.s.
	Specific hyphae length (m mg^{-1} DW of ERM)	●	n.s.	n.s.
	Number of spores (number mg^{-1} DW of ERM)	●	n.s.	n.s.
ERM from RC	Hyphae length density (m cm^{-3} substrate)	n.s.	n.s.	n.s.
	Spore density (number cm^{-3} substrate)	n.s.	n.s.	n.s.
	Specific hyphae length (m mg^{-1} DW of ERM)	n.s.	n.s.	n.s.
	Number of spores (number mg^{-1} DW of ERM)	n.s.	n.s.	n.s.

3.4.3 Nitrogen concentration and content in the extra-radical mycelium

At the end of the experiment AM fungal tissue N concentration and contents were estimated. The AM fungal extra-radical mycelium (ERM) showed significantly decreased nitrogen concentration and total N content when growing in compartments with *rmc* compared to compartments with WT donor roots (Fig. 3.3), an effect that was significantly related to the donor genotype but not to the disturbance treatment (Table 3.9). N status of ERM harvested from the receiver root compartments did not significantly differ due to any of the experimental factors (Fig. 3.3 and Table 3.9).

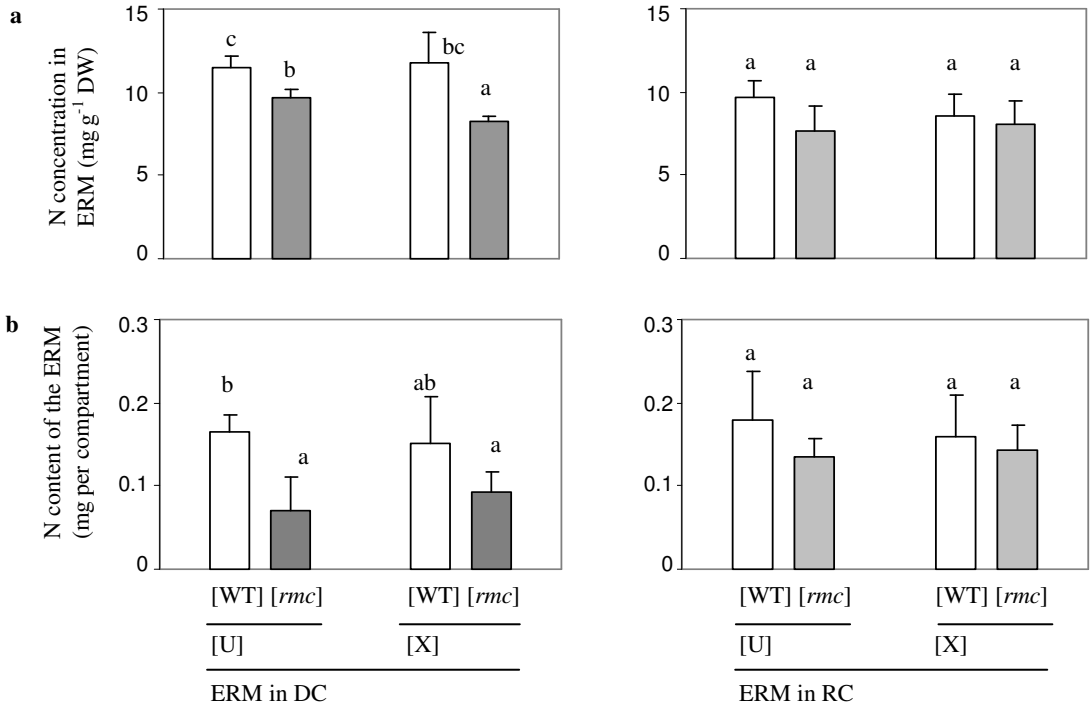

Fig. 3.3: Nitrogen status of the ERM from the fungal compartments harvested at the end of the experiment. Shown are **a.** N concentration and **b.** N content in the ERM from either donor (DC; figures left) or receiver (RC; figures right) root compartments. For abbreviations and statistics see Figure 3.2.

Table 3.9: Two-way-ANOVA for the N status of the ERM from fungal compartments located in either the donor compartment (DC) or the receiver compartment (RC). For abbreviations and statistics see Table 3.8.

				Interaction
		G	T	G x T
Mycelium from DC	N concentration	●	n.s.	n.s.
	N content	●	n.s.	n.s.
Mycelium from RC	N concentration	n.s.	n.s.	n.s.
	N content	n.s.	n.s.	n.s.

3.4.4 Dry weight and nutrient status of the receiver plants

3.4.4.1 Receiver plant dry weight and P status

Across all treatments, the receiver plant dry weight averaged 15.9 ± 0.5 g per plant. Total plant biomass and the ratio of shoot-to-root DW (data not shown) were not affected by donor plant genotype, donor treatment or AM fungal root colonisation. The total P content of receiver plant

tissue did not differ due to the neighbour plant's genotype or substrate treatment (Tables 3.10 and 3.11). When inoculated with AM fungi the shoot and root P concentration as well as the total plant P content in receiver plants were significantly increased compared to non-inoculated plants (Table 3.10 and 3.11).

Table 3.10: Phosphorus concentration and total P content in the receiver plant tissue. Receiver plants were cultivated with their root system neighboured to a ^{15}N labelled donor plant wild-type [WT] or [*rmc*] mutant root system. Both plants were either inoculated with *Glomus intraradices* [+AM] or non-inoculated [-AM]. After the ^{15}N labelling period the donor shoots were removed and the substrate in the donor root compartment was disrupted (treatment [X]) or was left undisturbed (treatment [U]). Different letters indicate significantly different mean values (Tukey-test; $p < 0.05$) due to the treatments.

		Shoot P concentration (mg g^{-1} DW)		Root P concentration (mg g^{-1} DW)		Plant P content (mg per plant)	
Donor treatment	Donor genotype	+AM	-AM	+AM	-AM	+AM	-AM
[U]	[WT]	1.55 b ± *0.05*	1.29 a ± *0.06*	2.04 b ± *0.09*	1.53 a ± *0.11*	25.91 b ± *1.65*	20.84 a ± *2.36*
	[*rmc*]	1.52 ab ± *0.13*	1.31 a ± *0.10*	2.23 b ± *0.17*	1.62 a ± *0.08*	26.39 b ± *2.97*	21.44 a ± *1.71*
[X]	[WT]	1.53 b ± *0.03*	1.18 a ± *0.09*	2.28 b ± *0.17*	1.47 a ± *0.11*	26.72 b ± *1.25*	19.73 a ± *2.11*
	[*rmc*]	1.49 ab ± *0.20*	1.29 a ± *0.08*	2.20 b ± *0.19*	1.50 a ± *0.06*	26.54 b ± *2.00*	20.56 a ± *1.71*

Table 3.11: Three-way ANOVA results for the receiver plant phosphorus status. A significant effect of AM fungal inoculation (M), donor genotype (G), or donor substrate treatment (T) is indicated with a black dot (n.s. = not significant).

				Interaction			
	G	M	T	G x M	G x T	M x T	G x M x T
Shoot P concentration	n.s.	●	n.s.	n.s.	n.s.	n.s.	n.s.
Root P concentration	n.s.	●	n.s.	n.s.	n.s.	n.s.	n.s.
Plant P content	n.s.	●	n.s.	n.s.	n.s.	n.s.	n.s.

3.4.4.2 Receiver plant status of total nitrogen and ^{15}N

Shoot N concentration (Table 3.12) and also total shoot N content (data not shown) were not significantly affected by any of the treatments. When the neighbouring donor plant was an undisturbed *rmc* plant, a significantly higher N concentration (Table 3.12) and content (data not shown) were recorded in [+AM] receiver roots compared to the [–AM] treatment. However, the total N content of the receiver plant was similar among all the treatments (Tables 3.12 and 3.13).

Table 3.12: Nitrogen concentration in shoot and root and total plant N content of the receiver plants. For abbreviations and statistics see Table 3.10.

		Shoot N concentration (mg g^{-1} DW)		Root N concentration (mg g^{-1} DW)		Plant N content (mg per plant)	
Donor substrate treatment	Donor genotype	+AM	-AM	+AM	-AM	+AM	-AM
[U]	[WT]	13.1 a *± 0.6*	13.1 a *± 0.2*	14.2 a *± 0.3*	14.1 a *± 0.4*	208.5 a *± 15.1*	206.6 a *± 23.6*
	[*rmc*]	13.3 a *± 0.4*	12.9 a *± 0.4*	15.3 b *± 0.6*	13.8 a *± 0.6*	215.3 a *± 8.9*	203.9 a *± 11.3*
[X]	[WT]	13.6 a *± 0.7*	12.9 a *± 0.2*	15.2 ab *± 0.5*	13.6 a *± 0.5*	219.4 a *± 8.6*	205.7 a *± 12.9*
	[*rmc*]	13.1 a *± 0.5*	13.5 a *± 0.9*	15.0 ab *± 0.5*	14.3 ab *± 1.0*	217.5 a *± 12.9*	210.9 a *± 5.6*

Table 3.13: Three-way ANOVA results for nitrogen concentration in shoot and root and total plant N content of the receiver plants. For statistics and abbreviations see Table 3.11.

				Interaction			
	G	M	T	G x M	G x T	M x T	G x M x T
Shoot N concentration	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root N concentration	●	●	n.s.	●	n.s.	n.s.	n.s.
Plant N content	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

^{15}N transfer from the donor to the receiver plant was clearly affected by the treatments: Significantly higher contents of ^{15}N were observed in AM fungal inoculated than in non-mycorrhizal receiver plants (Table 3.14). Only when AM fungal-inoculated, the quantity of ^{15}N

derived from WT plants clearly exceeded that from *rmc* donor plants. In undisturbed and AM fungal-inoculated treatments the quantity of ^{15}N in receiver plants originating from *rmc* mutant roots of donor plants was low and in a similar range to that of non-inoculated plants. After the disruption of the donor plant substrate [X] treatment, AM fungal-inoculated receiver plants obtained at least twice the amount of labelled N compared to the undisturbed [U] treatment, irrespective of the donor plant genotype (Fig. 3.4).

Table 3.14: ^{15}N content in shoot and root tissue of receiver plants. For abbreviations see Table 3.10. Different letters indicate significantly different mean values. Prior to multiple comparison Tukey-test ($p < 0.05$), data were square root transformed.

		^{15}N content in receiver tissue (µg per plant)			
		Shoot		Root	
Donor substrate treatment	Donor genotype	+AM	-AM	+AM	-AM
[U]	[WT]	8.7 ab ± *2.1*	0.3 a ± *0.4*	19.8 b ± *11.3*	1.8 a ± *1.5*
	[*rmc*]	0.2 a ± *0.4*	0.0 a ± *0.0*	6.2 a ± *3.4*	3.9 a ± *1.6*
[X]	[WT]	21.0 b ± *7.4*	0.0 a ± *0.0*	73.0 c ± *30.1*	8.4 a ± *2.1*
	[*rmc*]	3.3 a ± *2.8*	0.6 a ± *1.3*	25.5 b ± *9.1*	7.2 a ± *5.8*

The amount of total N transferred during the experiment ($\%N_{transfer}$; see Section 3.3.6; equation 1 and 2) was up to 1.5 ± 0.5% in WT plants and up to 0.5 ± 0.2% in *rmc* plants. The highest percentage of receiver total N content that derived from fungal transfer ($\%N_{dfT}$; equation 4 and 5) was found in WT treatments and amounted up to 0.4 ± 0.1% in the undisrupted [U] treatment and 1.1 ± 0.5% in the disrupted [X] treatment.

The %Root $N_{transfer}$ to receiver plants (equation 3) was significantly higher when donor roots were AM fungal inoculated [+AM] compared to the very low levels of non-inoculated [-AM] plants (Fig. 3.4). When AM fungal symbiosis was present, the average %Root $N_{transfer}$ from WT donor roots (3.4 ± 1.6%) clearly exceeded that from [*rmc*] roots (0.3 ± 0.4%). This effect was further enhanced by the disruption of donor roots: substrate disruption increased the amount of N transfer from AM fungal-inoculated roots of WT to 10.6 ± 4.8% and that of *rmc* plants to 3.8

± 1.5% (Fig. 3.4). The interaction between donor genotype and AM fungal inoculation was statistically significant (Table 3.15).

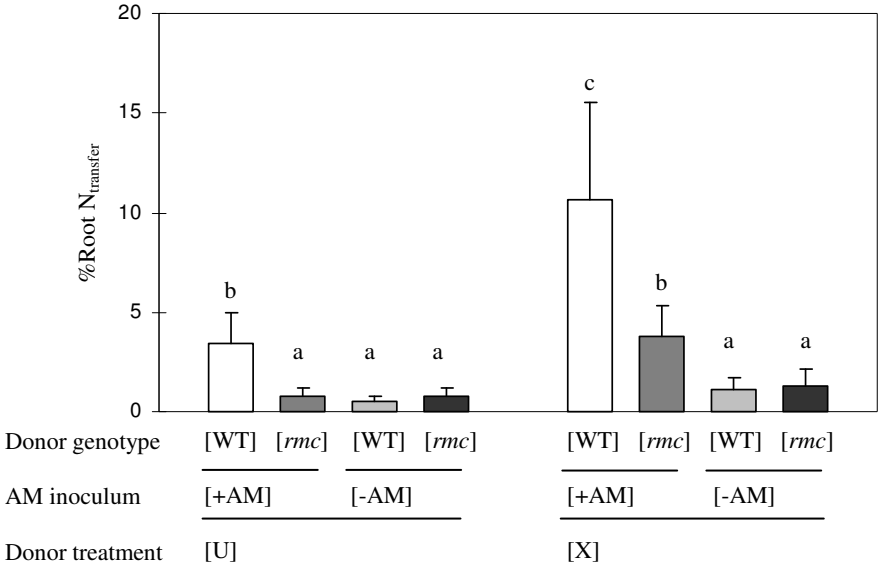

Fig. 3.4: %Root $N_{transfer}$ to receiver plants. For abbreviations see Table 3.10. Different letters indicate bars with significantly different means, estimated using the multiple comparison Tukey-test ($p < 0.05$). Prior to statistics, data were normalised by square root transformation.

Table 3.15: Three-way ANOVA results for receiver plant ^{15}N uptake (for data see Table 3.14 and Fig. 3.4). For abbreviations see Table 3.11. Significances of mean differences were calculated using the multiple comparison Tukey-test ($p < 0.05$) after data were normalised by square root transformation.

				Interaction			
	G	M	T	G x M	G x T	M x T	G x M x T
^{15}N content in receiver shoot	●	●	n.s.	●	n.s.	n.s.	n.s.
^{15}N content in receiver root	●	●	●	●	n.s.	n.s.	n.s.
$\%N_{transfer}$ to receiver	●	●	●	●	n.s.	n.s.	n.s.
%Root $N_{transfer}$ to receiver	●	●	●	●	n.s.	n.s.	n.s.

3.5 Discussion

3.5.1 Establishment of experimental conditions to quantify AM fungal derived interplant N transfer

Many tomato cultivars are unresponsive to AM fungi in terms of growth (Bryla and Koide 1990), including 'RioGrande 76R' used in the present experiment (Neumann and George 2005a). Furthermore, the use of the tomato *rmc* mutant allows quantifying the capacity of AM fungal mycelium to transfer N between roots which differed with respect to their ability to support mycorrhizal colonisation but without confounding effects of differences in plant biomass. In fact, neither the dry matter production nor the total N and P content of donor and receiver plants was significantly affected by the genotype of the donor. Therewith, all receiver plants had a similar nutrient demand when grown either adjacent to a wild-type or to an *rmc* mutant plant and on the other hand the donor plants all represented an N source of equivalent magnitude.

3.5.2 Symbiotic N transfer from mycorrhizal and non-mycorrhizal dead roots

As also revealed by Johansen and Jensen (1996), the volume of N transferred to a receiver plant from dead roots of a donor was significantly increased when the roots were mycorrhizal. The two root systems were physically isolated from one another by a nylon mesh which, nevertheless, allowed a limited extent of direct transfer between adjacent non-inoculated roots. For example, in undisrupted treatments direct transfer in the non-inoculated WT treatment was approximately 7% of that measured in the inoculated WT treatment. This form of direct N transfer is most likely to reflect the re-absorption of donor root N-losses by the receiver root, as also demonstrated by Li *et al.* (2009).

After a two week-period after shoot removal from donor plants, the amount of ^{15}N present in each receiver plants increased from 2-8 µg (not inoculated) to 30 - 90 µg per plant (inoculated with AM fungi). The proportion of the donor root N transferred ($\%RootN_{transfer}$) reached 13%. That was about one sixth of the donor root N content still available at the end of the experiment had been recovered by the receiver plants. Related to the total N content of receiver plants the proportion of N derived from fungal transfer ($\%N_{dfT}$) was <1%, irrespective of soil disturbance. Similar levels of N transfer between root systems connected by a common AM fungal mycelium have been reported by Johansen and Jensen (1996). This indicates that under the present experimental conditions the quantity of AM fungal N transfer from plant residues cannot be sufficient to have a positive impact on plant N nutrition compared to total plant N

uptake, presumably mostly by roots. Fresh plant residues in soil in many circumstances are rapidly mineralised (Nett *et al.* 2010), and hence are a direct source for N for subsequent and neighbouring plants. Also under the present experimental conditions N losses from donor roots would have increased with a longer time of ^{15}N exposure, as also shown by Ames *et al.* (1983) and Jalonen *et al.* (2009).

The contribution of AM fungi to plant N nutrition may be more important in a field situation, where mycorrhizal plants grow rather slowly and/or plant N demand exceeds its availability. This situation arises when, for example, N sources are present in an immobile form, or when drought stress limits the ability of roots to absorb nutrients from soil (Tobar et al. 1994; Subramanian and Charest 1999).

3.5.3 AM fungal mediated N transfer as affected by the presence of mycelium within the donor root

Possible sources of fungal-mediated ^{15}N uptake and transfer included (1) N in the substrate around donor roots, derived from rhizodeposition by live donor roots during the labelling period and from losses by root decay after shoot removal, and (2) N from inside the colonised donor root. The latter was accessible to AM mycelium connected to the receiver plant either directly from the cortex via the former intra-radical mycelium (IRM), or mobilised from fungal storage structures inside the root (vesicles). The use of the *rmc* mutant (lacking intra-radical colonisation) in the present experiment allowed for the separate quantification of N transfer based on the uptake via the pathway (1) (WT and *rmc* plants) and pathway (2) (WT plants only). Here it was shown that the extent of symbiotic N recapture was clearly determined by the donor plant's genotype - i.e., mycorrhizal (WT) as opposed to non-mycorrhizal (*rmc* mutant). Nearly three times more N was transferred from inoculated WT than from the corresponding *rmc* mutant donor root. Since the major source of transferred N was in the substrate released by dead donor roots, hyphal length close to the donor root may be a relevant factor. Note that the external mycelium in the *rmc* donor compartments was allowed to enter by means of the fungal window inserted between both neighbouring plants and therefore the fungus was likely in symbiosis with the receiver root. We observed that the fungal biomass and hyphae length in the WT compartments doubled that found in the *rmc* compartments. Based on isotope-labelled fertilisation of fungal compartments, it has been shown that hyphal length density in the soil is positively correlated with the capacity of the AM fungi to absorb and transfer both N (Ames *et al.* 1983) and P (Smith *et al.* 2004; Jansa *et al.* 2005). Therefore, the observed difference in N transfer between the WT and *rmc* roots may at least partly be

attributable to differences in hyphal density in donor root compartments, as parts of these hyphae were associated with receiver plants.

The pattern of root colonisation is important in the context of an N source derived from the internal structure of the root. The proportion of the WT root length successfully colonised by AM fungi following inoculation was 50-70%, while in the *rmc* root, AM fungi were restricted to the root surface (12-16%) and formed only appressoria. The extent of the *rmc* mutant root surface colonised by a mixture of *Glomus mosseae* and *Glomus intraradices* was of the same order (Neumann and George 2005a). Even after the demise of the *rmc* donor roots, the only intra-radical colonisation observed was the presence of a small number of intra-radical spores occupying not more than 2% of the root length. Thus, N transfer via the IRM from the inner root cortex could have been affected in the WT but not in the *rmc* mutant treatment. Root internal vesicles have a relevant potential to establish new root infection (Biermann and Linderman 1983), and represent a significant location for the storage of nutrient reserves (van Aarle and Olsson 2003), to be exported to the ERM as the fungus grows (Bago et al. 2002). In view of the differences in ERM density between the WT and the *rmc* donor root compartments, it remains unclear to what extent intra-radical fungal structures in colonised WT donor roots contributed to the quantity of N transferred. However, following the demise of the root, the former IRM may have been able to grow and later fuse with the symbiotic ERM originating from the receiver root compartment, facilitating the transfer of N also from root-internal fungal structures to the receiver.

3.5.4 Effect of soil disruption on N transfer to receiver plants

The effect of ERM disruption during the non-symbiotic growth of AM fungi is rather inconsistent. In some cases, a reduction in the capacity to colonise the host plant has been recorded after tillage in the field (Evans and Miller 1988; Jasper et al. 1989; Jasper et al. 1991), in some cases resulting into a decreased growth of the host plant (McGonigle et al. 1990). In contrast, effects due to the disruption treatment have not been observed (McGonigle and Miller 2000). Tillage also affects the AM fungal propagule density in the soil profile (Smith 1978; Kabir *et al.* 1998), and a high propagule density can compensate for the negative effect of tillage (Jasper et al. 1991; McGonigle and Miller 2000). The effect of disruption of the hyphae during the plant growth period and the resulting consequences for AM fungal nutrient transfer is less well explored. Periodic mechanical disruption of the ERM located in root-free and isotope-labelled fungal compartments has been shown to reduce the soil-to-plant transfer of

both N (Frey and Schüepp 1993) and P (Tuffen et al. 2002; Duan *et al.* 2011). Such a repeated and severe disruption of the mycelium network must reduce the capacity of the AM fungi to absorb nutrients, as also suggested earlier (Evans and Miller 1990). Here, mycelium was disrupted only once (as in a single mixing procedure) and root residues were used as N source (as they are usually present in vegetated soils). Under these conditions, the disruption in donor root compartments lead to higher ^{15}N contents in the receiver plants compared with undisrupted treatments. This effect was unexpected in light of earlier studies where disruption had decreased fungal nutrient transfer.

Two reasons may be responsible for the higher N transfer by hyphae after soil disruption in the present experiment. Firstly, root death can be followed by a substantial loss of nutrients from the root tissue due to autolysis (Wichern *et al.* 2007). For example, excised roots of rye grass incubated in soil for three weeks lose up to, respectively, 60% and 70% of their initial N and P (Eason and Newman 1990), and by such means lost nutrients rapidly become available to plant roots (Ritz and Newman 1985; Eissenstat 1990). Within a few days after mechanical disturbance, soil samples taken from a tilled field site showed a higher level of net N mineralisation accompanied by the continuous accumulation of nitrate susceptible to leaching than did soil sampled from an undisturbed site (Jackson et al. 2003). A similar contrast has been shown to apply in the comparison between sieved and non-sieved field soil samples (Calderon et al. 2000). The major effect of soil disruption in the present study included the fragmentation of the ^{15}N-labelled donor roots which very likely resulted in an increased root surface area exposed to microbial degradation thereby increasing N ad P losses from roots. Indeed, when the soil was disrupted P concentrations were reduced compared to undisturbed donor roots, suggesting that more nutrients were available to hyphae in disrupted soil perhaps because of leaching from damaged tissue. A better aeration in disrupted treatments may have additionally facilitated nutrient mineralisation processes in these pots.

Secondly, a single disturbance may be quickly overcome by hyphae of some AM fungi. Representatives of the *Glomus* family typically develop rapidly in the soil, and the hyphal network of *Glomus intraradices* appears to be quite insensitive to soil disruption with respect to following root colonisation (Duan et al. 2011). Mikkelsen *et al.* (2008) recorded a rate of advance of the hyphal front in soil of up to 3.8 mm per day, and Giovannetti *et al.* (1993) measured the elongation of germinated hyphae of up to approximately 5 mm per day. Injured hyphae of *Glomus* isolates are able to anastomose within minutes (de la Providencia et al. 2005), reflecting the species well-developed capacity to repair its ERM network following disturbance. Here, provided that the fungal mycelium was in continuous symbiotic association

with the (undisturbed) receiver plant, the two-week interval between soil disruption and harvest was apparently sufficient for the fungus to enter the donor root compartment. Spreading from the receiver compartment, the mycelium may have entered the donor root compartment, building linkages across the fragmented mycelium. This process would have enabled the ERM network to function once more with respect to N uptake and transfer, whether the donor was a mycorrhizal or a non-mycorrhizal plant. Note that the N concentration in ERM from fungal compartments in the *rmc* donor root compartments was significantly reduced by disruption. Attempts to grow again after the disruption and possible N losses from the fungal tissue might have lead to a dilution of nutrients within the fungal tissue. Since the ERM from WT treatments developed a significantly higher quantity of spores per unit hyphae length, it probably possessed larger N reserves for distribution within the tissue compared to the mycelium from *rmc* treatments. This may explain the higher extent of N dilution in *rmc* mycelium after disruption.

Together the anew establishment of the fungus in the donor compartment and a supposable increased availability of N from roots fragmented by soil disturbance could explain the higher AM fungal N transfer from both the inoculated WT and the *rmc* mutant donor roots compared with the non-inoculated treatments.

3.5.5 Conclusions

It has been possible to confirm that the quantity of N transferred between two root systems can be enhanced by the presence of mycorrhizal extra-radical mycelia. The quantity of N transferred during the short experimental duration was substantial compared to the total amount of N in the dead roots, but relatively small compared to the total N demand of a fast growing plant. Mycorrhizal N transfer from dying roots was further increased when these roots were AM fungal colonised before death. This difference can be reasoned by higher mycelium densities in the soil around the roots and in addition by the export of N reserves from root internal fungal structures through linkages to the receiver mycelium. The mechanical disruption of a soil containing dead roots can increase the availability of nutrients and therefore assist the process of mycorrhizal nutrient uptake and transfer. When associated with a living plant, *G. intraradices* appears to have a high potential to re-establish its network in the soil after disruption, and to function as a vehicle of N transfer. Agricultural practices, including reduced tillage may increase nutrient availability from plant residues and rather have a positive effect on AM symbiosis when involving fungi unsusceptible to a single mechanical disruption.

Chapter 4

Detached extra-radical mycelium networks of different AM fungi – Colonisation potential and plant growth promotion after mycelium disruption

4.1 Abstract

The aim was to study the potential of a detached extra-radical mycelium (ERM) network of different AM fungi to colonise a subsequent host plant. Therefore the horizontal and vertical distribution of the mycelium in soil was determined as well as the effect of mechanical disruption of the ERM in the context of the resulting re-establishment and contribution to the growth und P uptake of sweet potato plants.

A pot experiment was conducted where 'receiver' sweet potato plant cuttings were planted into compartments containing a previously established ERM of either *Glomus intraradices* BEG 110 [GI]; *Glomus mosseae* BEG 12 [GM]; or of AM fungi from an agricultural soil [AS]. At time of sweet potato planting the ERM network was separated from its 'nurse' plant where it has been established and then the ERM was either mechanically disrupted by soil mixing or left untreated.

All tested AM fungal inoculants effectively colonised the sweet potato plants within four weeks, leading to nearly double the quantity of biomass and P uptake compared to non-inoculated treatments, irrespective of the initial mechanical disruption of the ERM. Both the *Glomus* isolates produced the highest hyphae length and spore density in soil, and they colonised roots more intense in deeper soil sections. Accordingly, these fungi contributed more to both the nurse plant and the receiver plant growth as did AM fungi from the field soil in [AS] treatments. Although the latter developed much lower ERM densities in the substrate compared with [GI] and [GM] treatments, [AS] treatments increased plant growth and P uptake drastically in sweet potato plants, indicating a high nutrient uptake efficiency of these fungi. The results showed that an established, detached AM fungal ERM network can efficiently colonise host plants in absence of any mycorrhizal root fragments. A high spatial distribution and density of ERM in soil and high fungal specific nutrient uptake efficiency may ascertain fast fungal root colonisation and early contribution to plant nutrient uptake. Moderate soil disturbance such as applied in reduced tillage systems may not reduce the infection potential of AM fungi.

4.2 Introduction

Different AM fungal species possess diverse characteristics, such as specific life-cycles (Gavito and Olsson 2008), exploration patterns in soil (Boddington and Dodd 1998) and P uptake efficiencies of their mycelium (Drew *et al.* 2003). Accordingly, AM fungal species can contribute differently to plant P uptake during the period of the symbiosis, and the outcome of the plant-fungal relationship is depending on combination of both partners. An important attribute characterising fungal species is their foraging pattern in bulk soil. The ERM network can be located predominantly near the colonised root, or can have an extensive spatial distribution away from the root (Smith *et al.* 2000) bridging horizontal distances of about 15 cm (Mikkelsen *et al.* 2008). The fungal ERM not only spreads horizontally, but to some extent also follows root growth into deeper soil layers. The velocity at which the ERM spread into the soil after the establishment of the symbiosis is diverse (Mikkelsen *et al.* 2008). AM fungal species that have been shown to spread faster and further into soil (*Glomus intraradices*) are able to contribute early to plant P uptake by hyphal exploration of P resources distant from plant roots (Avio *et al.* 2006). Within the first weeks of colonising a host plant, AM fungi with a slow ERM spread development (e.g. *Gigaspora* spp.) seem to contribute less to plant P nutrition and growth compared to faster developing species, such as *Glomus* spp. (Smith *et al.* 2004), at least in the short term.

After the termination of a life-cycle, the ERM network can serve as an inoculum for a following plant. Depending on the species, infective AM fungal propagules may also be predominantly those structures present in mycorrhizal roots, such as intra-radical vesicles and intra-radical spores. The extra-radical mycelium functions as an important infective unit in that it produces stable and long lasting spores being important propagules (Biermann and Linderman 1983). Representatives of the *Glomeraceae* family are known to infect new roots also by means of hyphae, while members of *Gigasporaceae* likely depend on spores only and lack the ability to infect by means of external hyphae (Klironomos and Hart 2002). The existing spatial spread and density of the ERM in soil must therefore be crucial for the infection potential. So far, the infectivity of an excised ERM in relation to its spatial distribution with respect to horizontal and vertical mycelium proliferation in the soil has not been quantified in the absence of infective mycorrhizal roots. The first aim in this study was to determine the infective potential of an excised ERM network, previously established on a ‘nurse’ plant and thereafter re-establishing on a ‘receiver’ plant. It was hypothesized that the AM fungi with the

highest extent of vertical and horizontal proliferation in the soil prior to mycelium detachment will also have the highest infective potential and growth promoting effect on the following plant. The horizontal mycelium spread was studied in terms of hyphae length-density and spore density in soil distant from the nurse plant root. Moreover, vertical AM fungal spread was measured, characterised by the soil depth up to where root colonisation occurred, and the resulting outcome of the symbioses in terms of plant P uptake and growth was quantified. To exclude that the outcome of the symbiosis with the tested AM fungal inocula may be plant species specific, two different plant species were used to follow one another as a 'nurse' (Maize) and a 'receiver' (sweet potato) plant. Sweet potato was chosen for receiver plants as it is an important tuber crop produced in the tropics even on marginal land (Woolfe 1992) and can achieve significant growth benefits in symbiosis with AM fungi (Sieverding 1991).

As the AM symbiosis is a common and widely distributed association it is important to study effects of agricultural practices on its development. Soil disturbance, such as tillage has been reported to reduce AM fungal root colonisation, leading to reduced fungal contribution to plant P uptake (Kabir *et al.* 1997; McGonigle *et al.* 1999). Tilled field soils have been observed to have reduced sporulation of some species and AM fungal community structures dominated by *Glomus* species (Jansa *et al.* 2002). Fast spreading species may better compensate for hyphae disruption and therefore have been observed to dominantly occur in disturbed agricultural field sites (Oehl *et al.* 2003). Thus, the destruction of hyphae networks not always have a negative effect on fungal colonisation and the resulting growth benefit of the colonised plant (see McGonigle and Miller 2000; Duan *et al.* 2011). Previous studies attributed negative effects of mechanical disturbance to a decrease of the fungal P uptake effectiveness due to hyphae fragmentation and the resultant necessity to re-establish a mycelium network by means of mycorrhizal root fragments or spores (McGonigle and Miller 2000). However, earlier reports on the effects of soil disturbance on the functioning of AM symbiosis remained inconsistent and the underlying mechanisms have not been fully understood. Possibly, AM fungi that typically spread fast and intensive will be less affected in terms of re-establishment on a following host plant after being disrupted than would species colonising less intensive. The scarce knowledge about this issue lead us to the second aim of this experiment which was to obtain certainty about the effect of soil disturbance on the infection potential of a mycelium network containing spores and hyphae only. It was hypothesized that soil disturbance reduces the total infectivity of an excised AM fungal ERM and therefore reduces fungal contribution to plant P uptake and growth of a following crop.

4.3 Materials and methods

In the present experiment, using special rhizoboxes with two root compartments divided by a hyphae permeable mesh membrane, it was possible to separate the root system of a maize nurse plant from that of a sweet potato receiver plant. This allowed for the study of AM fungal colonisation and plant growth unaffected by interferences of root soil occupation or nutrient absorption by the nurse plant. Using rhizoboxes of 40 cm depth, root growth and root colonisation rate in relation to soil depth could be measured for three different AM fungal isolates.

4.3.1 Production of experimental plants

Shoot cuttings of sweet potato (*Ipomoea batatas* L.) plants were rooted and grown for 30 days in a nutrient solution (pH 6.8) composed of the following: 5 mM N (half $Ca(NO_3)_2$, half NH_4NO_3); 0.7 mM P (KH_2PO_4); 4 mM K (KH_2PO_4 and K_2SO_4); 2.5 mM Ca ($Ca(NO_3)_2$ and $CaSO_4$); 1 mM Mg ($MgCl_2$); 4 mM S ($CaSO_4$ and K_2SO_4); 10 µM Fe (Fe-EDTA); 10 µM B (H_3BO_4), 5 µM Mn ($MnSO_4$); 1 µM Zn ($ZnSO_4$); 0.7 µM Cu ($CuSO_4$); 0.5 µM Mo ($(NH_4)_6Mo_7O_{24}$). The cuttings were transplanted into experimental planting units at the 4th leaf stage with an adventitious root length of about 20 cm.

4.3.2 Preparation of rhizoboxes, substrate filling and AM fungal inoculation

To observe root growth and development of the fungal ERM in the substrate, specific two-compartmented rhizoboxes were used (Fig 4.1 a-e). This allowed a young plant to grow into an already existing AM fungal ERM in absence of the maize plant roots. One such planting unit was constructed from 8 mm thick PVC plates (bottom, sides) and removable, transparent acryl glass plates (front and back) and measured inside 18 x 9 x 39 cm (length, width, height) resulting in a total volume of 3.3 L. The planting unit was halved vertically by a porous 0.4 cm thick PVC plate resulting to two adjacent and attached equal sized soil compartments of 4.3 cm width. To allow hyphae but not roots to grow through the barrier, one side of the PVC plate was covered with a nylon membrane of 30 µm mesh size (Sefar AG; Switzerland) sealed using silicone (Probau, Bauhaus AG, Germany). Each soil compartment was filled to 3 cm below the top with 3600 g dry soil-substrate (for properties, preparation and fertilisation, see Chapter 2.1) at a bulk density of 1.3 g cm^{-3}, to a soil depth of 36 cm. AM fungal inoculum was added according to the treatment only to the soil compartment (Cpt A) of the nurse plant by mixing it homogenously into the substrate, while the neighbouring soil compartment (Cpt B) of the subsequent receiver plant was not inoculated (Fig 4.1 e).

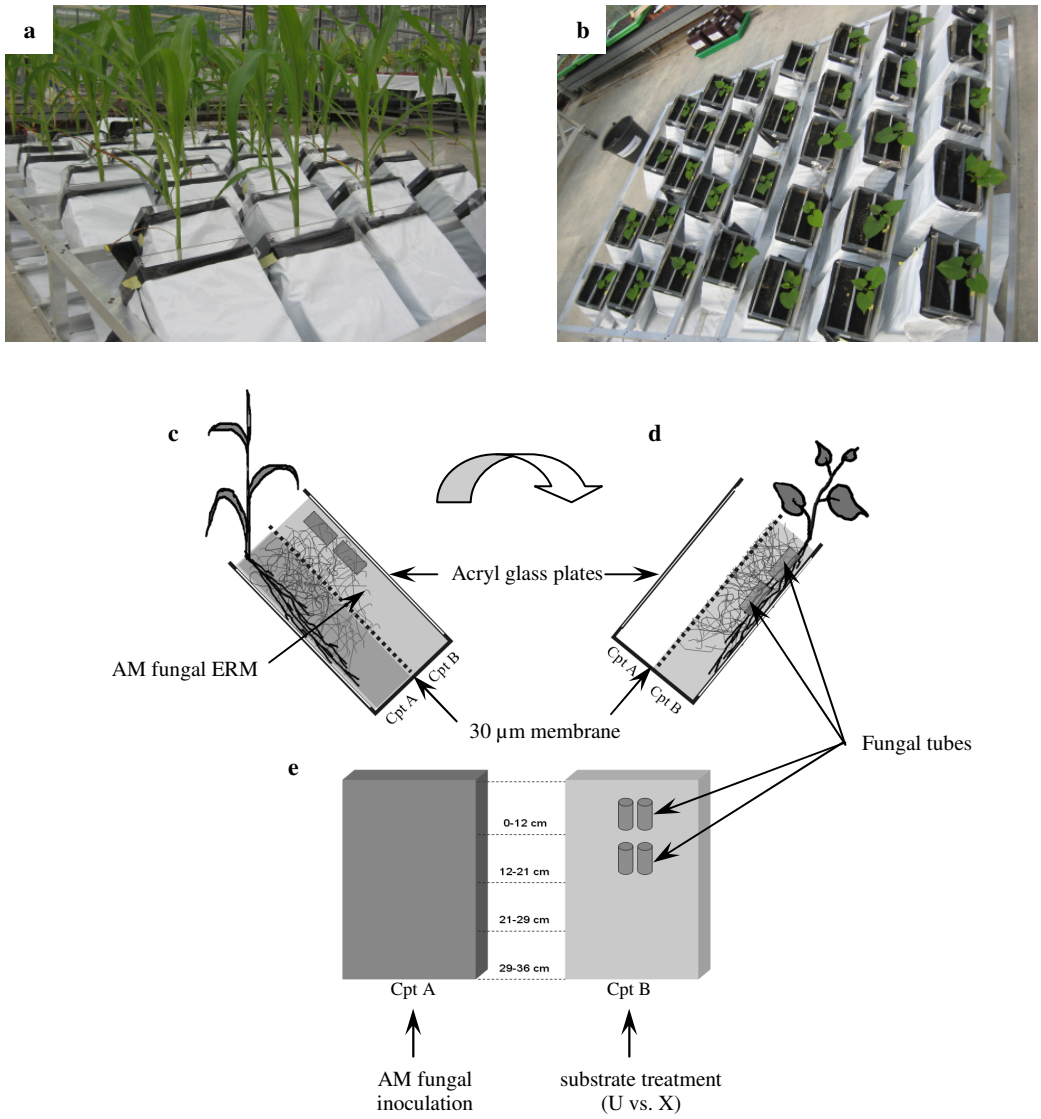

Fig. 4.1: a Photograph of the soil boxes in experimental phase 1 with maize plants in Cpt A, 25 days after sowing (DAS) and **b:** experimental phase 2 with sweet potato plants in Cpt B, 6 DAP. **c. & d:** Vertical cross-sectional view of a rhizobox, separated into Cpt A and Cpt B by a 30 µm hyphae permeable membrane. The front sides were covered by an acryl glass plate. The rhizoboxes were kept in an angle of 45° to encourage root growth towards and along the acryl glass plate and visible roots were recorded weekly. To establish an ERM network, a maize plant inoculated or not with AM fungi was grown in Cpt A and AM fungal hyphae had access to both compartments. **d & e:** Subsequent to the removal of the maize plant from Cpt A, the substrate in Cpt B was either disrupted (X) or was left untreated (U) and following the sweet potato cuttings were planted therein. **e:** Root growth was studied in four different soil depths as indicated centred between both compartments. Two fungal tubes were inserted into Cpt B at two different soil depths each.

Three different inoculum types were used in Cpt A: i) *Glomus intraradices* BEG 110 [GI]; ii) *Glomus mosseae* BEG 12 [GM], both self propagated on a similar substrate and iii) a sample

from the top layer of an agricultural soil [AS] including different AM fungal species, see Chapter 2.5. To ensure similar substrate conditions in all treatments, Cpt A received always all three inoculum types, but for each AM fungal treatment only one inoculum type was used alive while the other two inoculum types consisted of sterilised inoculum. In non-inoculated [-AM] treatments, the root compartments obtained sterilised AM fungal inoculum as a mixture of all used inoculum types and a filtrate as described in Chapter 2.6.

4.3.3 Preparation and insertion of fungal tubes

Fungal tubes (FT) were constructed from 25 ml (6.3 cm length and 2.2 cm diameter) plastic cylinders with a latticed wall. The outer surface of the plastic cylinder was covered with a nylon membrane having a mesh width of 30 µm (Sefar AG; Switzerland) that allowed hyphae but not roots to grow into the tubes. The nylon membrane was fixed to the plastic cylinders using silicone (Probau, Bauhaus AG, Germany). The FT substrate was prepared as described in Chapter 2.3 and fertilised at the same rate and with the same compounds as the rhizobox substrate. Four fungal tubes were inserted vertically into the substrate of Cpt B (Figure 4.1 d and e). Two FT were placed at 4-10 cm depth, while the remaining two were placed at 12-18 cm depth. All FT were horizontally centred and were located close to the acryl glass plate of the rhizoboxes. This permitted the study of the ERM development in two different soil depths at two different harvest times.

4.3.4 Planting, experimental set-up and growth conditions

The experiment was divided into two phases. i) The experimental phase 1 served for the establishment of the AM fungal extra-radical mycelium in the [+AM] treatments (Fig. 4.1 a). Two seeds of *Zea mays* (L.) 'Golda' were germinated within the substrate in the central position of Cpt A. The water content of the substrate was maintained at 18%. After the emergence of the second leaf, seedlings were reduced to one per rhizobox. The sides of the rhizoboxes were covered with an opaque plastic wrapping to prevent light exposure to roots. The surface of the substrate in the compartments was covered with a foil to reduce water loss by evaporation. The rhizoboxes were arranged randomised on a shelf and inclined at an angle of 45° with the planted compartment downwards to encourage root growth towards and along the acryl glass plate, where root growth could be traced weekly. After seven weeks maize plants were harvested, substrate was removed from Cpt A and soil disturbance treatment in Cpt B was conducted: The acryl glass plates were opened and the substrate was cut into squares of approximately one cm edge length. The substrate was then mixed with a spatula respecting the

vertical sections of the soil profile such that no dislocation between the depth sections occurred. During the disturbance process fungal tubes were removed from the boxes and were re-installed afterwards. ii) Experimental phase 2: Directly after the conduction of soil disturbance the boxes were closed again and a rooted sweet potato (*Ipomoea batatas* L.) cutting was planted into the neighbour compartment Cpt B (Fig. 4.1 b). Sweet potato cuttings were prepared as described in Section 4.3.1. The experiment consisted of 32 two-parted rhizoboxes including four different inoculum treatments [GI], [GM], [AS] and [-AM], combined with two different substrate treatments, where the substrate was untreated [U] or was disturbed by soil mixing [X], see Table 4.1.

Table 4.1: Overview of the treatments in the experimental phase 2 with sweet potato plants. Treatments were replicated four times.

Substrate treatment	[U]	[X]
AM fungal inoculum	[GI]	[GI]
	[GM]	[GM]
	[AS]	[AS]
	[−AM]	[−AM]

[GI]: *G. intraradices*
[GM]: *G. mosseae*
[AS]: Agricultural soil
[−AM]: Non-inoculated treatment
[U]: Substrate non-disturbed
[X]: Substrate disrupted

The experimental plants were grown in a glasshouse. Maize plants were grown for 50 days from August to September and sweet potato plants for 28 days during October. Throughout the growth period, average day and night temperatures in the glasshouse were 27°C and 21°C, respectively. The relative air humidity averaged 65%. During the last 21 days of the experimental period, the plants received additional light during 6 h with 350 µmol photons m^{-2} s^{-1} at plant height, provided by 400 W lamps (SON-T Agro; Philips, Germany). The plant water uptake was estimated gravimetrically twice a week and replaced with deionised water, applied to the top of the rhizobox. In between the gravimetric estimations, a calculated amount of the expected water uptake was given to all plants as deionised water. Root growth was monitored once a week by tracing roots growing along the acryl glass plate with a permanent marker using different colours for each week. The length of roots was later estimated using a digital map reader (Wayfinder MR H; Huger Electronics, Germany).

4.3.5 Harvest and analysis of plant and AM fungal material

Maize shoots and roots were harvested 50 DAS and sweet potato plants 28 days after planting and shoot fresh weight (FW) was recorded. For maize, the biomass of the reproductive organs

(flowers) was estimated separated from the residual shoot parts. Sweet potato plants had not developed any tubers at time of harvest. The plant root system was divided into four sections: 0-12, 12-21, 21-29 and 29-36 cm depth within the growth substrate. Root material from each section was washed free from the substrate, FW determined, and representative samples (0.5 g) of fresh roots were collected and stored in 15% ethanol. These were thereafter used to determine the AM fungal root length colonisation rate, as described in Chapter 2.7. All plant parts were dried at 65°C for three days and the dry weight (DW) was recorded. Root DW from the different soil depth was added to result in total root DW. The fungal tubes (FT) were harvested sequentially. One FT from each placement depth was harvested at time of sweet potato planting (t_0), and the other at harvest of sweet potato plants (t_1). The ERM in the FT was extracted and freeze-dried and spore number and hyphae length were assessed as described in Chapter 2.4.

4.3.6 Nutrient analysis and statistics

Subsamples of 200 mg ground plant material (shoot or root) were digested and P concentration was analysed as described in Chapter 2.8. For maize shoot nutrient analysis, all shoot parts including stem, leaves and flowers were pulverised. Four replicates per treatment were used. Provided that results passed the test for normal distribution (Kolmogorov-Smirnov test; $p > 0.05$) and homogeneity of variance (Levene test; $p > 0.05$), data were subjected to two-way ANOVA. In cases where the ANOVA indicated a significant effect of any factor, the multiple comparison Tukey-test was used to estimate differences between means of all treatments. *P* values below 0.05 obtained in both tests were interpreted as indicating significant effects. Statistic calculations were conducted using SigmaStat software, version 3.5 (Systat Software, Inc., USA). Results in tables and figures are presented as treatment means ± standard deviation.

4.4 Results

4.4.1 Maize plants in experimental phase 1

At harvest, the dry weight (DW) of maize flower, total shoot and total plant differed according to the mycorrhiza treatment, i.e. [GI] and [GM] inoculated plants showed approximately double the amount of that observed in [AS] and [-AM] treatments (Table 4.2). On the other hand, the root biomass was unaffected by the AM fungal treatment. This lead to a significantly higher shoot-to-root ratio of DW in [GI] and [GM] plant compared to [AS] and [–AM] plants. Almost double the amount of shoot P concentration was found in [GI] and [GM] plants compared with

[AS] and [–AM] plants (Table 4.2). Root P concentration was significantly higher in all AM inoculated plants compared with [–AM] plants. All mycorrhizal treatments showed significantly higher P contents compared with the [–AM] treatment, and the highest amount of total plant P content was observed in [GI] and [GM]. In inoculated treatments, total AM fungal colonised root length as the average from all soil depths, significantly differed between mycorrhiza treatments and was increased in the order of [AS] < [GM] < [GI] (Table 4.2).

Table 4.2: Maize plant biomass, total AM fungal root colonisation rate and P status, 50 DAS. Shown are means ± *SD*. For the pre-experimental phase, maize plants were grown during 50 days in Cpt A, inoculated with *Glomus intraradices* [GI], *G. mosseae* [GM], agricultural field soil [AS] or were non-inoculated [-AM]. AM fungal colonised root length (%) measured in the different soil depths was averaged to the total AM fungal root colonisation rate. Means within a row followed by different letters are significantly different (Tukey-test; $p < 0.05$; n = 8).

	[GI]	[GM]	[AS]	[-AM]
Plant DW (g per plant)	12.42 b ± *2.22*	11.44 b ± *2.04*	7.83 a ± *1.41*	6.41a ± *1.03*
Flower DW (g per plant)	1.19 b ± *0.31*	1.10 b ± *0.26*	0.43 a ± *0.12*	0.32 a ± *0.05*
Shoot DW (g per plant)	9.14 b ± *1.63*	8.49 b ± *1.61*	5.38 a ± *0.94*	4.08 a ± *0.42*
Root DW (g per plant)	3.28 a ± *0.89*	2.67 a ± *0.38*	2.32 a ± *0.52*	2.34 a ± *0.91*
Shoot-to-root ratio	2.82 ab ± *0.58*	3.17 b ± *0.22*	2.36 a ± *0.11*	1.94 a ± *0.59*
Total AM fungal colonised root length (%)	64.27 b ± *3.67*	59.93 b ± *4.48*	24.81 a ± *4.09*	0.00
Plant P content (mg per plant)	26.83 c ± *4.23*	25.61 c ± *4.04*	10.12 b ± *1.41*	6.76 a ± *1.18*
Shoot P concentration (mg g^{-1} DW)	2.55 b ± *0.13*	2.66 b ± *0.10*	1.46 a ± *0.10*	1.26 a ± *0.11*
Root P concentration (mg g^{-1} DW)	1.14 c ± *0.04*	1.12 c ± *0.08*	0.95 b ± *0.01*	0.69 a ± *0.06*

In the soil depths down to 29 cm, AM fungal root length colonisation in [GI] and [GM] treatments ranged from approximately 60 to 90%, and exceeded that from [AS] treatments more than twofold (Fig. 4.2). In the soil depth of 21-29 cm, the root colonisation rate was significantly increased in the order of [AS] < [GM] < [GI]. In the lowest soil depth (29-36 cm), less than 20% colonised root length was observed, and the values were highly variable and not significantly different among the mycorrhiza treatments. Roots from all mycorrhiza treatments,

[AS], [GM] and [GI] were observed to be colonised by AM fungal arbuscules (data not shown). Plant roots from the non-inoculated treatment [-AM] were free from AM fungal colonisation.

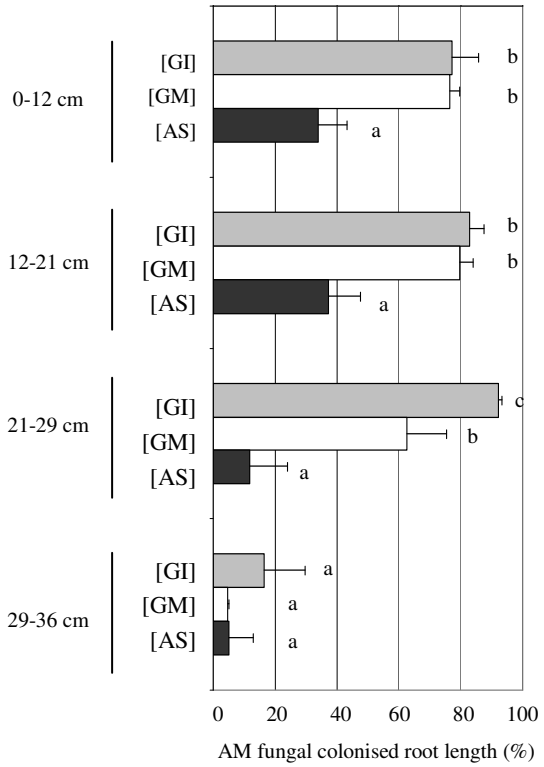

Fig. 4.2: AM fungal colonised root length (%) of maize plants, in the soil depths of 0-12; 12-21 cm; 21-29 cm and 29-36 cm. Shown are means ± *SD*. Maize plants were inoculated with *Glomus intraradices* [GI], *G. mosseae* [GM] or agricultural field soil [AS] or were non-inoculated [-AM]. Non-inoculated plants were not colonised by AM fungi. Within each depth category, means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; n = 8).

Total root lengths of maize plants traced on the acryl glass plate were not significantly different among all mycorrhizal treatments up to the time of 45 DAS (Fig. 4.3). During the growth period higher root lengths were measured in [GI] compared to non-inoculated [-AM] treatments and this effect was increased to a significant extent until 50 DAS. Root lengths from plants of [GM] and [AS] treatments were by trend higher compared to [-AM] but the values did not gain a significant difference during the growth period. During the cultivation period, in all treatments the total root length traced on the glass plates was about 760-900 cm per plant.

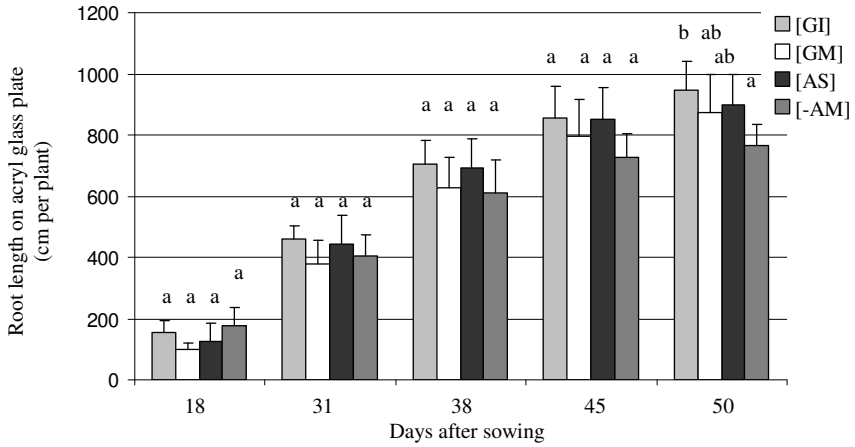

Fig. 4.3: Total root length (cm) of maize plants traced on the acryl glass plate after 18, 31, 38, 45 and 50 DAS. Shown are means ± *SD*. Maize plants were inoculated with *Glomus intraradices* [GI], *G. mosseae* [GM], agricultural field soil [AS] or were non-inoculated [-AM]. For each date, means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; n = 8).

4.4.2 Sweet potato plant biomass, AM fungal root colonisation rate and P status

At harvest, twenty-eight days after planting (DAP), sweet potato plant DW was higher in [GI] and [GM] compared with [AS] and [-AM] treatments (Tables 4.3 and 4.4). This was due to increased shoot but not root DW. According to the increased shoot growth, the shoot-to-root ratio was significantly higher in mycorrhizal compared with [-AM] treatments. The shoot DW in [AS] treatments ranged between that of [-AM] and [GI] as well as [GM] treatments (Table 4.3). Soil disturbance had no significant effect on plant biomass (Table 4.4).

Sweet potato plant roots from the non-inoculated [-AM] treatment were free from AM fungal colonisation. In mycorrhizal and undisrupted treatments, AM fungal root colonisation rate was similar in [GM] and [AS] plants, and compared to these, it was significantly higher when inoculated with [GI] (Table 4.3). When the substrate was disrupted, AM fungal colonisation rate was significantly decreased in [GI/X] plants compared with [GI/U] treatments (Table 4.3 and 4.4). Roots from all mycorrhiza treatments, [AS], [GM] and [GI] were frequently colonised with AM fungal arbuscules (data not shown).

Table 4.3: Sweet potato plant biomass and AM fungal root colonisation rate after harvest. Shown are means ± *SD*. AM fungal mycelium was established on maize plants in the neighbour compartment, inoculated with *G. intraradices* [GI], *G. mosseae* [GM] or agricultural field soil [AS]. At time of sweet potato planting, the substrate in Cpt B was untreated [U] or disturbed [X]. AM fungal colonised root length (%) measured in the different soil depths was averaged to the total AM fungal root colonisation rate. Non-inoculated plants [-AM] were not colonised by AM fungi. Means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; n = 4).

	[GI]		[GM]		[AS]		[-AM]	
	[U]	[X]	[U]	[X]	[U]	[X]	[U]	[X]
Plant DW (g per plant)	1.45 b ± *0.31*	1.63 b ± *0.52*	1.54 b ± *0.23*	1.57 b ± *0.60*	1.17 ab ± *0.38*	1.16 ab ± *0.37*	0.83 a ± *0.09*	0.89 a ± *0.20*
Shoot DW (g per plant)	1.11 b ± *0.23*	1.21 b ± *0.38*	1.17 b ± *0.19*	1.14 b ± *0.44*	0.82 ab ± *0.28*	0.83 ab ± *0.31*	0.56 a ± *0.06*	0.59 a ± *0.17*
Root DW (g per plant)	0.34 a ± *0.09*	0.42 a ± *0.14*	0.37 a ± *0.05*	0.42 a ± *0.16*	0.35 a ± *0.11*	0.33 a ± *0.07*	0.27 a ± *0.04*	0.31a ± *0.03*
Shoot-to-root ratio	3.30 b ± *0.37*	2.90 b ± *0.22*	3.20 b ± *0.37*	2.69 b ± *0.22*	2.32 ab ± *0.26*	2.47 ab ± *0.60*	2.09 a ± *0.24*	1.89 a ± *0.45*
Total AM fungal colonised root length (%)	35.62 b ± *4.89*	25.90 a ± *3.19*	22.41 a ± *1.47*	24.09 a ± *5.05*	25.84 a ± *7.16*	19.19 a ± *8.46*	/	/

Table 4.4: Two-way-ANOVA results for biomass and AM fungal colonisation rate of sweet potato plants after harvest (see Table 4.3). A significant effect of AM fungal inoculum (M) or disturbance treatment (D) is indicated with a black dot (n.s. = not significant).

			Interaction
	M	D	M x D
Plant DW (g per plant)	●	n.s.	n.s.
Shoot DW (g per plant)	●	n.s.	n.s.
Root DW (g per plant)	n.s.	n.s.	n.s.
Shoot-to-root ratio	●	n.s.	n.s.
AM fungal colonised root length (%)	●	●	n.s.

Sweet potato root DW was largest in the soil depth of 12 – 21 cm (Fig. 4.4). This resulted in about 35-45% root DW of total root biomass in this depth layer. In this depth, the root DW percentage was significantly higher in [GI] and [GM] compared to [AS] and [–AM] treatments. In the upper and the two deepest layers, not more than 25% of total root DW was developed in each layer, irrespective of the AM fungal treatment. In the deepest root zone (29-36 cm) a significantly lower percentage of root DW was present in [GI] and [GM] compared to [AS] and [–AM] treatments (Fig. 4.4). Over all soil depths, the disturbance treatment [X] had no

significant effect on the percentage root DW distribution compared with undisturbed [U] treatments, irrespective of the mycorrhiza treatment (data not shown).

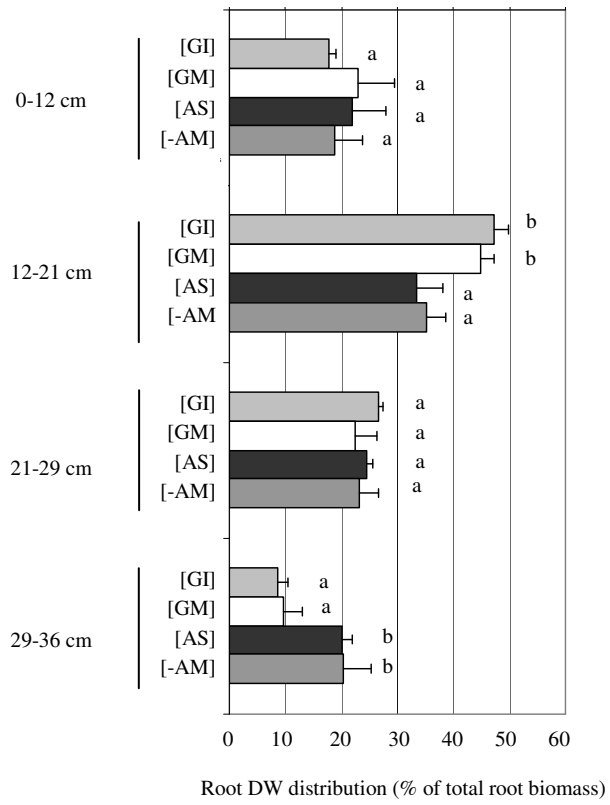

Fig. 4.4: Root DW distribution of sweet potato plant in % of total root biomass in the soil depths of 0-12 cm; 12-21 cm; 21-29 cm and 29-36 cm. Data are averaged over disturbance treatments. Prior to the cultivation of sweet potato plants, AM fungal mycelium was established on maize plants in the neighbour compartment, inoculated with *G. intraradices* [GI], *G. mosseae* [GM], agricultural field soil [AS] or non-inoculated [-AM]. Data was square root transformed before being analysed statistically. Within each depth category, means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; $n = 8$).

The AM fungal root colonisation rate in different soil depths was highest in 12-21 cm depth (Fig. 4.5). In a depth of 21-29 cm the mycorrhizal treatments differed significantly. In this depth root colonisation rates increased in the order of [AS] < [GM] < [GI], and the fungal colonisation in the [AS] treatment was very low. Roots from all mycorrhiza treatments were observed to be colonised by AM fungal arbuscules (data not shown). No colonisation was found in roots located deeper than 29 cm. A significantly decreased root colonisation rate with

G. intraradices in depths of 21-29 cm was observed in disturbed [GI/X] compared to untreated [GI/U] treatments (Fig. 4.5).

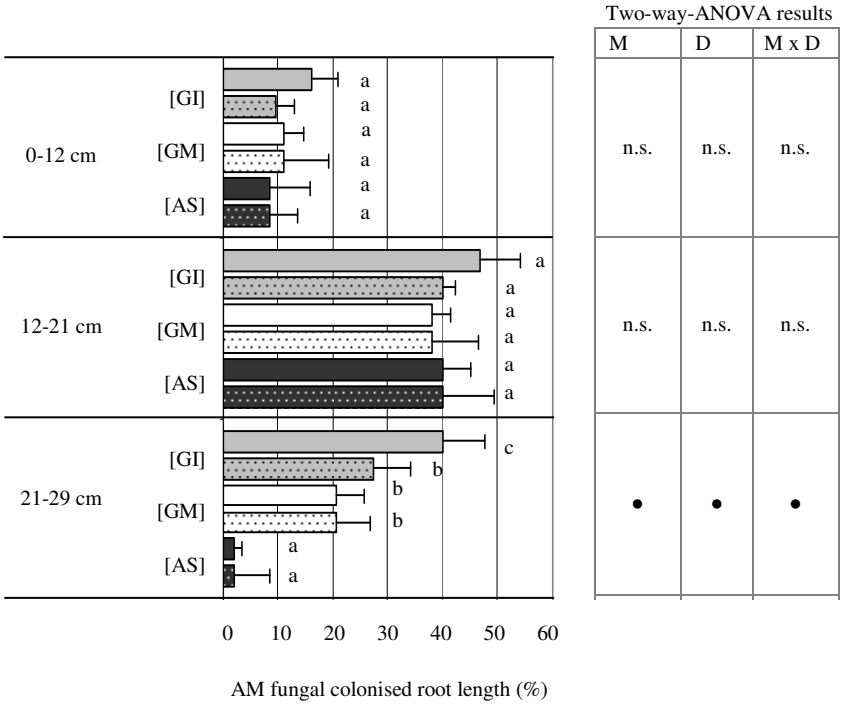

Fig. 4.5: AM fungal root colonisation rate (%) of sweet potato plants, observed in the substrate depths of 0-12; 12-21 cm; 21-29 cm and 29-36 cm. Shown are means ± *SD*. AM fungal mycelium was established in the planted nurse plant compartment, inoculated with *G. intraradices* [GI], *G. mosseae* [GM] or agricultural field soil [AS]. At time of sweet potato planting, the substrate was untreated [U] (plain bars) or disturbed [X] (dotted bars). In the soil deeper than 29 cm, no AM fungal root colonisation occurred. Within each depth category, means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; n = 4). Columns on the right show the two-way-ANOVA results for the AM fungal root colonisation rate. For abbreviations and statistics see Table 4.4.

Shoot and root P concentration in sweet potato plants was lowest in [-AM] treatments; [GI] and [GM] plants showed about double the shoot P concentration compared to [-AM] treatments (Figs. 4.6 a, b; Table 4.7). [AS] plant shoot P concentration remained between the [-AM] treatment and the treatments inoculated with either [GI] or [GM]. The P concentration in roots was similar compared to that of the shoots. Plant total P content in the different mycorrhizal treatments was not significantly different but was much higher than that of non-inoculated treatments. Soil disturbance had no significant effect on P concentration or content in any plant part, irrespective of the inoculation treatment (Fig. 4.6 a, b, c; Table 4.7).

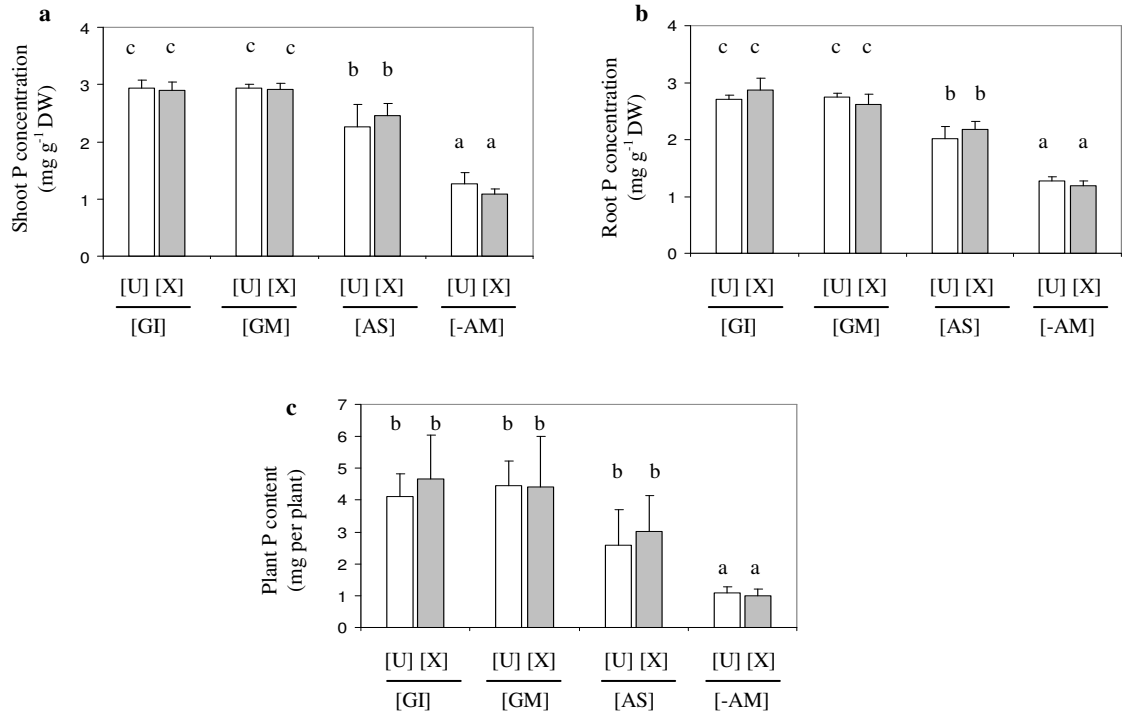

Fig. 4.6: Sweet potato plant P status after harvest. a. Shoot P concentration, **b.** Root P concentration and **c.** Total P content in the plant. Prior to the cultivation of sweet potato plants, AM fungal mycelium was established in the planted neighbour compartment, inoculated with *G. intraradices* [GI], *G. mosseae* [GM], agricultural field soil [AS], or non-inoculated [-AM]. At time of sweet potato planting, the substrate was untreated [U] or disturbed [X]. Means followed by different letters are significantly different from each other (Tukey-test; $p < 0.05$; n = 4).

Table 4.5: Two-way-ANOVA results for P status of sweet potato plants after harvest (see Fig. 4.6). A significant effect of AM fungal inoculum (M), or disturbance treatment (D) is indicated with a black dot (n.s. = not significant).

			Interaction
	M	D	M x D
Plant P content (mg per plant)	●	n.s.	n.s.
Shoot P concentration (mg g^{-1} DW)	●	n.s.	n.s.
Root P concentration (mg g^{-1} DW)	●	n.s.	n.s.

4.4.3 AM fungal ERM development in fungal tubes

When plants were non-inoculated [-AM], no AM fungal hyphae or spores were observed in fungal tubes (FT) from both studied profile depths (4-10 and 12-18 cm). At the time of sweet potato planting (t_0), the ERM DW from [GI] and [GM] from the upper depth averaged from 4.4

to 5.8 mg per FT, this was significantly higher compared to that of [AS] treatment (Table 4.6). The ERM DW in FT from the [AS] treatment was less than half a milligram. At the final harvest date (t_1), ERM DW in FT of [GI] and [GM] treatments was at a similar magnitude compared with that of the earlier harvest date (t_0), and still significantly exceeded that of the [AS] treatment. In the [AS] treatment, in the upper soil depth mycelium DW was increased at t_1 compared with t_0. As shown by the two-way-ANOVA, the disturbance treatment as a main factor did not affect the ERM DW significantly at harvest time (t_1) (Table 4.6; right side), though fungal DW was significantly lower in the deeper FT of [GM/X] compared with [GM/U] treatment (Table 4.6; left side).

Table 4.6: AM fungal extra-radical mycelium DW (mg per 25 ml FT). Fungal tubes from the receiver compartment from 4-10 cm and 12-18 cm soil depth and were exerted at time of sweet potato planting (t_0) or harvest (t_1), respectively. Prior to the cultivation of sweet potato plants, AM fungal mycelium was established in the planted neighbour compartment, inoculated with *G. intraradices* [GI], *G. mosseae* [GM] or agricultural field soil [AS]. At time of sweet potato planting, the substrate was untreated [U] or disturbed [X]. Means within a row followed by different letters are significantly different (Tukey-test; $p < 0.05$; $n = 4$). The right-sided columns of the table show the results of the statistical analysis using two-way-ANOVA, for abbreviations and statistics see Table 4.5. Data was square root transformed before being analysed statistically. In case means belong to the sampling date t_0, the factor disturbance (D) was not included in the ANOVA-analysis, since data were obtained before the set-up of the disturbance treatment.

		[GI]		[GM]		[AS]				Interaction
Date	Soil depth	[U]	[X]	[U]	[X]	[U]	[X]	M	D	M x D
t_0	4 - 10 cm	4.73 b ± *1.85*	3.99 b ± *0.80*	5.79 b ± *0.76*	4.39 b ± *0.83*	0.06 a ± *0.08*	0.16 a ± *0.06*	•	-/-	-/-
	12 - 18 cm	3.74 b ± *1.41*	3.22 b ± *1.61*	3.12 b ± *0.90*	2.51 b ± *0.99*	0.03 a ± *0.02*	0.00 a ± *0.00*	•	-/-	-/-
t_1	4 - 10 cm	5.72 c ± *1.24*	6.37 c ± *1.08*	4.71 bc ± *1.02*	2.91 b ± *1. 20*	0.60 a ± *0.78*	0.45 a ± *0.65*	•	n.s.	n.s.
	12 - 18 cm	3.08 c ± *2.04*	3.79 c ± 1.*95*	4.16 c ± *1.81*	1.88 b ± *1.14*	0.01 a ± *0.02*	0.08 a ± *0.07*	•	n.s.	n.s.

At the time of sweet potato planting (t_0), the hyphae length, spore density and number of spores per unit hyphae length in the substrate of the FT obtained from the soil depth of 4-10 cm was similar in both *Glomus* inoculated treatments and was significantly higher when compared with the [AS] treatment (Fig. 4.7 a, b, c; Table 4.7).

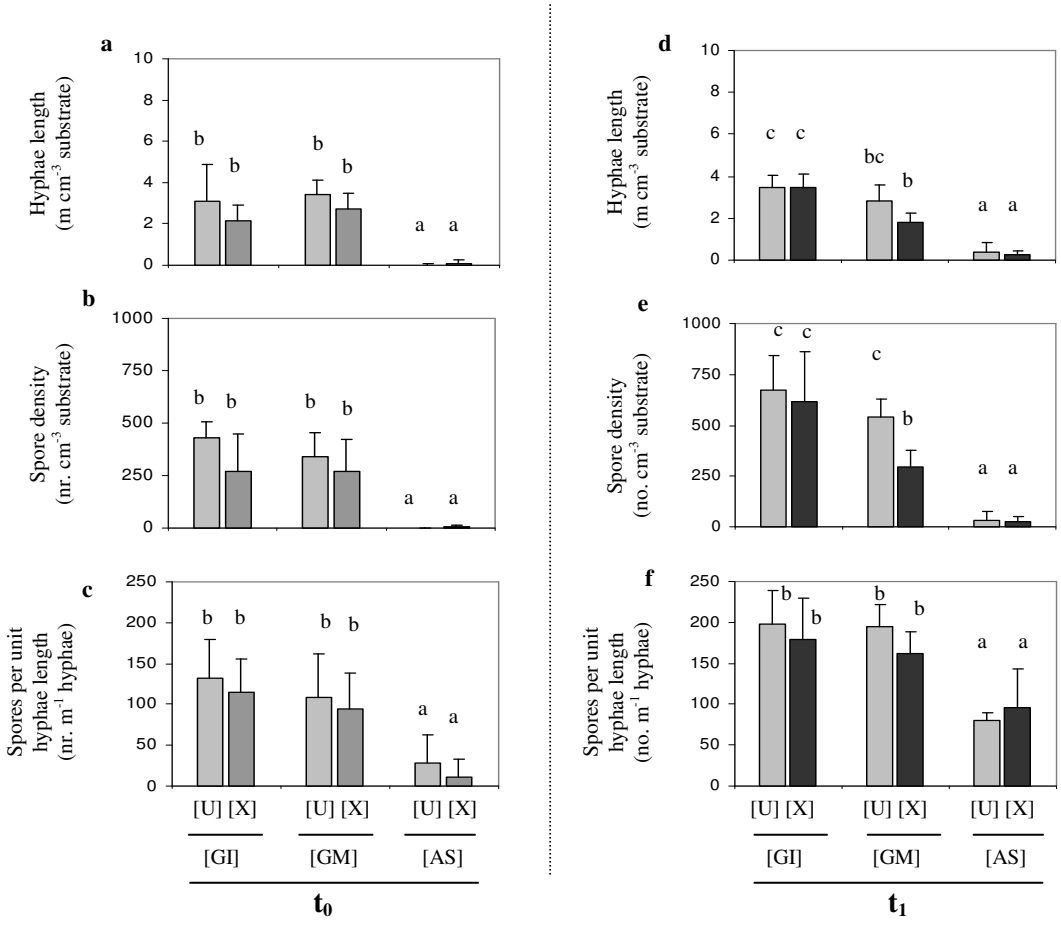

Fig. 4.7: AM fungal ERM development in fungal tubes obtained from the upper 4-10 cm of soil in the receiver compartment. Fungal tubes were sampled before set-up of disturbance treatments at time of sweet potato planting (t_0; figures left) or four weeks after the conduction of disturbance treatments at sweet potato harvest (t_1; figures right). Shown are hyphae length density (**a.** and **d.**), spore density in the substrate (**b.** and **e.**) and number of spores per unit hyphae length (**c.** and **f.**). For abbreviations and statistics see Fig. 4.6. Data was square root transformed before statistical analysis.

At time of sweet potato harvest (t_1), the hyphae length density in FT was about 3.4 m cm^{-3} in [GI] and about 2.8 m cm^{-3} in [GM] inoculated treatments (Fig. 4.7 d) and thereby comprised significantly higher hyphae length densities compared to [AS] treatments with up to 0.4 m cm^{-3}. A similar difference between fungal treatments was shown for spore densities in the FT (Fig. 4.7 e). At (t_1) the number of spores in FT of [AS] inoculated rhizoboxes averaged 33 ± 41 [U] and 27 ± 23 [X] spores cm^{-3}. The number of spores per meter hyphae length was similar between [GI] and [GM] but much higher compared with that of the [AS] treatment (Fig. 4.7 f). Soil disturbance did not have a significant effect on the total hyphae length, but significantly decreased spore density in FT of the [GM] treatment (Fig. 4.7 d, e; Table 4.7). A significant

interaction between AM fungal inoculum and soil disturbance occurred in the case of spore density in fungal tubes (Table 4.7) which was related to a significantly decreased spore density in FT after disturbance of the [GM] treatments only (Fig. 4.7 e).

Table 4.7: Two-way-ANOVA results for the ERM development in fungal tubes from the upper 4-10 cm at planting (t_0) or at harvest (t_1) of sweet potato plants (see Fig. 4.7). For abbreviations and statistics see Table 4.5. Data was square root transformed before being analysed statistically. In case means belong to the sampling date t_0, the factor disturbance (D) was not included in the ANOVA-analysis, since data were obtained before the set-up of the disturbance treatment.

				Interaction
		M	D	M x D
Hyphae length (m cm^{-3} substrate)	t_0	●	-/-	-/-
	t_1	●	n.s.	n.s.
Spore density (nr. cm^{-3} substrate)	t_0	●	-/-	-/-
	t_1	●	●	n.s.
Number of spores per unit hyphae length	t_0	●	-/-	-/-
	t_1	●	n.s.	n.s.

4.5 Discussion

4.5.1 Maize plant colonisation and growth in experimental phase 1

During experimental phase 1 (for ERM establishment on maize plants), large functional differences were observed between the inocula utilised. The extent of root length colonisation was clearly higher in [GI] and [GM] treatments (60%) compared with [AS] plants which had a low AM fungal root colonisation rate of about 25%. Similar results were shown by Douds *et al.* (1993), who reported AM fungal colonisation rates of about 30% of field-soil inoculated, four-week old maize plants grown in the greenhouse. The observed higher colonisation rates of [GI] and [GM] compared with [AS] inocula might indicate that the *Glomus* inocula had a development advantage due to a better adaption to the experimental soil conditions wherein the fungi have been propagated before. It might also reflect the species specific growth pattern of the respective fungi in soil and in roots, determining the inoculum potential of a fungus (McGee *et al.* 1999; van der Heijden *et al.* 2006).

AM fungal inoculation with [GI] and [GM] increased maize plant growth and total P content about two- and three-fold, respectively, compared with non-inoculated maize plants. [AS] plants showed a similar biomass production but contained significantly more P compared to the

non-inoculated plants. In the last week of cultivation, maize root growth on the acryl glass plates of [GI] was significantly higher and that of [GM] and [AS] plants was by trend higher compared with non-inoculated plants (see Fig 4.3). Also, P concentration in roots and plant total P uptake was increased in the mycorrhizal plants (see Table 4.2). These findings indicate that the P uptake of the maize plants was increased by all used sources of AM fungal inoculation compared with non-inoculated plants after the time period of seven weeks, and that the *Glomus* inocula increased plant growth more effectively compared to field soil inoculation [AS].

After the pre-cultivation phase (t_0), the extent of vertical distribution of colonisation rate and ERM abundance in fungal tubes in the receiver compartment depended on the utilised AM fungal inocula and decreased in the order [GI] < [GM] < [AS]. Differences in fungal development lead to distinct distribution patterns and spread intensities of the mycelium within the receiver root compartments before the maize root compartment was emptied. The following paragraphs will discuss the consequences for sweet potato AM fungal colonisation pattern and mycorrhizal contribution to plant growth after the mycelium had been detached from the former host by maize plant removal.

4.5.2 Detached excised extra-radical mycelium as a source of AM fungal colonisation

The present experiment allowed for the build-up of AM fungal colonisation from detached extra-radical mycelia (ERM), and this successfully enabled AM root colonisation of sweet potato plants. As being removed from the neighbour compartment before planting of sweet potato plants, AM colonised roots of maize plants were not present during the experimental phase 2. Therefore, intra-radical vesicles were absent, structures that are considered as important propagules for *Glomus* species (Tommerup and Abbott 1981; Biermann and Linderman 1983) and usually are present in soil-based AM fungal inoculum (Ijdo *et al.* 2011). Thus, the excised ERM in this study consisted only of spores and hyphae. Consequently, root colonisation was limited by the use of such structures serving as propagules. Not all fungal species are able to establish new root colonisation using hyphae fragments only: Representatives of the order *Glominae* were shown to establish from hyphae fragments as well as spores, while members of *Gigasporaceae* depend solely on spores (Klironomos and Hart 2002). However, the present study does not allow a distinction, whether hyphae fragments or spores were more important for subsequent colonisation of sweet potato roots. Knowledge about species related establishment from extra-radical propagules in absence of mycorrhizal

roots is scarce, and the number of studies on infectivity rates of individual AM fungal structures still is limited (Tommerup and Abbott 1981; Biermann and Linderman 1983; Klironomos and Hart 2002). Nevertheless, the excised mycelium allowed a considerable colonisation rate of about 20-35% after four weeks in sweet potato roots. This rate was similar for both the *Glomus* as well as the [AS] treatment. Considering the short time period, this colonisation rate is high compared to that observed in the study of O'Keefe and Sylvia (1993), who obtained similar values more than eight weeks after planting of sweet potato in the field. In the present study, colonisation by means of the excised ERM in all used AM fungal inocula was followed by a clear improvement of sweet potato plant growth. Therefore, it can be stated that the infection potential of the excised ERM studied here was high.

4.5.3 The growth response and P uptake of sweet potato plants in relation to AM fungal colonisation

About one third of the sweet potato plant root length was colonised irrespective of being inoculated with the *Glomus* strains [GI], [GM] and with field soil [AS]. The results revealed a high response of sweet potato plants to mycorrhizal root colonisation, since all AM fungal inocula increased dramatically the growth of the host plant. Total P content of the plant as well as P concentration in shoot and root was increased by AM fungal inoculation (approximately two-fold in [GI] and [GM] and approximately 1.8-fold in the [AS] treatment). Accordingly, shoot P concentration in the dry matter ranged between 0.25 and 0.30% in mycorrhizal sweet potato plants compared to about 0.12% in non-inoculated plants. This indicates that non-inoculated sweet potato plants were clearly P-deficient (crop plants are characterised as P-deficient with shoot P concentrations below 0.2% (Marschner 1995)). The significant enhancement of tissue P concentration from an insufficient to a sufficient nutritional P status due to AM fungal colonisation reflects the high mycorrhizal dependency of sweet potato plants. A clear increase of net P uptake in inoculated plants was achieved within only four weeks in an early plant growth stage. Under conditions of restricted P availability in soil, AM fungal colonisation may be very beneficial for sweet potato growth. Additional P stored in plant tissue may serve as a resource for later plant growth, especially at phases where plant nutrient demand is high, e.g. during rapid plant growth or at storage-root formation (O'Keefe and Sylvia 1993).
In the present study, AM fungal arbuscules were observed in roots of [GI], [GM] and of field soil [AS] inoculated treatments, underlining the presence of functional AM colonisation in all mycorrhizal treatments. Both *Glomus* inocula lead to clearly higher root colonisation rates and showed a more thorough extension of root colonisation into the deeper soil sections compared

to plants inoculated with field soil [AS]. Accordingly, AM fungal contribution to sweet potato plant growth and net P uptake was higher for both *Glomus* compared with [AS] treatments. A similar pattern of AM fungal colonisation and resulting plant growth promotion by the respective AM fungal inocula was also observed with the preceding maize plants. Besides other indicators, the total degree of hyphal extension in the soil volume is likely reflected by the root colonisation pattern according to depth. Therefore, an improved contribution to host plant growth and nutrient uptake might be attributed to a higher absorptive surface area of the fungal mycelium in the soil. Underlining this, AM fungal mediated improvement of host plant P nutrition can not be predicted directly by the percentage of total colonised root length (Jones *et al.* 1998; Burleigh *et al.* 2002).

4.5.4 The growth pattern of the AM fungal extra-radical mycelium

In order to estimate the extent of mycelium spread in the 'receiver' substrate, fungal tubes (FT) were inserted into the Cpt B in two different soil depths. A considerable amount of ERM (2.4 to 6.3 mg per 25-ml-FT) was harvested from the *Glomus* inoculated plants. Hyphae length was between 2.8 and 3.4 m cm^{-3} substrate which was within the range reported by Hawkins and George (2001) and Hart and Reader (2002). The FT from the [AS] treatment contained relatively low amounts of spores (up to 30 per cm^3), similar to the amounts reported for agricultural field soil (Hayman 1970; Gosling *et al.* 2010) and for natural grasslands (Oehl *et al.* 2003). Here, AM fungi of both *Glomus* inocula produced significantly higher amounts of spores per unit hyphae length compared with fungi of the [AS] treatment. Typically, *Glomus* species (also termed as ‚r-strategists') have a higher specific spore density in the mycelium compared to the representatives of *Gigasporaceae* (de la Providencia *et al.* 2005). It is likely that AM fungi of the latter family were also part of the field soil inoculum [AS] used in the present study. Representing C sources, spores may crucially support later fungal proliferation into soil during the long term continuation of the symbiosis, as reported for *Scutellospora* isolates (family *Gigasporaceae*) (Gavito and Olsson 2008). However, long-term effects or strategies of ERM formation in different AM fungi were not studied in the present experiment.

During the experimental phase 1, before being detached from their host plant, AM fungi proliferated into the substrate and into FT of the plant-free root compartment Cpt B. The ERM in FT harvested at the first sampling date (t_0) represented the spatial spread into the substrate as it was achieved in absence of sweet potato plants. At time t_0, ERM DW, hyphae length and spore number recovered from FT of *Glomus* inoculated compartments was approximately two-

fold compared with those in the [AS] treatment. These differences between the AM fungal inocula were consistent also at the second harvest (t_1) when AM fungi have been associated with sweet potato plants since four weeks. ERM amounts from FT estimated at time t_0 and t_1 were not clearly different. However, mycelium usually gets lost by means of turnover (Staddon *et al.* 2003), and the proportion of viable mycelium is unknown because it was not estimated here. It is also possible that fungal growth after the planting of the sweet potato plant was predominantly concentrated on the colonisation of roots at the expense of the proliferation into the substrate. A simultaneous support of both activities would cost an inappropriate energetic effort for the fungus. After sweet potato roots became present in the receiver root compartment, the mycelium spread in FT (located next to the roots) may have declined to avoid the close vicinity of roots. A preferred mycelium proliferation into bulk soil that is several centimetres away from the rhizosphere has been demonstrated earlier (Mikkelsen *et al.* 2008).

It has been reported that the extra-radical growth pattern and resulting P uptake strategies are different among AM fungal species. Using labelled P the external mycelium of a *Glomus* isolate (family *Glomeraceae*) was shown to have taken up much P from root-distant fungal compartments and contributed most to plant P supply, while an isolate of *Scutellospora* (family *Gigasporaceae*) obtained P predominantly from soil close to the host plant root (Smith *et al.* 2000). AM fungal P delivery to host plants is often highest for such AM fungi that have the highest amount of ERM in root-free soil (Jakobsen *et al.* 1992; George *et al.* 1995; Smith *et al.* 2000). In addition, AM fungal contribution to plant P uptake was also positively correlated to hyphae length in specific fungal compartments when comparing different AM fungal species (Avio *et al.* 2006) or individual strains of one AM fungal species (Munkvold *et al.* 2004; Smith *et al.* 2004). Although the ERM proliferation into the fungal tubes was low as observed in the present study, [AS] inoculated sweet potato plants reached two-third of the biomass and total P uptake compared with that for the *Glomus* inoculated plants. It is possible that AM fungi contained in the [AS] inoculum had a higher P uptake efficiency compared to both *Glomus* species, so that all mycorrhizal treatments resulted in a clearly improved plant growth compared with the non-inoculated treatment.

4.5.5 The effect of soil disturbance on the infectivity of the excised ERM

Directly before sweet potato planting, in treatment [X] the substrate of the 'receiver' compartment was disrupted once by means of cutting and horizontal mixing of the substrate. During this process, all pre-defined vertical soil sections were maintained in the respective depth.

The AM fungus showing the highest ERM DW in fungal tubes was [GI]. Despite a partly decreased root colonisation rate, this fungus was apparently almost unaffected by the disturbance treatment, as was shown at harvest (t_1) where ERM DW and hyphae length in fungal tubes (FT) were similar irrespective of the disturbance treatment. In contrast, the inoculation with [GM] yielded a lower amount of ERM DW in fungal tubes after soil disruption: ERM DW in deeper soil layers and spore density in [GM] treatments were significantly decreased. Concerning the formation potential of the external mycelium subsequent to soil disturbance, in pot experiments Duan *et al.* (2011) showed that *Glomus intraradices* is relatively insensitive, and the authors have attributed the tolerance to a very rapid establishment of ERM spread from propagules in the soil. In the present study, the [GI] isolate showed a higher extent of spatial spread in soil than the [GM] isolate, as expressed by a deeper root colonisation of both experimental plants. Possibly, more pronounced fungal proliferation behaviour leads to a lower susceptibility to soil disturbance. This may explain the reduction of mycelium DW in FT in [GM] treatments subsequent to disruption, while the effect was lacking for [GI] treatments.

Nutrient uptake by the ERM from soil is the most direct contribution of AM fungi to plant growth (Smith and Read 2008). According to Olsen *et al.* (1999), a mechanical soil disruption induces a reduced infection potential of an AM fungal network, because the establishment by means of a disrupted extra-radical mycelium might need more C expenditures from the following plant compared with an intact mycelium. In the present experiment it was assumed that without soil disturbance ([U] treatments) the intact ERM would contribute more to plant P uptake than would disturbed fungi in the treatment [X]. This was not observed, i.e. sweet potato plants colonised by the different AM fungal inocula showed similar biomass production and P uptake irrespective of being disrupted [X] or not [U]. The results therefore show no indication of distinct differences in ERM establishment from an intact compared with a disrupted ERM network when inoculated with either *Glomus* species. The amount of the external mycelium in fungal tubes in [AS] was very low, so that an ERM disruption effect could perhaps not be expected in this treatment. Former studies reported reduced AM fungal root colonisation rates and ERM growth after the external mycelium had been disrupted by sieving the experimental substrate through meshes of 4 mm size or smaller (Fairchild and Miller 1990; Jasper *et al.* 1991; Hart and Reader 2004), or by soil ploughing in field studies (Kabir *et al.* 1997; Jansa *et al.* 2002). However, negative effects on the AM fungal colonisation rate that have been reported after ploughing may also be explained by the resulting effect of turning the soil

vertically. It is possible that deeper soil sections, comprising lower microbial activity and fungal abundance, might overlay the top soil as the main location of plant root associated AM fungal structures. The main abundance of AM fungal structures has been observed in the top first centimetres of the field soil profile (Oehl *et al.* 2005). This corresponds with Kabir *et al.* (1998) who found AM fungal populations to be greatest in up to 15 cm depth, and the authors stated that ploughing to more than 15 cm depth reduces propagule density in the rooting zone by dilution, and therewith also reduces mycorrhiza formation. Less intensive operations, where soil is loosened but not turned are management methods such as disking, which is a common method used in reduced tillage systems in agriculture of the temperate zones (Cannell 1985). In the present study, disturbance was conducted moderately by mixing the substrate by hand, such that lower or upper sections were not dislocated or diluted. This operation in some respect simulated a cultivation method typically used in reduced tillage systems. The results of the present study indicate that all used inocula comprised a high potential to overcome moderate mechanical soil disturbance, and the outcome of the AM symbiosis was not affected by this intervention.

In cases where the density of viable spores in soil is low, differences in spore numbers may become more important for those AM fungal species that are fully dependant on spores as propagules. An insufficient presence of infective spores might occur after certain crop management activities such as ploughing or after crop plant harvest at an early stage of AM symbiosis, where AM fungal hyphae proliferation could still be higher than spore formation. In such situations, mycorrhizal infectivity may depend more on the presence of infective mycorrhizal root fragments or on an intact ERM as has been suggested earlier (Jansa *et al.* 2002; Hart and Reader 2004). Furthermore, after repeated soil disturbance and cultivation of crops that mature within short time periods, AM fungal species with a characteristic late and less intense spore development might lose competitiveness compared with species that complete their life cycle within a shorter time period. This matches with the field study of Oehl et al. (2003), who demonstrated in intensively managed agro-ecosystems a selection for species forming spores rapidly, and also the intensity of land use has been negatively correlated with AM fungal species richness. Accordingly, *Glomus* species were predominantly present in intensely tilled fields, while representatives of the *Gigasporaceae* family were more prevalent in non-tilled soils (Jansa *et al.* 2002).

4.5.6 Root distribution with depth

Besides the total plant root biomass of sweet potato, the root DW distribution in four different vertical soil sections was measured. A changed shoot-to-root DW-ratio towards higher shoot biomass was observed, as it usually occurs due to mycorrhizal infection (Berta et al. 1995), but also root morphological features were changed. In non-inoculated plants, root DW was about 50% both in the upper 0 - 21 cm and bottom 21 - 36 cm soil depths, respectively. In these treatments, roots were more evenly distributed across all depths compared to those of [GI] and [GM] inoculated plants with their root DW predominantly located in the upper half of the substrate (about 70%). Concomitantly, in the bottom soil (29-36 cm) fungal root colonisation was clearly low in [GI] and [GM] treatments, and root biomass was significantly lower than that of non-inoculated plants. Apparently, the inoculation with both *Glomus* isolates shifted the vertical root distribution towards the upper soil sections, possibly because the upper 21 cm contained the main part of the AM fungal colonised root length. A continuous P delivery through the fungal network to roots may have occurred mainly in this soil section and therefore induced root branching therein. This pattern of response is consistent with the suggestion made by Helgason and Fitter (2009): When AM fungi transfer P to the plant across the arbuscular membrane, there will be a local increase in P concentration in the root. Thus, the plant will unlikely distinguish fungal mediated transfer from that taken up by the plant itself via the root epidermis. Therefore, the plant will respond by differential transport of hexoses to the site of increased P uptake, and consequently the root branching and growth in the section will be increased (Helgason and Fitter 2009). However, an exact experimental proof for this interpretation is still lacking. At least it is known that an individual plant root system increases branching and consequently also the number of lateral roots within soil patches containing higher P concentration levels (Drew 1975; Lyons et al. 2008).

4.5.7 Conclusions

Directly after detachment from a former host plant the ERM of the studied AM fungi showed a high inoculum potential on new plant roots, reflected by an early root colonisation of sweet potato plants. After a relatively short cultivation period, sweet potato cuttings benefited enormously from AM fungal colonisation, as all AM fungi lead to a dramatic increase of plant growth and P uptake.

According to the outlined hypothesis, the fungal species that showed the highest spatial ERM spread in soil contributed most to the growth of a subsequent plant after the establishment of the symbiosis. The inoculation with *G. mosseae* and *G. intraradices* showed the highest extents

of external mycelium abundance in root distant substrate, and the outcome of the symbiosis with both *Glomus* isolates was more beneficial to the host plant in terms of plant P uptake and growth promotion than with the AM fungi from the field soil inoculum.
Despite their lower extension of ERM in soil, the AM fungi from the field soil caused a similar extent of total root colonisation rate in sweet potato compared with the *Glomus* species, and significantly increased plant growth, showing a high specific P uptake and transfer efficiency. The results support former studies, indicating that a beneficial outcome of the association with a certain AM fungus can be predicted rather by the AM fungal specific P transfer efficiency than by the extent of the total root colonisation rate.

It has been hypothesized that mechanical disturbance of an excised AM fungal mycelium reduces the subsequent fungal contribution to plant growth or P uptake compared with an intact mycelium. In contrast to the outlined hypothesis, soil disturbance in most instances did not affect root colonisation and in no case affected nutrient uptake or growth of a newly colonised plant. This model study indicates that as long as the density of fungal propagules in soil is sufficient, non-turning, moderate soil management practices used in reduced tillage systems may not affect the inoculum potential of AM fungi and their following establishment.

Chapter 5

AM fungal sporulation within dead trap roots – Spore quantities and distribution pattern

5.1 Abstract

The pattern and density of AM fungal sporulation within dead roots ('trap roots') excised from different plant genotypes (host or non-host) was estimated in regard to root diameter and to thickness of trap root layers.

Experiments were conducted by inserting trap root compartments into the substrate of pre-cultivated maize 'nurse' plants inoculated with *Glomus mosseae* to obtain an infective AM fungal extra-radical mycelium [viableAM]. Compartments contained either trap roots, or an empty space as a control, and were covered with a 30 µm mesh membrane to allow fungal mycelium but not plant roots to enter. Non-infective [deadAM] treatments were obtained by killing the mycorrhizal, pre-cultivated nurse plants by shoot removal before trap root compartments were inserted into the pots.

Trap roots from the non-infective [deadAM] treatments were free from AM fungal structures. In the infective [viableAM] treatments, after a two-week incubation period, AM fungal spores were observed on the surface and inside the cortex of trap roots, irrespective of the genotypic origin. The calculated spore density per unit trap root volume was up to 18000 spores per cm^3. It surmounted that of a similar volume of the colonised substrate by more than hundred-fold. No sporulation occurred in the space between empty nylon meshes of control compartments. When similar total length of coarse and fine trap roots were provided, a higher percentage of coarse trap root length (diameter > 150 µm) contained spores compared with finer roots. AM fungal hyphae and spores were observed in trap root layers of up to 5 mm in thickness.

The results indicate that dead roots can attract AM fungal growth and sporulation, possibly because they function as a nutrient source or supply a protected space. This demonstrated ability to yield spores within dead roots may represent a potential technique to obtain AM fungal spores in a low-weight, organic carrier material. In addition to this discovery, a method for the fast and simple quantification of spores and vesicles contained in trap roots was developed.

5.2 Introduction

Arbuscular mycorrhizal fungi form a symbiotic association by colonising the cortical cells of a plant partner, however the symbiosis is not restricted to the intra-radical space, with hyphae extending out through the rhizosphere and beyond into the distant soil habitat. Once into this extra-radical space, the fungal mycelium spreads and branches within the bulk soil producing spores continuously during the whole growth period. Formed from cytoplasm and storage lipids and protected by a thick-walled cell membrane, spores have a longer life-span than hyphae and are able to last several years in the soil and overcome adverse abiotic and biotic conditions (Brundrett 1991). By these means, spores play an important role as supportive structures for the establishment of new colonies. To a great extent, AM fungal development is influenced by the nutritional status of the host plant. An elevated plant phosphorus demand (while other nutrients are not limited) can lead to increased fungal root colonisation, higher development of extra-radical mycelium (ERM) and consequently elevated spore production (Verkade and Hamilton 1983; Douds and Schenck 1990; Douds 1994; Saito *et al.* 2011). Moreover, AM fungal spore production during the symbiosis could be modulated directly according to carbon derived from the host plant (Ijdo *et al.* 2010). Thus, irrespective of the host plant carbon supply, different AM fungal species have specific extra-radical colonisation strategies. For example, members of the suborder *Glomineae* establish colonisation from spores, vesicles and hyphae fragments, while most members of *Gigasporineae* were observed to use only spores as propagules (Biermann and Linderman 1983; Klironomos and Hart 2002).

Besides sporulation in bulk soil, different *Glomus* species were also observed to sporulate within empty seed cavities and glass capillaries (Taber 1982; Rydlova *et al.* 2004), in dead spores of AM fungi (Koske 1984), in nematode cysts (Francl and Dropkin 1985), in nodules of legumes (Vidal-Dominguez *et al.* 1994) and in root fragments (Daniels-Hetrick 1984). Concentrated AM fungal spores can usually be found within the decomposing root fragments contained in commercial inoculum (own observation). However, it could not be determined whether these spores in the root residues were emplaced there directly or whether they were former vesicles, later transformed into spores after the death of the host plant. Presently, it is not well understood what factors might stimulate or induce sporulation into hollow bodies. Rydlova *et al.* (2004), who observed spore agglomeration within glass capillaries or empty seed cavities, suggested that AM fungi may seek shelter to elude predators such as soil insects. Dead roots are ubiquitous in soils, for example as residues from root turnover in natural ecosystems or as post-harvest remains of main and cover crops in agricultural fields. When deposited

within root fragments, AM fungal spores may be better protected against some unfavourable abiotic conditions or against insect feeding. To date, neither AM fungal spore colonisation pattern nor spore colonisation quantities within root fragments ('trap roots') have been described, the present study thus aims to fill this knowledge gap.

The first objective of this study was to assess the effect of the genotypic origin of trap roots (host vs. non-host species) on sporulation intensity within the trap roots, and to compare this with sporulation within the bulk soil. It was hypothesised that trap roots from either host or non-host plants will be colonised to a similar extent by AM fungal spores and external hyphae. Therefore, the sporulation quantity within roots excised from non-host species (*rmc* tomato plants and of Pak Choi) was compared with that in host species (wild-type tomato and *Tropaeolum majus*). Under the assumption that root fragments serve as hollow spaces attractive to AM fungal proliferation, the trap root geometry was also taken into account. The different trap root genotypes differed to a significant extent in their volume per unit length and therefore this criterion was estimated and related to the sporulation intensity. It was hypothesised that a higher frequency of sporulation will occur in coarse compared to thin trap roots. A second objective in this study was to estimate AM fungal proliferation and sporulation intensity within different layers of trap root material. Mycelium growth has been reported to be more dense in substrates distant from the rhizosphere compared to substrates within the rhizosphere (St-Arnaud *et al.* 1996; Mikkelsen *et al.* 2008; Neumann *et al.* 2009), indicating mycelium growth into soil volumes likely occurs in the absence of living plant roots. Some authors observed AM fungal hyphae colonisation of different types of organic matter (Warner and Mosse 1980, Hepper and Warner 1983). Large agglomerations of dead root material occur, for example in grasslands where roots are dying-off for seasonal reasons (winter or drought period) or because of shoot removal by harvesting or animal grazing. In temperate mountain grasslands, within the first ten centimetres of soil depth, the biomass of dead roots can account for half of that of living roots (Pucheta *et al.* 2004). Accumulated dead roots may represent a considerable part of naturally abundant belowground organic matter and could possibly be colonised by the ERM. It was hypothesized that AM fungal hyphal growth and sporulation is not restricted to single root fragments scattered in bulk soil but also a proliferation of ERM occurs into dense layers of trap roots.

AM fungal colonisation within patches (compartments) filled with trap roots could be used to yield mycorrhizal hyphae and spores in an organic carrier material free from solid substrate. To test the hypotheses, compartments containing trap roots were constructed and inserted into the substrates of mycorrhizal plants. AM fungal ERM growth and sporulation intensity within trap

roots were compared with nurse plant root colonisation and spore density within the growth substrate. A method for a fast and convenient quantification of spores and vesicles contained in trap roots was developed.

5.3 Materials and Methods

5.3.1 Nurse plant pre-cultivation and AM fungal inoculation

Experiment 1: Seeds of *Zea mays* (L.) 'Golda' were germinated in the dark in saturated $CaSO_4$ solution. Seedlings with a fully established primordial leaf were transplanted into one litre plastic planting pots (TEKU-Tainer; Pöppelmann, Germany) containing 1.3 kg of heat sterilised (85°C for 48 h) dry substrate (for soil properties, preparation and fertilisation see Chapter 2.1). One plant was grown per pot. All plants were inoculated by mixing 10% (w/w) AM fungal inoculum of *Glomus mosseae* (Glm IFP S/08; INOQ GmbH, Schnega, Germany) with the substrate. After planting, water content in the substrate was maintained at 18% (w/w) by irrigation with deionised water. Once a week water loss was calculated gravimetrically and from this measurement daily water loss was estimated. The plants were grown under greenhouse conditions for 49 days between April and May. Throughout the growth period the day/night temperature averaged 24/19°C and the mean relative air humidity was 64%.

Experiment 2: Seeds of *Zea mays* (L.) 'Golda' were germinated in the dark in saturated $CaSO_4$ solution. Three weeks after germination seedlings were transplanted into 2 L planting pots (TEKU-Container BC 17; Pöppelmann, Germany) containing 3.4 kg of washed and heat sterilised (85°C for 48 h) dry quartz sand (particle size 1-2 mm). Plants were fertilised once a week with a nutrient solution (pH 6.8) containing the following elemental concentration: 9 mM N ($Ca(NO_3)_2$ and NH_4NO_3); 0.7 mM P (KH_2PO_4); 6 mM K (K_2SO_4); 3 mM Ca ($Ca(NO_3)_2$ and $CaSO_4$); 1.2 mM Mg ($MgCl_2$); 4 mM S ($CaSO_4$ and K_2SO_4); 80 µM Fe (Fe-EDTA); 40 µM B (H_3BO_4), 7 µM Mn ($MnSO_4$); 6 µM Zn ($ZnSO_4$); 0.7 µM Cu ($CuSO_4$) and 0.05 µM Mo ($(NH_4)_6Mo_7O_{24}$). Water content in the substrate was maintained at 20% (w/w) by irrigation with deionised water. Two maize plants were grown per pot. All plants were inoculated by mixing 5% (w/w) AM fungal inoculum of *Glomus mosseae* (Glm IFP S/08; INOQ GmbH, Schnega, Germany) with the growth substrate. The plants were grown under greenhouse conditions for 95 days between June and August. Throughout the growth period the day/night temperature averaged 25/19°C and the mean relative air humidity was 70%.

Experiment 3: Seeds of *Zea mays* (L.) 'Golda' were germinated and pre-cultivated as described in Experiment 1. All plants were inoculated by mixing 10% (w/w) AM fungal inoculum of self-

propagated *Glomus mosseae* BEG 12 (Schenck & Smith) with the growth substrate. The single strain inoculum used for plant inoculation was self-propagated on maize using the same experimental substrate (see Chapter 2.5) and consisted of AM fungal colonised roots with a surrounding growth medium containing spores and hyphae. After planting, water content in the substrate was maintained at 18% (w/w) by irrigation with deionised water. Once a week water loss was calculated gravimetrically and from this measurement, daily water loss estimated. The plants were grown under greenhouse conditions for 45 days between March and April. Throughout the growth period the day/night temperature averaged 22/17°C and mean relative air humidity was 68%.

5.3.2 Production and preparation of trap roots

Experiment 1 and 2: To obtain 'trap' root material, seeds of *Solanum lycopersicum* (L.) cv. RioGrande 76R (WT); mycorrhiza-defective (*rmc*) mutant tomato (Barker *et al.* 1998); *Tropaeolum majus* (L.) ('Monks Cress'); *Brassica rapa* (L.) ssp. *chinensis* ('Pak Choi') and *Chloris gayana* ('Rhodes grass') were germinated in the dark in saturated $CaSO_4$ solution. Seedlings were transferred to an aerated nutrient solution (pH 6.8) containing the following elemental concentration: 5 mM N (half $Ca(NO_3)_2$, half NH_4NO_3); 0.7 mM P (KH_2PO_4); 4 mM K (KH_2PO_4 and K_2SO_4); 2.5 mM Ca ($Ca(NO_3)_2$ and $CaSO_4$); 1 mM Mg ($MgCl_2$); 4 mM S ($CaSO_4$ and K_2SO_4); 10 µM Fe (Fe-EDTA); 10 µM B (H_3BO_4), 5 µM Mn ($MnSO_4$); 1 µM Zn ($ZnSO_4$); 0.7 µM Cu ($CuSO_4$) and 0.5 µM Mo ($(NH_4)_6Mo_7O_{24}$). The nutrient solution was exchanged twice a week. Prior to the experimental use, the average specific root length of four subsamples (1 g fresh weight each) was determined by a modified line intercept method (Newman 1966). The average root diameter was measured by means of ten randomly chosen fragments within these subsamples. Results are shown in Table 5.1. Subsamples were dried at 85°C for 48 h to estimate the dry weights necessary to determine the specific root length.

Table 5.1: Average trap root diameter and specific trap root length of the trap root material prior to experimental use. Root material was obtained from different plant species, grown for 50 days. Shown are means ± *SD,* estimated on subsamples of four replicates.

	Plant species	**Average root diameter** (µm)	**Specific root length** (m g^{-1} DW)
Experiment 1	*S. lycopersicum* '76R'	332 ± *120*	65 ± *11*
	S. lycopersicum 'rmc'	313 ± *137*	66 ± *8*
	T. majus	338 ± *122*	53 ± *6*
	B. rapa ssp. *chinensis*	171 ± *68*	130 ± *21*
Experiment 2	*C. gayana*	221 ± *155*	162 ± *41*

To check for fungal contamination, roots of each plant species were analysed microscopically prior to experimental use. Four subsamples of the freshly harvested root material were stained with trypan blue according to the procedure explained in Chapter 2.7 and examined microscopically (150 x magnification). All root samples were free from fungal colonisation. The roots were dried (60°C; 48h) and stored at room temperature until application. Before application, the plant roots were cut into approximately 2 cm long pieces, sterilised by transfer into 70% ethanol for 30 seconds, and remaining ethanol was removed by blotting the root surface with paper towels. Prepared roots were inserted into the trap root compartments as described in the following section (Section 5.3.3).

Experiment 3: To obtain homogenously grown plant roots free from fungal colonisation, roots of hydroponically grown cucumber plants (*Cucumis sativus* L.) were used (De Kreij *et al.* 1997). The cucumber plants were obtained from the Institute for Ornamental Crops in Großbeeren where they were produced from February to April 2010 in nutrient film channels supplied with a standard nutrient solution. After three months growth in a nutrient film, the root systems of the cucumber plants had formed long, flat and intensely interwoven layers within the channel. Sections (0.18 x 1 m) were cut from areas of the roots which appeared to have the most homogenous growth pattern (Fig. 5.2 c). Harvested roots were washed carefully in tap water, air dried at 40°C for 12 h and stored at room temperature for further use. Five subsamples of the harvested fresh root material were stained with 0.05% trypan blue (according to the procedure explained in Chapter 2.7) and microscopically examined at 150 x magnification. All root samples were free from fungal colonisation. Using four replicate samples of cucumber root material, the average root diameter and specific root length (according to the line intercept method of (Newman 1966) were estimated to be 248 ± 9 µm and 267 ± 41 m g^{-1} DW, respectively.

5.3.3 Preparation and filling of trap root compartments

Experiment 1: Trap root bags were constructed from a nylon membrane (30 µm mesh size), sealed with silicone (Probau, Bauhaus AG, Germany). This construction allowed AM fungal mycelium but not plant roots to grow into the bags. Using strips of silicon, each bag was closed at the edges and subdivided into twelve compartments of similar sizes (3 x 4 cm each; see Fig. 5.1 c). The compartmented trap root bags were 52 cm in length so that they could fit around the circumference of the transplanted maize plant root. When preparing the trap root compartments, 100 mg (DW) of roots were randomly selected and put into each compartment. To test whether sporulation and hyphae growth occur inside air gaps between synthetic

surfaces, a 'control compartment' was created by combining three layers of a nylon mesh (2 mm mesh size; 3 x 4 cm) and sealing it similar as the trap root compartments. After filling, all compartments (trap root and controls) had a thickness of approximately 3 mm. Each trap root bag contained eight trap roots compartments of all four genotypes and four control compartments (Fig. 5.1 c). Because of the higher specific root length of *Brassica rapa* (L.) ssp. *chinensis* (see Table 5.1), only 50 mg (DW) of root fragments were used to ensure consistency across treatments. The prepared trap root bags were inserted immediately into the planting pots as described in the following section (Section 5.3.4).

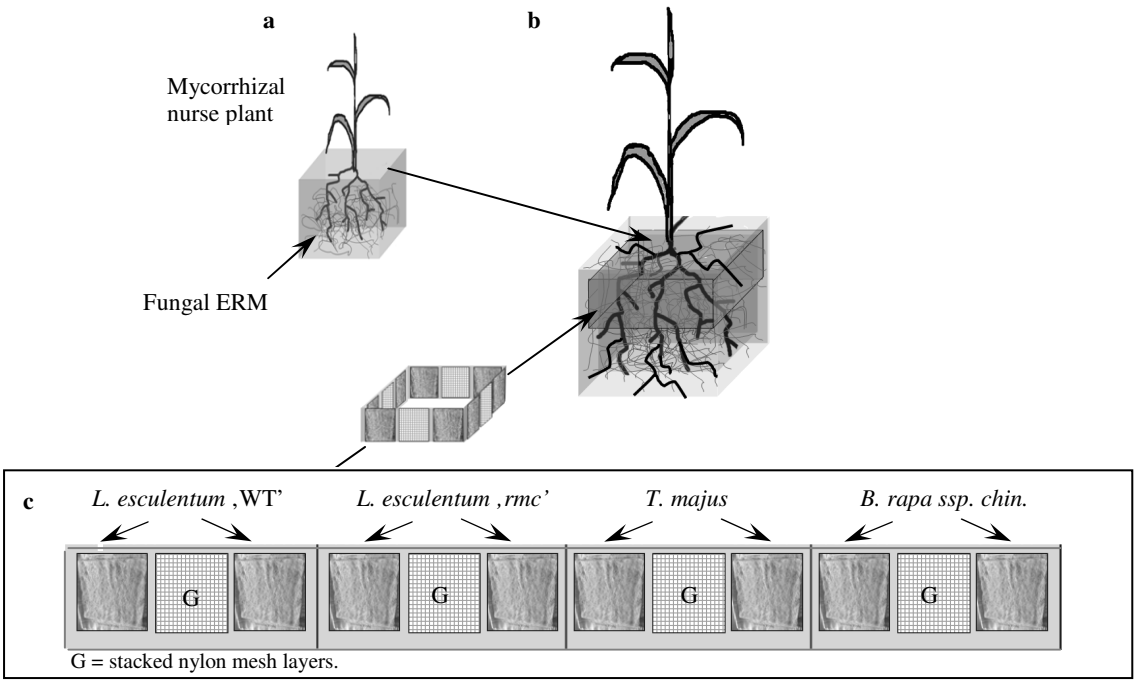

Fig. 5.1: Illustration of the experimental planting units including a trap root bag (experiment 1). a. AM fungal inoculated maize plants were pre-cultivated to establish an ERM network on their root system. **b.** Colonised maize plants were transplanted into bigger pots where the upper part of the transplanted root system was surrounded by the compartmented trap root bag. **c.** Trap root bags containing trap roots of all different genotypes (as indicated) or control (stacked nylon mesh layers; G).

Experiment 2: Single trap root compartments, measuring 3 x 4 x 0.3 cm each, were constructed from a 30 µm mesh size nylon membrane sealed with silicone, as described in experiment 1. A mass of 50 mg of dry *C. gayana* root fragments (prepared as described in Section 5.3.2) was filled into each compartment. Each compartment had a thickness of about 3 mm.

Experiment 3: Trap root compartments were constructed from plastic frames measuring 11 cm in length and 3 cm in width. Three different compartment volumes (S_c1, S_c2 and S_c3) were constructed by varying the frame depth which was 0.3 cm, 1 cm or 1.6 cm (Fig. 5.2 b). Both the front and back sides of the open frames were covered with a nylon mesh (1 mm) and a nylon membrane (30 µm) (Sefar AG; Switzerland) allowing hyphae but not roots to grow into the trap root compartments. The membrane was fixed with a fungicide-free silicone sealant (Probau, Bauhaus AG, Germany). The compartments were filled with layers of cucumber roots (prepared as described in Section 5.3.2). Prior to application, roots were sterilised by transferring into 70% ethanol for 30 seconds and retaining ethanol was soaked with paper towels from the root surface. The root mats were cut to fit the frame size of 11 x 3 cm. To prevent the two root layers from sticking together, they were separated by a single layer of glass beads (Ø 1-2 mm). The total dry weights of the trap root material inserted into the [S_c1], [S_c2] and [S_c3] compartments, were 0.32, 2.0 and 3.2 g, respectively, and this translated into trap root densities of 8, 25 and 60 mg (DW) cm^{-3}, respectively.

5.3.4 Experimental set-up and growth conditions

Experiment 1: Forty-nine days after germination, when roots were tested positive for AM fungal colonisation, the root system together with the attached substrate of the pre-cultivated maize nurse plants (Fig. 5.1 a) were removed from their former planting pots and transplanted into 2 L planting pots (TEKU container MXA 17; Pöppelmann, Germany) containing 2 kg of heat sterilised (85°C for 48 h) dry substrate. For substrate properties and preparation see Chapter 2.1. One plant was grown per pot. Transplanting the nurse plants required 3 distinct steps. First, one third of the new substrate was filled into the bottom of the planting pots to serve as underlayment for the transplanted root system of a nurse plant. Next, one filled trap root bag was wrapped horizontally around the upper four centimetres of the plant root system and fixed with a stainless steel needle. Finally, the gap between the root system and the pot wall was filled with the remaining substrate (Fig. 5.1 b). Fourteen pots were produced.

Experiment 2: Two compartments filled with trap roots were inserted into the upper 4 cm of each pot substrate when the nurse plants were 95 days old. Ten replicates were produced per treatment.

Experiments 1 and 2: To obtain a treatment containing dead AM fungi [deadAM] but with a composition of microorganisms similar to the mycorrhizal treatment [viableAM], the shoots of the nurse plants of four prepared pots were removed one day prior to the trap root insertion.

Experiment 3: After AM fungal root colonisation was detected, the pre-cultivated maize plants (nurse plants) were transplanted into black, round 2 L plastic planting pots (TEKU container MXA 17; Pöppelmann, Germany) containing 2.4 kg of heat sterilised (85°C for 48 h) dry substrate (for substrate properties and preparation, see Chapter 2.1). Two plants were grown per pot. During pot filling, a plastic rectangular-shaped 'dummy compartment' was placed vertically into the upper 6 cm of the substrate, between the two maize plant root systems, in order to create a space that would later be filled with the trap root compartments. Trap root containing compartments were fitted twenty eight days after transplantation and each compartment size was replicated five times. To obtain a treatment containing dead AM fungi [deadAM] but with a composition of microorganisms similar to the viable mycorrhizal treatment, four extra nurse plant pots were prepared in a similar way to the [viableAM] treatments with the smallest trap compartment size [S_c1], but nurse plants were killed by shoot removal one day prior to the trap root insertion.

Experiment 1, 2 and 3: After their insertion into the experimental pots, the trap root compartments were incubated for 14 days during continued cultivation of the nurse plants. Water content in the pot substrate was maintained at 18% (w/w), and daily water loss to be replaced was estimated gravimetrically twice a weak.

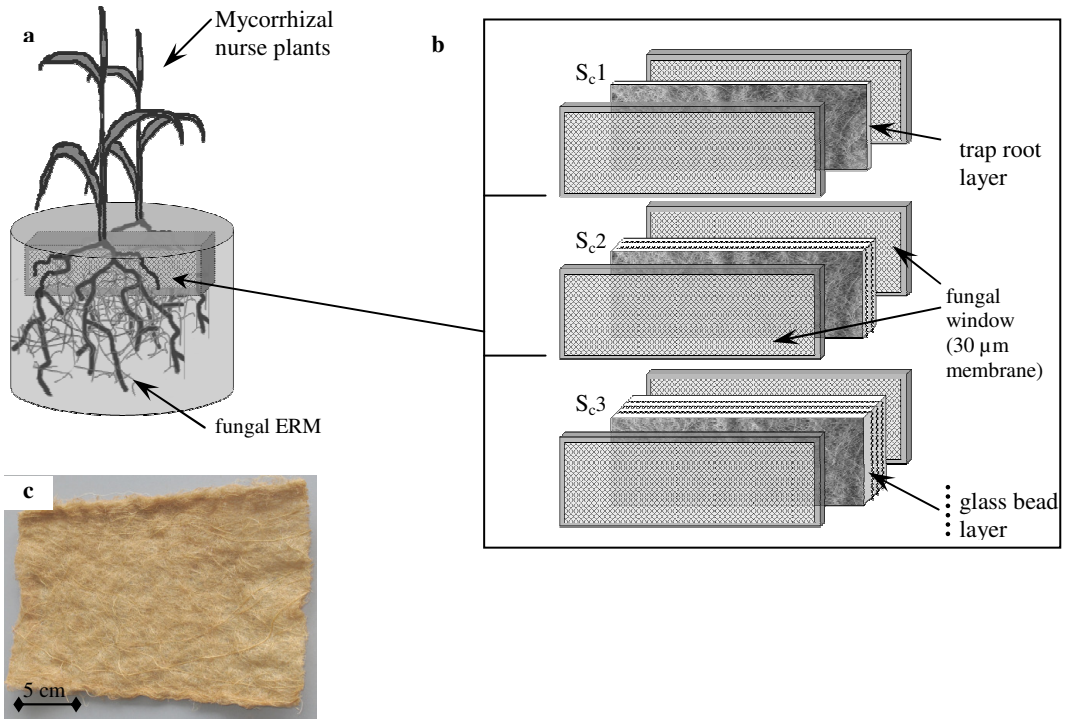

Fig. 5. 2: View of the planting units, trap root compartments and the raw root material used in experiment 3. a. Side view of a 2 L planting unit containing two maize plants and one trap root compartment buried in the substrate, treatment [viableAM]. **b.** Individual components of the compartments fillings. In each case, one glass bead layer separated two trap root layers. Single or stacked trap root layers were filled into the compartments to a total thickness of 0.3 cm [S_c1], 1 cm [S_c2] and 1.6 cm [S_c3], respectively. Both sides of the trap root compartments were covered by a 30 µm, hyphae permeable membrane. **c.** Photograph (top view) of the flat mat formed by hydroponically grown, interwoven cucumber roots used as trap root material. These mats were cut into segments so as to fit into the inner frame area of the compartment.

5.3.5 Harvest and quantification of AM fungal propagules in roots

Experiment 1 and 2: Trap root compartments were removed 14 days after insertion and at the same time, nurse plant roots were also harvested for analysis. Nurse plant roots and trap roots were prepared and stained as described in Chapter 2.7.

The stacked nylon mesh layers from each control compartment were also stained by the same method before being examined under the microscope for any signs of AM fungi. For both the nurse plant roots and trap roots, the percentage of AM fungal colonised root length was quantified using a modified gridline intersection method, as described in Chapter 2.7. Spore density per unit root trap root length was determined by counting the spore number within ten randomly chosen trap root segments of 3 mm length (using 50 x magnification). The percentage of trap root length un-colonised and colonised with intra-radical AM spores was estimated in relation to three root diameter classes: <150 µm, 150-300 µm and >300 µm. Four

replicates of each trap root genotype, consisting of three pooled trap root compartments, taken randomly, were examined. The samples were distributed homogenously onto a glass plate containing an underlying grid (0.5 cm squares). When a plant root was found to intersect the underlying grid lines, it was at this point examined. For each sample, about 150 intersections were examined under a stereo microscope with transmitted illumination (100 x magnification). At each intersection the root was positioned underneath a hairline micrometer, located in the ocular, and the size measured. Concurrently, the percentage of trap root length colonised with intra-radical spores was estimated separately for each diameter class. In addition, all intersections were classified separately as roots 'without' (when free from AM fungal spores) or 'with' spores (when containing AM fungal intra-radical spores). At least 20 intersections per diameter class were examined.

Experiment 3: Nurse plants were harvested 14 days after the insertion of trap root compartments into the growth substrate. Nurse plant roots as well as trap root material from the compartments were analysed for occurrence of spores. Trap root top (T) and central (C) positioned layers of the trap root compartments [S_c2] and [S_c3] were extracted and analysed separately for each replicate. AM fungal abundance in trap roots was quantified using the following methods:

i) Gridline intersection counting: Prior to the estimation of colonisation rate and spore density, roots were air-dried at 60°C, weighed, and subsequent to staining, examined under a microscope as described for experiments 1 and 2 in Section 5.3.5. In addition, the proportion of intra- to extra-radical spores was quantified. To achieve this, during estimation of spore density, spores found within the cortex were counted separately from those attached to the cortex surface by hyphal connections. Nurse plant (maize) root samples (approximately 1 g, n = 4) were taken randomly from each root system and after staining with trypan blue, AM fungal colonisation was quantified (procedure as described in Chapter 2.7).

ii) Filtration method: The propagule quantity of trap roots and nurse plant roots were estimated after breaking up and filtrating samples. Stained roots obtained from the gridline intersection counting (see above) were shred into pieces of less than 0.5 mm length, in 300 ml of water, using a blender (Waring Blender 7009G, Waring, USA). By shredding, root cells were fractured and AM fungal propagules contained therein were released. The resultant suspension could easily be mounted on a membrane filter which was used for subsequent microscopy, as described in Chapter 2.4.

For experiments 1 and 3, representative subsamples from the substrate were taken (approximately 200 g, n = 4). From these subsamples, spores were extracted by wet sieving (40

µm mesh) and then separated from foreign particles by centrifugation (2000 rpm for 2 minutes) in a 70% sucrose solution, following the methods of Gerdemann and Nicolson (1963). After staining with trypan blue for 24 h, the spores were mounted on a membrane filter with a 3 mm squared grid and counted as described in Chapter 2.4.

5.3.6 Statistical analysis

Provided that results passed the test for normal distribution (Kolmogorov-Smirnov test; $p > 0.05$) and homogeneity of variance (Levène test; $p > 0.05$), data were subjected to a one-way ANOVA. The multiple comparison Tukey-test was used to estimate differences between means. In both tests, p values below 0.05 were interpreted as indicating significant effects. Data which did not show homogeneity of variance was subjected to the Kruskal-Wallis-test ($p < 0.05$). Statistic calculations were conducted using SPSS software, version 15.0 (SPSS Inc., USA). Results in tables and figures are presented as treatment means ± standard deviation.

5.4 Results

5.4.1 Nurse plant root AM fungal colonisation and spore density in pot substrate

Experiment 1: Maize plant root biomass after harvest averaged 4.2 ± 0.8 g DW, with a root density in the substrate of 14 ± 5 cm per cm^3, and a specific root length of 72 ± 3 m g^{-1}. The percentage of the total root length colonised with *Glomus mosseae* averaged 83 ± 15%, with 24 ± 6% containing arbuscules, and 20 ± 5% containing intra-radical vesicles. The estimated spore density within the planting pots averaged 51 ± 11 spores per cm^3 of substrate.

Experiment 2: Maize plant root biomass after harvest averaged 18.4 ± 2.1 g DW per pot. The percentage of root length colonised by *Glomus mosseae* averaged 78 ± 26%, with 31 ± 9% containing arbuscules and 25 ± 3% intra-radical vesicles.

5.4.2 AM fungal colonisation and sporulation in trap roots

Experiment 1 and 2: The compartmented trap root bags were harvested 14 days after insertion. Nurse plant roots did not cross the 30 µm membrane so no root growth was observed in the trap root compartments. The trap roots which were inserted into the [deadAM] pots were free from AM fungal structures. In mycorrhizal [viableAM] treatments, the control compartments containing only nylon mesh layers were free from AM fungal spores, but some AM fungal hyphae growth across the nylon mesh layers was observed (Fig. 5.3 h, see arrow).

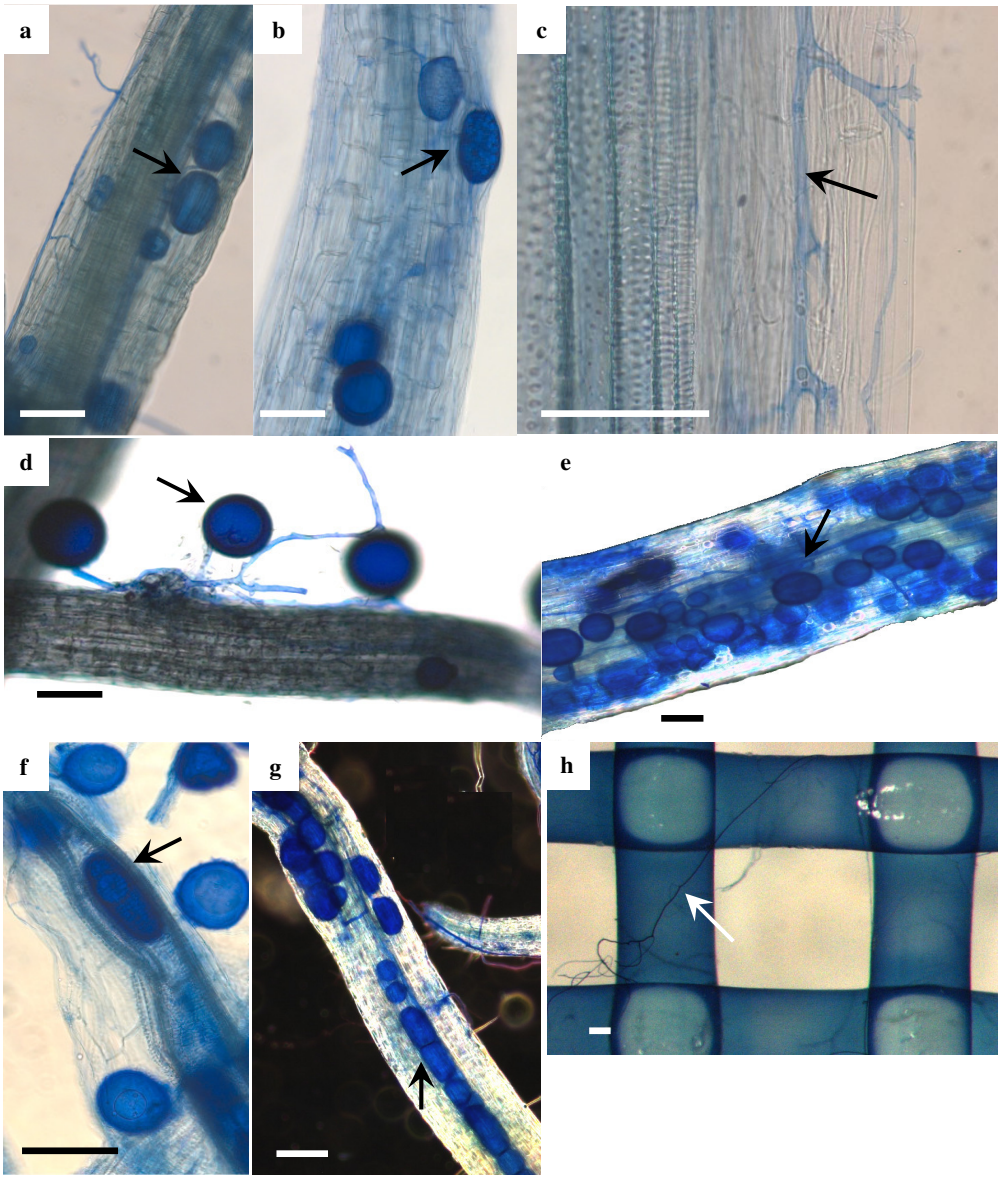

Fig. 5.3: Microphotographs of trap roots after 14-days incubation within the substrate of pre-cultivated maize plants inoculated with *Glomus mosseae* in [viableAM] treatments (experiment 1 and 2). Lateral hyphae growth and differently sized spores found between cortical cells of: **a.** '*Rmc*' tomato, and **b.** *Tropaeolum* trap roots. **c.** Intercellular hyphae growth longitudinal to the trap root cortex of *T. majus*, as generally found for all studied trap root genotypes. **d.** Trap roots originated from 'Pak Choi' with diameters smaller than 150 µm, colonised extra-radically with spores and hyphae. **e.** A coarse trap root of *C. gayana* colonised intra-radically by AM fungal spores. Stelar cylinders were frequently colonised by AM fungal spores, shown in a dissected *Tropaeolum* sample **(f)** and in an intact *C. gayana* sample **(g). h.** Top view of a nylon mesh layer, excised from a stained mesh control. Coarse and finely branched hyphae were observed to cross the mesh surface but no spores were found. All trap roots were free from any fungal colonisation before being used for incubation. Bars indicate 100 µm.

In [viableAM] treatments, hyphae growth of *G. mosseae* (diameter of hyphae 3 to 15 µm) was observed on the surface and along the trap root main axis (Fig. 5.3 a, see arrows), as well as between cortical cells (Fig. 5.3 c). Hyphae were spread all through the trap root tissue and showed branching, and occasionally, inter-connection by h-bridges. *G. mosseae* developed thick-walled, oval or globose shaped spores (diameter up to 150 µm) within the cortex (Fig. 5.3 a, b and e). To a lesser extent globose shaped spores were also observed outside of the trap root tissue (Fig 5.3 d) and within the stelar cylinder (Fig 5.3 f and g). No arbuscules were found in trap roots.

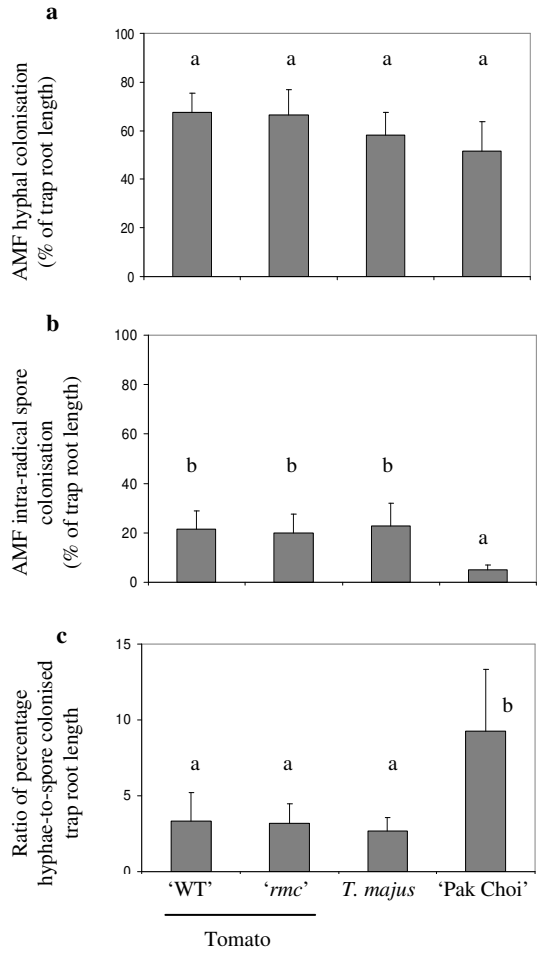

Fig. 5.4: Percentage of AM fungal colonised trap root length and ratio of percentage hyphal-to-spore colonisation (Experiment 1)**.** Trap roots from different plant genotypes (named on x-axis) were inserted for a 14-day period into a substrate containing a pre-cultivated maize plant inoculated with the AM fungus *Glomus mosseae*. **a.** Percentage of root length with AM fungal hyphae colonisation. **b.** Percentage of root length colonised with intra-radical spores. Here, spores outside of the root cortex were not counted. **c.** Ratio of percentage hyphal-to-spore colonised root length. Bars represent means ± SD. Different letters indicate significant different means (multiple comparison Tukey-test, $p < 0.05$; $n = 4$).

Trap roots originated from wild-type tomato (host), *Tropaeolum* (host) and *rmc* mutant tomato (non host), did not differ in colonisation rates and averaged between 55 - 80% for hyphal colonisation and between 20 - 30% for intra-radical spore colonisation (Fig. 5.4 a and b). For 'Pak Choi' (non host), the trap root length colonised with spores was up to 12% which was significantly lower (Fig. 5.4 b) than for the other trap root genotypes. In addition to this, the pattern of colonisation in 'Pak Choi' was different than in the other genotypes. The cortex of 'Pak Choi' trap roots were colonised with many spores and hyphae (see Fig 5.3 d), while intra-radically, hyphae dominated and relatively few spores were present. This pattern was also reflected in the high ratio of hyphae-to-spores in 'Pak Choi' roots (Fig. 5.4 c).

The spore density per unit length of trap roots originated from 'Pak Choi' was lower than for the other trap root genotypes (Fig. 5.5 a). A similar result was also observed for spore density per unit trap root DW (Fig. 5.5 b).

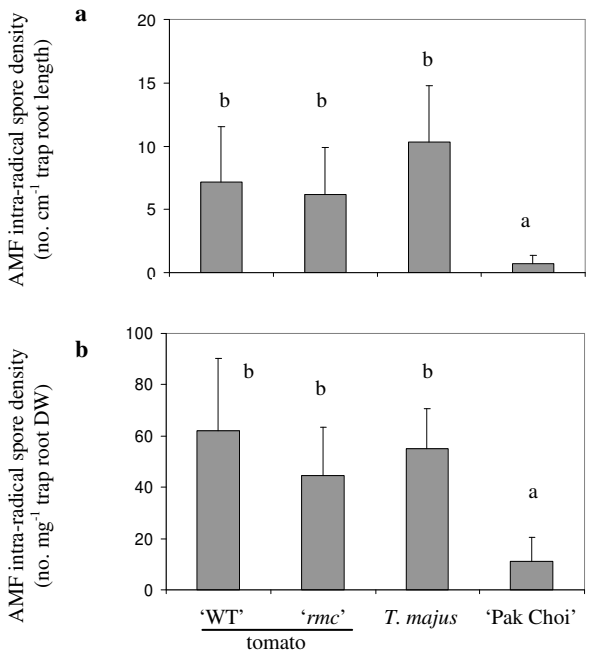

Fig. 5.5: Intra-radical spore densities per unit trap root (Experiment 1), estimated as **a**. per cm trap root length and **b.** per mg trap root DW. Bars represent means ± SD. Different letters indicate significant different means (multiple comparison Tukey-test, $p < 0.05$; $n = 4$).

In [viableAM], the trap roots of *C. gayana* had a specific root length of 221 ± 155 m g^{-1} and an average total length of 11 ± 0.3 m per compartment. The total AM fungal colonised length of trap roots averaged 39 ± 4%, with 13 ± 2% intra-radical spores.

Within distinct root diameter size classes, the total trap root length in the compartment, the trap root length colonised with AM intra-radical spores, and the trap root length not colonised with AM intra-radical spores, were estimated for each genotype used in experiments 1 and 2. Since both wild-type and *rmc* tomato trap roots were similar in their features and were colonised in a similar way with AM fungal structures, data for *rmc* roots are not shown any further. Roots were separated into three diameter size classes, defined as <150 µm (fine), 150-300 µm (intermediate) and >300 µm (coarse) (Fig 5.6 a-d). Tomato roots predominantly consisted of diameters larger than 150 µm (Fig 5.6 a) with a very low proportion of roots being thinner than 150 µm. This trend could also be observed in *T. majus* trap roots (Fig 5.6 b) which were completely lacking the finest root class. In contrast to this, trap roots originated from Pak Choi and *C. gayana* showed a relatively high proportion of thin diameter classes (37% and 30% of total length in compartment; Fig. 5.6 c and d). *C. gayana* trap roots exhibited a relatively homogenous distribution pattern of all size classes. The percentage of trap root length colonised with intra-radical spores (Fig 5.6 e-h) was low in fine trap roots and markedly increased with increasing diameter size. This trend was observable in all studied genotypes, except for *T. majus* trap roots which lacked of the thinnest diameter size class (Fig. 5.6 f), but was most pronounced in Pak Choi and *C. Gayana* (Fig. 5.6 g and h). Both these genotypes, who exhibited root size classes in approximately the same dimensions, showed that up to four times more coarse trap root length was colonised with intra-radical spores than for the fine class.

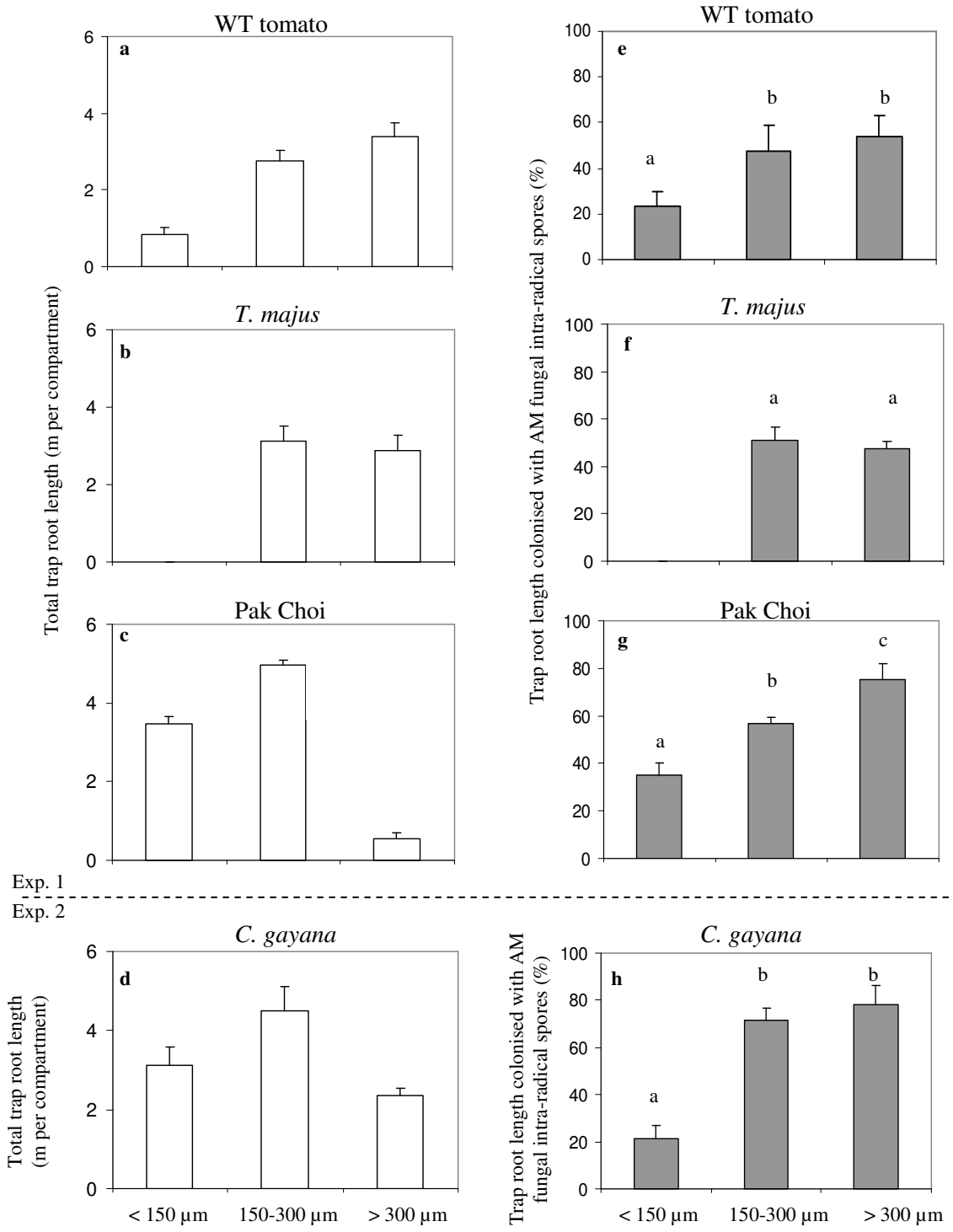

Fig. 5.6: Total trap root length (m per compartment) (figures on the left) **and percentage of trap root length colonised with AM fungal intra-radical spores** (figures on the right). Values were estimated for each root diameter size (see x-axis). Trap roots were excised from the plant species indicated on top of the respective diagram. Bars represent means ± SD. Different letters (figures on the right) indicate significantly different means (multiple comparison Tukey-test, $p < 0.05$; $n = 4$). Data were square root transformed prior to statistical analysis.

Experiment 3

5.4.3 Nurse plant root AM fungal colonisation and spore density in pot substrate

At harvest 14 days post trap root insertion into substrate, nurse plant root dry matter averaged 10.4 ± 0.7 g per pot. The percentage of root colonisation with *Glomus mosseae* averaged 71 ± 4% of which 21 ± 2% included arbuscules and 19 ± 3% intra-radical spores and vesicles. The estimated spore density within the pot substrate averaged 34 ± 14 spores per cm^3.

5.4.4 AM fungal colonisation and sporulation in trap roots

Subsequent to the incubation within the pot substrate, as intended, no nurse plant roots penetrated the fungal windows covered by the 30 µm membrane. The trap root material inserted into the control pots containing the killed nurse plants [deadAM] was free from fungal structures such as spores or hyphae. In mycorrhizal treatments [viableAM], hyphae of *G. mosseae* were found on surfaces and within the trap root cortex, and spores were found within and outside of the cortex (Fig. 5.7 a-d). More than double the amount of spores were located inside the inner root tissues compared with outside. Irrespective of the trap compartment size [S_c1-3], the ratio of extra- to intra-radical spores averaged 0.34 ± 0.09 in the top layer. Spores were up to 120 µm in diameter and were shaped differently according to their location, e.g. round spores were developed outside of roots, round or oval spores were observed between cortical cells and only oval spores were observed within stelar cylinder tissue (Fig. 5.7 d). No arbuscules were found in the trap roots.

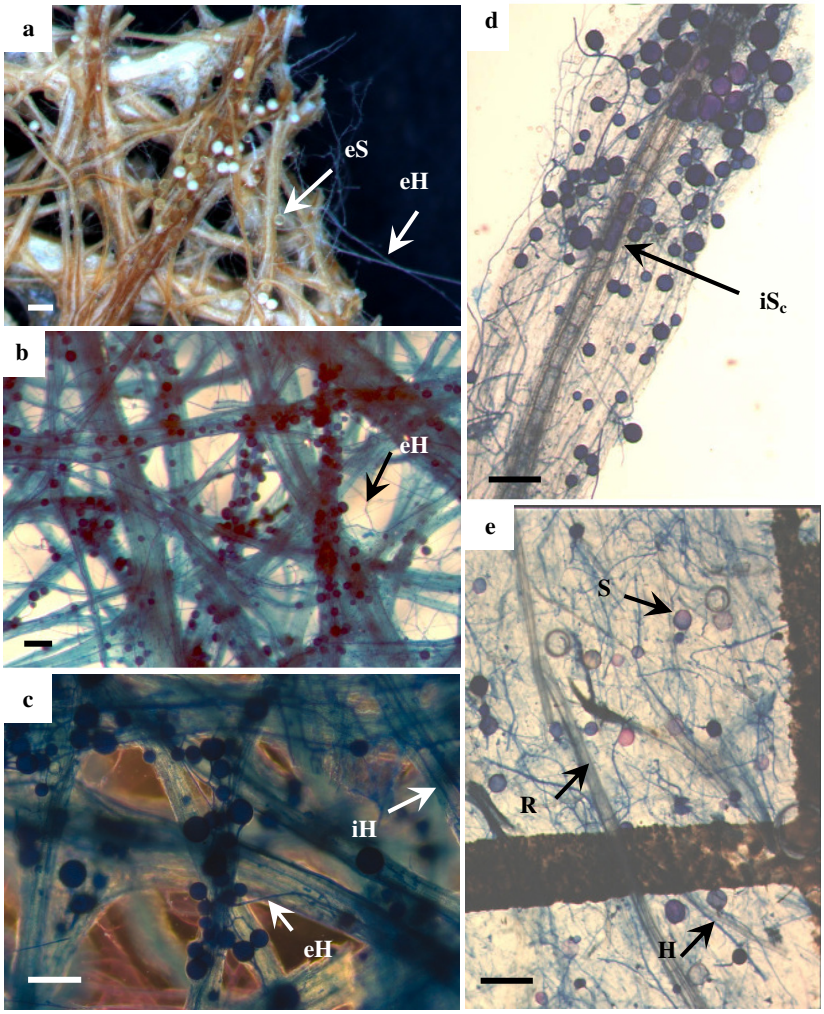

Fig. 5.7: Microphotographs of the cucumber trap roots after 14-days incubation within the substrate of mycorrhizal maize plants inoculated with *Glomus mosseae,* [viableAM] treatments (experiment 3). Trap roots before (**a**.) and after (**b**.) staining showed intense colonisation with AM fungal spores and hyphae. Frequently, extra-radical spores (eS) and hyphae (eH) were observed on the surface of trap roots. **c**. Stained trap roots showed intra-radical hyphae (iH) growth longitudinally along the root axis, and external hyphae (eH) connections between trap roots. **d**. Spores were also located within the stelar cylinder (iS$_c$) of cucumber roots. Spore diameter varied and was up to approximately 120 µm. **e**. Using the filtration method for spore quantification, stained trap roots were fragmented in a blender and thereafter, extracted spores (S), hyphae (H) and root cell residues (R) were mounted on a gridded membrane filter. All trap roots were free from any fungal colonisation before being used for incubation. Bars indicate 200 µm.

The percentage root length colonised with AM was estimated via the gridline intersection counting method. Top (all compartments) and central layers (only [S$_c$2] and [S$_c$3]) were counted separately. (Fig. 5.8 a and b). Therefore, the top layers (T) were taken from the first 3 mm of the inserted root material in every case, while the central layers (C) were obtained from 5 mm [S$_c$2] and 8 mm [S$_c$3] depth. Approximately half of the root length was colonised with AM fungal hyphae in (T) and this did not differ significantly between compartment sizes (Fig.

5.8 a). Differing from this trend is the hyphae colonised root length in the (C) layer of [S_c3] which was just below that of the (T) layer but and significantly lower than that of the (C) layer of [S_c2] (Fig. 5.8 a). The internal spore colonised root length ranged between 3 - 25%, with the significantly lowest rate also being found in the (C) layer of [S_c3] (Fig. 5.8 b). Within (C) layers of [S_c2] and [S_c3], the reduction in spore colonised root length was much more pronounced than in the case of the hyphae, as demonstrated by a significantly higher ratio of hyphal-to-spore colonised root length (Fig. 5.8 c).

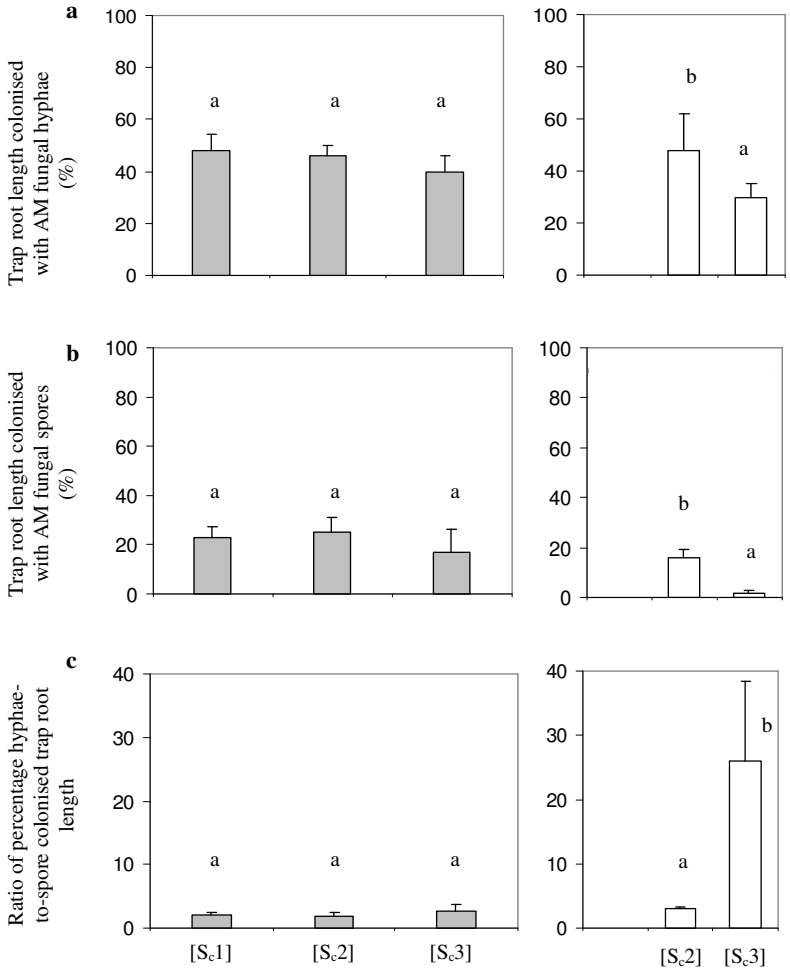

Fig. 5.8: Percentage of AM fungal colonised trap root length and ratio of percentage hyphae-to-spore colonisation, estimated via gridline intersection counting; Experiment 3. Trap root material with three different thickness classes (S_c1-3) was inserted during a 14-day period into substrate containing maize nurse plants inoculated with the AM fungus *Glomus mosseae*. Trap root material in the compartment sizes [S_c2] and [S_c3] were analysed separately for the top (grey bars; left side) and the central (white bars; right side) layers, respectively. **a.** Percentage of root length with AM fungal hyphae colonisation. **b.** Percentage of root length colonised with intra-radical spores. **c.** Ratio of percentage hyphal-to-spore colonised root length. Bars represent means ± SD. Data of top layers was analysed separately from that of central layers, using the Kruskall-Wallis-test ($p < 0.05$; $n = 5$).

The filtration method as a possible tool for fast spore quantification was tested and the results were compared with those achieved by the gridline intersection counting method. When estimated using the filtration method, the AM fungal spore density per mg trap root DW from the top layers (T) averaged 250-280 spores in [S_c1] and [S_c2] compartments and did not exceed 100 spores in [S_c3] (Fig 5.9 a). The gridline intersection counting method produced results with a similar pattern to the filtration method but with approximately 30-40% higher values in all compartment sizes (Fig. 5.9 b). When [S_c3] was compared with [S_c1] and [S_c2], significantly lower spore densities were found in [S_c3] when both quantification methods were used. As calculated by both methods, spore density within the central layers of [S_c3] were significantly lower than that in [S_c2] (Fig. 5.9 a and b).

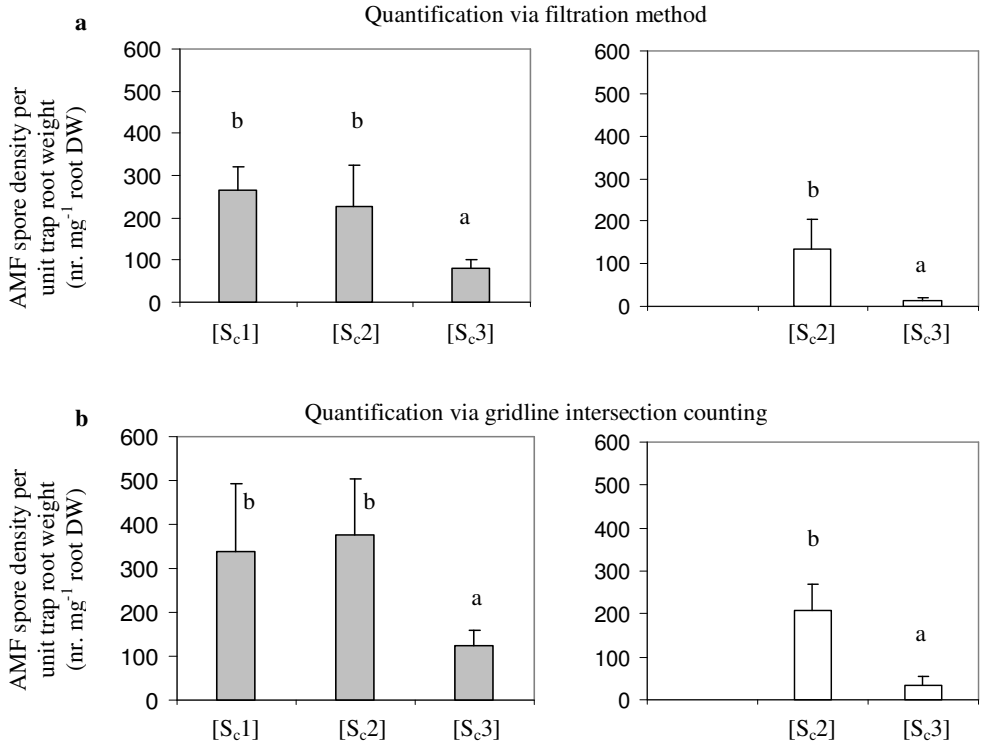

Fig. 5.9: Spore density per mg trap root, estimated using the filtration method (a) and gridline intersection counting (b), experiment 3. Bars represent means ± SD. Data of the top layers (grey bars; left side) and the central layers (white bars; right side), tested by the Kruskall-Wallis-test ($p < 0.05$; $n = 5$). Data were square root transformed prior to statistical analysis.

The average spore densities per mg trap root in the compartments were put in contrast with the average vesicle and spore density in nurse plant roots, both results were obtained using the filtration method (Fig. 5.10 a and b). Nurse plant roots contained on average 70 vesicles per mg

dry weight (Fig. 5.10 a). A similar level of spore abundance was found in the largest compartment [S_c3] which had up to 80 spores per mg trap root. In contrast, [S_c1] and [S_c2] had significantly higher quantities of spores, double to three-fold the amount of [S_c3] (Fig. 5.10 b).

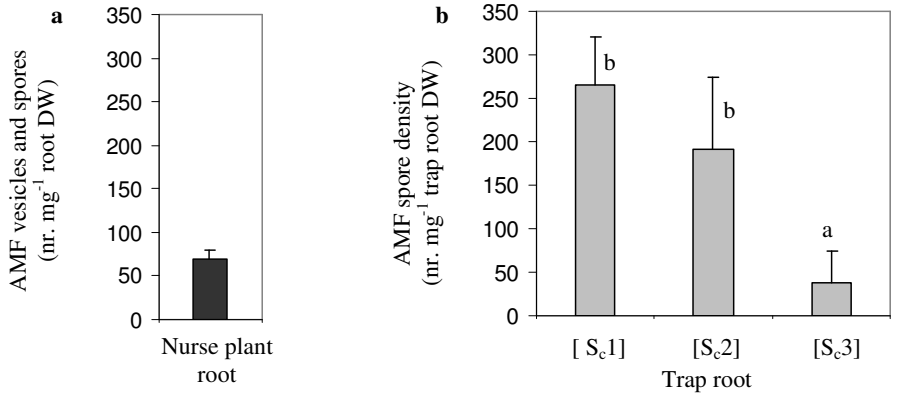

Fig. 5.10: Density of propagules in maize nurse plant roots (a) and in trap roots (b); Experiment 3. AM fungal spore density in trap roots from S_c2 and S_c3 compartment sizes were calculated from the average spore density in top and central layers. The propagule quantification was conducted using the filtration method in every case. Different letters indicate significant mean differences between trap root colonisation (Kruskal-Wallis-test; $p < 0.05$; $n = 5$).

Table 5.2 gives an overview of the quantity of spores calculated per unit volume of trap roots and of the respective substrate (in experiments 1 and 3). When thin-layered trap roots were inserted into the substrate, the spore number obtained in one cubic centimetre of trap roots was several hundred times higher than that of one cubic centimetre of substrate.

Table 5.2: Calculated average number of spores per cubic centimetre of trap roots and of corresponding substrate. Spore densities of trap roots were estimated from trap root layers of about 3 mm thickness. Shown are means ± *SD*.

Origin of trap roots (plant genotype)	Number of spores	
	cm^{-3} trap root	cm^{-3} substrate
T. majus (Experiment 1)	14699 ± *6888*	51 ± *11*
C. sativus (Experiment 3)	17884 ± *3727*	34 ± *14*

5.5 Discussion

5.5.1 Experimental conditions and AM fungal sporulation pattern in trap roots

The number of *Glomus mosseae* spores present within trap roots excised from host and non-host plant species was quantified in relation to root length and diameter characteristics. Where plant shoots have been removed to kill the AM fungus prior to insertion of trap roots [deadAM], as intended all trap compartments were free from AM fungal structures and contamination by other fungi. In mycorrhizal treatments where the AM symbiosis with the nurse plant was intact [viableAM], AM fungal hyphae (3 to 15 µm diameter) as well as thick-walled, round spores were observed inside and outside the cortex of trap roots. These spores were between 30 to 150 µm which corresponds to the size range typical for spores produced by *G. mosseae* (Giovannetti *et al.* 2003). Consequently, it can be assumed that the observed structures were from the AM fungus used in this study. Note that in terms of shape and size, spores observed in trap roots could be confused with vesicles which are also produced by *G. mosseae*. In general, vesicles are defined as hyphal swellings developed only by the intra-radical mycelium during symbiotic interaction with live host roots (Sieverding 1991). Since trap roots were dead, they could have only been colonised by means of the extra-radical mycelium. Therefore, it can be assumed that all globose structures located within the trap roots but developed by the external mycelium were AM fungal spores.

The experimental setup allowed colonised nurse plant roots to have close contact with the compartment holding the trap roots. Nurse plant root systems in all experiments were densely distributed within the substrate and highly colonised by *G. mosseae* (up to 80% colonised root length, including intra-radical hyphae, arbuscules and vesicles). Consequently, AM fungal multiplication can be considered to have been very successful in this nurse plant substrate, with densities of approximately 50 spores per cm^3. The control nylon mesh layers did not contain AM fungal spores, while a few hyphae have traversed the nylon mesh layers. In contrast, trap roots were colonised with considerable amounts of AM fungal structures. Hyphae growth was observed longitudinal to the trap root main axis, on the surface, as well as between cortical cells. Fungal hyphae were branched and occasionally even connected by hyphal bridges. A small portion of the spores were attached to mycelium outside of trap root tissue, but most were located inside between the cortical cells. Spores of *G. mosseae* normally continuously increase in size until maturity, when they can measure up to 260 µm (Giovannetti *et al.* 2003). Marleau *et al.* (2011) observed continuous spore size increases for more than 30 days during the

maturing process. According to the incubation period of the trap roots in the present study, *G. mosseae* spores were not older than 14 days, a time period possibly not sufficient for fungal spores to reach full maturity, as most did not exceed a size of 150 µm.

Interestingly, several hundred times more spores were obtained per unit trap root volume than when compared with the similar volume of colonised nurse plant substrate (see Table 5.2). From the results in the present experiment, it is clear that *G. mosseae* preferentially sporulates inside trap roots over the bulk substrate or the spaces of nylon mesh layers. Representing a form of organic matter, the dead roots used here as trap roots have certain properties of which some are as follows:

a) Chemically, dead roots

a 1) could represent a source of nutrients;

a 2) can contain repellent or antifungal compounds, depending on the genotype of the root

b) Microbiologically, dead roots represent a source of nutrients for other soil-borne microorganisms, whose activity may affect AM fungal development and

c) Physically, dead roots provide enclosed space in which AM fungi might be protected.

In the following sections, AM fungal trap root colonisation is discussed with respect to these properties, as they are important considerations for AM fungal development within trap roots.

5.5.2 Trap roots as a possible source of nutrients

In regard to nutritional benefits, it is possible that the trap root enhanced ERM growth and spore production by acting as an organic source for nutrients. Elevated ERM proliferation and sporulation into patches rich in organic matter have been demonstrated earlier (Hodge *et al.* 2001; Gryndler *et al.* 2002; Gryndler *et al.* 2003; Hodge and Fitter 2010; Quilliam *et al.* 2010). Subsequent to microbial decomposition, soil organic matter releases nutrients readily taken up by AM fungi, as have been shown for nitrogen (Hodge *et al.* 2001; Hodge and Fitter 2010) and phosphorus (Duan *et al.* 2011). Nitrogen might be an important nutrient for AM fungal development: In sterile cultures the growth of the external mycelium and spore production have been enhanced in fungal compartments supplied with nitrate compared to non-fertilised ones (Bago *et al.* 2004). However, the results obtained in studies investigating the role of organic matter on the sporulation of AM fungi remain inconsistent (Gryndler *et al.* 2002; Gryndler *et al.* 2003). This is probably a consequence of the varying nutrient composition of the organic matter used in the experiments and the compounds released by microorganisms during organic matter decomposition (Gryndler *et al.* 2009). In the presence of saprophytic fungi or bacteria

(Ames *et al.* 1989; Tylka *et al.* 1991; Azcon 1987), and also AM fungal spore-associated bacteria (Mayo *et al.* 1986) synergistic effects on AM fungal ERM production have been reported. Although these microbial interactions are poorly understood, microbial degradation of organic matter might also stimulate AM fungal ERM growth. Thus, with respect to the relatively short incubation period of trap roots in the present experiment, it remains unclear if the observed intense ERM proliferation towards, and sporulation into, trap roots can be attributed to an increased availability of nutrients for the fungus. External hyphae growth of two *Glomus* species was demonstrated on *Sphagnum* leaf fragments embedded within a sand substrate (Warner 1984). There, hyphae were attached firmly to the cell surface by rudimentary appressoria and they also grew inter-cellular. This finding supports the assumption that AM fungal extra-radical mycelium growth is increased in the presence of plant residues.

5.5.3 Sporulation intensity per trap roots of different origin

Root length containing AM fungal hyphae and spores was similar in trap roots of host plant origin. In contrast to this, Pak Choi (non-host) trap roots held significantly lower spore densities than the other studied genotypes. The spore number per unit trap root weight in tomato and *T. majus* was two- to three-fold higher than in Pak Choi trap roots. Note that different from all other genotypes studied here, roots of Pak Choi and *T. majus* contain glucosinolates (Verkerk *et al.* 2009). Glucosinolates are secondary plant compounds produced by different members of *Brassicaceae*, of which some are supposed to function as repellents against insects and phytopathogens (Bones and Rossiter 1996). Many types of glucosinolates exist and their form and concentrations vary depending on the plant species and organ in which they are synthesised. A certain group of chemicals derived from glucosinolates, the isothiocyanates were shown to have a fungitoxic or fungistatic effect. For example, El-Atrach *et al.* (1989) and Vierheilig *et al.* (1995) reported that isothiocyanates reduced AM fungal external hyphae development. Extracted from roots of diverse *Brassicaceae* species and carrot, isothiocyanates reduced the axenic spore germination of *Glomus etunicatum* (Schreiner and Koide 1993). Isothiocyanates are stable, biologically active degradation products of glucosinolates and are synthesised when glucosinolates located in the cell vacuole are exposed to degradative enzymes (myrosinases) after the cell wall has been damaged (Bones and Rossiter 1996). This enzymatic biosynthesis could also be enabled in the root fragments used in this study, since dead roots cells may lose their cell wall stability. It is likely that both, the Pak Choi and *T. majus* trap roots still contained glucosinolates after harvest. Representing host plants for AM fungi (Vierheilig *et al.* 2000) *T. majus* is not supposed to include antifungal

isothiocyanates in their glucosinolate spectrum, which in contrast are produced by non-host species of the genus *Brassica* (Vierheilig *et al.* 2000). Therefore, in the present study *T. majus* trap roots were not expected to have a detrimental effect on AM fungi. Accordingly, the results showed that trap roots of *T. majus* contained spore densities similar to those of tomato plants. Because being a member of the *Brassicaceae,* Pak Choi was rather assumed to potentially produce antifungal glucosinolates. In fact, lower spore densities were observed but the percentage of trap root length of Pak Choi colonised by hyphae was unaffected compared with other genotypes. This may underline that Pak Choi roots rather did not contain antifungal compounds and to date there is no evidence for that in the literature. It seems that AM fungal colonisation was not generally inhibited, but sporulation was less induced within the Pak Choi trap roots. As shown by microscopy, tissues of Pak Choi roots consisted of very small sized cells compared with roots of tomato plants and especially of *T. majus*. It is assumable that small sized cells in Pak Choi trap root tissue have a firm cell wall structure and/or a dense cell compound which may be more resistant to the fungal penetration and the enlargement of spores.

5.5.4 Sporulation quantity according to trap root diameter

Pak Choi trap roots were found to be distinctly thinner than those of tomato or *T. majus* plants. Since the trap root diameter represents the cross-sectional space which can potentially be colonised by AM fungal spores, spore quantities in relation to the trap root diameter size (fine, <150 µm; intermediate, 150-300 µm and coarse, >300 µm) became the focus. When both fine and coarse trap roots were present, the percentage of the trap root length containing spores was significantly higher within the coarse fraction, compared with the fine fraction. That was shown for Pak Choi, as well as for *C. gayana,* trap roots. The results indicate that sporulation occurred dominantly in trap roots with diameters larger than 150 µm. Different from the typical symbiotic root colonisation pattern, where AM fungal organs are only located in the cortex (Sieverding 1991), AM spores in trap roots were not only abundant in the cortex but also in the xylem tissue of the stelar cylinder. These sections represent large, encapsulated cavities/free spaces within trap roots and that may be attractive to AM fungi for sporulation purposes. Fine roots in general have smaller cortical cross-sectional space, when compared to coarse roots, and thus fungal spore development may be reduced due to the restricted space. Via microscopic examination of trans-sectioned ryegrass roots, Waid (1957) illustrated that cortical cells left behind large volumes of free space after having been partly degraded. According to studies by Campbell and Drew (1983) on the cortex of excised maize roots, those gas-filled spaces can

extend to more than 100 µm in diameter. Michael *et al.* (1999) also showed considerable gas volume fractions located in intercellular spaces within the cortex of excised young maize roots. Trap roots applied in this experiment were exposed to cell degradation due to their excision. Therefore, it can be assumed that considerable space was present in trap roots in the cortex, and also within the xylem vessels, and that coarse trap roots may provide more space for an AM fungus to shelter than compact fine roots.

5.5.5 Sporulation in trap root layers of different thickness

AM fungal hyphae growth and spore density was highest in the top layers of trap roots, irrespective of the total thickness of the compartments. The fungus showed a strong capacity to colonise thin layers of trap root material with hyphae and spores. In the colonised top layers, about twice as many spores were observed within the trap root cortex than were located extra-radically, indicating a preference for the use of dead root cortex as housing. The capacity of the fungi to explore the thickest trap root compartment was limited, i.e. spore density per unit trap root weight was significantly decreased with increasing trap root layer thickness. Furthermore, spore abundance was almost non-existent in trap root layers located more than 5 mm from the top layer. Possibly, hyphae elongation into the deeper layers of the root traps was not necessary, or the duration of the experiment did not provide sufficient time for the fungus to colonise such a significant root surface area. Another possibility is that the top layers contained more nutrients, since it can not be excluded that, due to the watering process, a small proportion of nutrients might have been flushed from the substrate into the top layers of trap root compartments. AM fungi preferentially proliferate into a patch of different types of organic matter (Warner and Mosse 1980, Hepper and Warner 1983; Hodge *et al.* 2001; Hodge and Fitter 2010). Hodge *et al.* (2001) postulated that nutrients were taken up by the extra-radical mycelium either directly from organic matter (ERM), or indirectly subsequent to microbial decomposition. However, only little is known about a possible saprophytic capability of AM fungi. In several pot experiments, small amounts of cellulolytic and other hydrolytic enzymes were observed in AM fungal external mycelium and colonised roots (Garcia-Garrido *et al.* 1992; Rejon-Palomares *et al.* 1996; Garcia-Garrido *et al.* 2000; Vela *et al.* 2007). However, the production origin of these enzymes could not clearly be attributed to the AM fungus, and it has been speculated that hydrolytic enzymes are involved in the softening of invaded root cell walls during mycorrhizal root colonisation process (Vela *et al.* 2007). Usually present in the rhizosphere of land plants, many saprophytic fungi belonging to the orders *Basidiomycota* and *Ascomycota,* produce large quantities of enzymes which are capable of

degrading the structural components of organic matter such as lignin and cellulose (Osono and Takeda 2006). Thus, recent studies provide no evidence that AM fungi might forage for nutrients from organic matter in the way common to soil-borne saprophytic fungi do. Here, only a few spores were observed in the centre of the largest trap root compartments [S_c3] what may underline the inability of AM fungi to undertake saprophytic foraging.

Results from this study showed that high spore densities can be yielded per unit trap root weight, which in some cases clearly exceeded the density of intra-radical vesicles in nurse plant roots, and also that of spores within the substrate. Comparing different trap root layer thicknesses, a maximal spore yield per unit trap root weight was achieved by the insertion of 3 mm layers. Therefore spores encapsulated in or attached to the trap root surface possibly represent a suitable source for AM fungal propagules in cases where non-sterile AM fungal inoculum is demanded. Sporulation patterns are diverse among different AM fungal species. For example, members of the genus *Scutellospora* depended more on spore originated resources for foraging than *G. intraradices* (Gavito and Olsson 2008), representatives of the genus *Gigaspora* depend exclusively on their spores as propagules, while *Glomus* and *Acaulospora* species in addition to their spores, also establish new colonies by means of extra-radical hyphae (Klironomos and Hart 2002). It remains to be tested, if the infectivity of the spores entrapped within trap roots is as high as those obtained from substrate, since a two-week trap root insertion may not be sufficient for spores to fully mature.

The method of filtrating trap root material post maceration was a method developed for spore extraction from soils (Hanssen *et al.* 1974), but in this study it was modified to quantify the amounts of spores per unit trap root dry weight. This filtration method was revealed to be less time consuming when compared with the commonly used gridline intersection counting method (Kormanik and McGraw 1982). The quantification of spores contained in and attached to trap roots resulted in almost similar values for both methods. Spores were not destroyed by the blender´s maceration of the root material. Senoo *et al.* 2007 used the maceration procedure in a blender to extract arbuscules from roots for further metabolic studies, indicating that spores could also be extracted by means of blending for further use as inoculum. The spore and hyphae encapsulated inside the trap roots prior to use as inoculum may be also extracted by means of enzymatic root cell wall destruction: Saito (1995) extracted intra-radical hyphae from roots using enzymes for plant cell digestion and the metabolic activity within the fungal material was not significantly reduced. Compared to common substrate-based inocula, in this way extracted spores and attached hyphae could be advantageous with respect to their applicability (Ijdo *et al.* 2011). AM fungal structures after extraction from trap roots could be

made into a suspension and then applied in a liquid medium. Such an application form might be of special interest since there is still a demand for a low-weight inoculum easy to transport and simple to apply for commercial plant production or re-vegetation activities in the field.

5.5.6 Conclusions

This study showed that spores of the AM fungus *Glomus mosseae* were located on the surface, between cortical cells and within the stelar cylinder of trap roots, irrespective whether roots have been excised from host or non-host plant species. Distant from host plant roots, the interior of trap roots represented a suitable environment for AM fungi to deposit spores and these roots were preferred over free spaces in the substrate. Dead roots may attract AM fungal growth by supplying a protected space, as well as being a source of nutrients from organic matter. The spore containing root length of coarse trap roots (thicker than 150 µm diameter) was higher than that of finer roots, supporting the theory that the dead roots provide a physical shelter for the fungi.

The AM fungus showed a considerable capacity to colonise thin trap root layers of up to 5 mm in thickness with hyphae and spores. When comparing the number of spores entrapped in the trap roots with the number in the substrate, per unit volume the trap roots contained a significantly higher quantity. This indicates that this spore aggregation method may represent a potential technique to obtain AM fungal spores in a substrate-free, low-weight carrier material. Further investigations should test the inoculum potential of spores yielded this way.

Chapter 6

General discussion

6.1 Inter-plant N transfer through a common arbuscular mycorrhizal mycelium network

Plants and free living microbes usually compete for available N pools under conditions where sufficient C is available. Moreover, the N-cycling including the decomposition of organic matter is mainly driven by microbial activities and their interaction (Beare et al. 1992; Leff et al. 2012), and soil microbes, including mycorrhizal fungi are progressively recognised to have functional importance in regulating ecosystem functioning (Herman et al. 2012).

There is still a demand for experiments that allow studying N transfer via the AM fungal mycelium between a source and a target plant. A lack of knowledge especially exists with regard to AM fungal N transfer, when N is provided by the dying off of plants. The resulting agricultural and ecological significance for the interaction between mycorrhizal and non-mycorrhizal plants in the competition for nutrients is not understood. Available evidence on the potential effects of AM fungi on N-cycling processes varies considerably, and compared with studies on P, still relatively little has been done on N transfer with microcosm studies (Veresoglou *et al.* 2012). Therefore, attempts to quantify inter-plant N transfer mediated by AM fungi are rare and still need further attempts to improve the experimental technique that achieves adequate non-mycorrhizal controls with respect to plant biomass and composition of microbial community. By using a special experimental set-up, the present study in fact provides a new insight into the transfer of N from dying roots to a receiver plant when the N has been taken up directly or indirectly from dying roots that either shared a common AM fungal mycelium network or did not. The main findings are discussed below.

6.1.1 Relevance of AM fungal N transfer for the receiver plant growth

In several cases, high quantities of soil-to-plant N transfers via the AM fungal ERM network have been reported (Ames *et al.* 1983; Johansen *et al.* 1992; Mäder *et al.* 2000; Hodge *et al.* 2001), suggesting that receiver plants might benefit significantly from AM fungal N supply,

whereas the use of N-enriched patches that are only accessible to the AM fungus may not lead to realistic conditions compared to soils where plant residues are available.

As shown with the present study, AM mediated N transfer from plant roots significantly surmounted that of direct transfer via solute movement or diffusion. However, N transfer from dying roots did not increase N content or biomass production of the receiver tomato plants. Similar results have also been reported in other studies showing that plant-to-plant N transfer is too low to be considered for the growth of mycorrhizal plants, especially when the supplier plant is alive (Ikram et al. 1994; Johansen and Jensen 1996; Jalonen et al. 2009; Li et al. 2009). Here, despite receiver plants had a high demand for N (as shown by a low N concentration in the shoot) and the fact that the extra-radical mycelium in the experimental substrate was dense, a two-week period of N capture still lead to only a small transfer by the fungus from dead roots (equivalent to less than 1 mg N per day, given the uptake is constant during the time). Compared with the plant root N uptake of for example 6-8 mg per day in maize plants (Polisetty and Hageman 1982), the fungal transfer rate observed here was low. Taking into account that plant uptake of N is about ten times greater than that of P, the fungal transfer can significantly supply a fast growing plant with P but unlikely with N. The present study confirms a lacking significance for crop plant N nutrition by AM fungi, as speculated earlier (Smith and Read 2008). However, it has often been stated that under some conditions (different from those achieved here), AM fungal colonisation may bring important advantage for the host plant: Among the inorganic N forms AM fungi preferentially take up NH_4 (Tanaka and Yano 2005; Govindarajulu *et al.* 2005), and clearly deplete NH_4 in the soil environment (Johansen *et al.* 1992). Moreover, proliferation into NH_4-rich, transient patches by plant roots may be more costly to the plant than to the fungus, since hyphae have a relatively short turnover time compared to roots (Veresoglou *et al.* 2012). Compared with the abundant nitrate, ammonium is a relatively immobile form of N. Therefore plants grown in soils dominated by heterogeneously distributed but profitable NH_4 patches might benefit most from the colonisation with AM fungi because the latter may have an improved ability to assimilate NH_4 compared to plants (Veresoglou *et al.* 2012).

6.1.2 Relevance of AM fungal N transfer for N-cycling

Plant root turnover as occurring in vegetated soils is a considerable part of the below-ground N pool (van der Krift *et al.* 2001). Organic N derived from fresh plant residues in most conditions is readily mineralised to inorganic N within a short time (Nett *et al.* 2010) and thereafter N is captured by AM fungal hyphae (Hodge and Fitter 2010). The widespread, finely structured AM

fungal extra-radical hyphae make them adapted to proliferate within the soil and organic matter (Ames *et al.* 1983; Hodge *et al.* 2001) to absorb inorganic N (as NH_4^+ or NO_3^- ; Tanaka and Yano 2005), or organic N (as amino acids; Hawkins *et al.* 2000). Following, all forms of N taken up are assimilated into amino acids (especially arginine which accumulates to high concentrations) to be further distributed within the fungal tissue and transferred to the host plant mainly as ammonium (Govindarajulu *et al.* 2005; Jin *et al.* 2005).

In the present study, a substantial amount of up to about 13% of the donor root N was transferred to receiver plants via the AM fungal network. That even occurred within the short time period of two weeks, suggesting an ecological relevance of AM fungi in the N-cycling. For example, in low N-fertilised agricultural fields the role of AM fungi that take up and assimilate N may represent one important factor reducing N losses by leaching. Soil mineral N levels in agricultural systems vary depending on plant species, management system, and the amount and source of N fertilisation. Compared with conventional managed systems, organically managed and low-input agricultural systems can have greater N pools (Poudel *et al.* 2002). The authors have assumed lower N mineralisation rates due to chemically and physically relatively stable soil organic matter compared to conventionally managed fields, and they conclude from this a resulting lower risk of N losses to deeper soil layers by leaching (Poudel *et al.* 2002). Accordingly, low-input management systems are more and more recommended for future implication in crop production (Kirchmann *et al.* 2002; Poudel *et al.* 2002; Plenchette *et al.* 2005). In temperate zones agricultural soils fertilised with less than 100 kg N ha^{-1} per year, leaching of inorganic N ranged between 10 and 40 kg N ha^{-1} per year (Bergström and Brink 1986), equivalent to 3 - 12 mg N per m^2 per day. In the pot experiment of the present study the amount ^{15}N taken up within a 14-day-period and transferred to mycorrhizal plants was up to 0.09 mg per pot (see Chapter 3) which would be equivalent to about 0.5 mg N per m^2 per day. Though this is a comparatively low value, with respect to the experimental soil conditions it may underestimate the true potential of AM fungi to prevent N losses. The donor compartment contained likely low quantities of N for several reasons: Firstly, with respect to small plant biomasses and N concentration donor roots probably represented a weak N source. Secondly, the used substrate itself contained only a small proportion of organic matter (not more than 0.4%, even including the dead root), and was certainly characterised by a low mineralisation rate. Moreover, following initial soil sterilisation the total microbial community was strongly reduced since it was forced to re-establish mainly originating from the AM fungal inoculum. An indication for a low availability of inorganic N in the planting units might have been the total N concentration observed in the AM fungal tissue (less than 1.5%).

This is not nearly matching the possible N accumulation capacity of AM fungal mycelium (about 5%; Hodge and Fitter 2010). It can therefore be assumed that the availability of N from the root was not sufficient to be stored in the fungal tissue as a reserve when it was simultaneously also delivered to the receiver plant. Among other factors, N mineralisation strongly depends on the litter quality but usually increases with litter N concentration during time (Jensen *et al.* 2005). Thereby, in the early phase of decomposition of fresh plant residues immobilisation of the limiting nutrient (mostly N) can occur so that N mineralisation can be delayed for several days (Kirchmann and Lundvall 1993; Nicolardot *et al.* 2001). It is likely that a higher concentration of soil organic matter, efficiently mineralised by microorganisms, would have represented a richer N source than that achieved in the experimental conditions presented here. A significant potential of AM fungal N capture leading to reduced N losses therefore should not be ruled out. This suggestion is underlined by the observation of Hodge and Fitter (2010), who demonstrated that AM fungi incorporate large amounts of N from soil patches enriched with organic material. Therewith, N concentration in the mycelium was up to seven times higher than that observed in the plant shoot. The authors ascribed the high level of N consumption to the need of the AM fungi for N to support the growth of the extra-radical mycelium (Hodge and Fitter 2010).

The function of AM fungi with respect to reduction of N in the soil solution has just recently begun to be studied. Testing three different plant species with various levels of nutrient supply, leachates contained a lower quantity of NH_4 when plants were AM fungal colonised (van der Heijden 2010). Another study also demonstrated lower N quantities in the leachate of mycorrhizal plants (Asghari and Cavagnaro 2012), but those results were influenced by the fact that the mycorrhizal plants had higher root biomasses than the non-mycorrhizal correspondents. However, due to the lack of experimental work that has been done so far, the mechanisms for AM fungi to reduce N losses in soil by means of hyphal uptake are not yet understood. Speculatively, organic N forms or NH_4^+ are directly taken up from dying, lysing plant cells and soil solution. Therewith, soil-borne microbes are denied access to the N and as a consequence the abundance of mineralised and easy leachable nitrate is reduced. Accordingly, a competition for NH_4^+ between AM fungi and weak ammonia oxidisers has been discussed when showing reduced nitrification rates in the mycorrhizosphere (Veresoglou *et al.* 2011). It may be safe to assume that at least the more efficient uptake of inorganic N by AM colonised plants may result in a decline of N in the soil solution and consequently also reduce N-losses through leaching (Veresoglou *et al.* 2012).

6.1.3 The common mycorrhizal mycelium network as an underground transport means affecting inter-plant communication and competition

The common mycorrhizal network connecting plants is increasingly recognised as an important determinant of plant community dynamics (van der Heijden *et al.* 2003; reviewed by Bever 2003). Hyphal networks of individual AM fungi usually show a low host specificity and the fungi form linkages between different plant species (van der Heijden *et al.* 2003) and transfer nutrients covering distances of more than half a meter (Walter *et al.* 1996). The extra-radical mycelium is a below-ground pathway for rapid inter-plant transport, as shown for N and other nutrients and solutes relevant for host plant growth, such as P (Wilson *et al.* 2006), root exudates and allelochemicals (Barto *et al.* 2011). Furthermore, the surface of AM fungal hyphae is colonised by diverse bacteria depending on the fungal species (Toljander *et al.* 2006), and the presence of AM fungi also changes the bacterial species community in soil including ammonia-oxidising bacteria (Amora-Lazcano *et al.* 1998) and other rhizosphere bacteria (Marschner *et al.* 2001; Toljander *et al.* 2006). The present study allowed for the quantification of AM fungal N transfer based either on fungal uptake of N from the rhizosphere only via the ERM (in case of *rmc* mutants, lacking intra-radical colonisation), or, in addition the uptake of N from inside of the root via the IRM (in case of wild type; see Chapter 3). By using the *rmc* mutants in the experimental set-up as a second non-mycorrhizal control this study truly contributes to a better understanding of N movement along AM mycelium networks connecting mycorrhizal or non-mycorrhizal plants with one another. Firstly, both the mycorrhizal and non-mycorrhizal plants had similar biomass and nutrient status. Secondly, different from treatments usually achieved with non-inoculation, in *rmc* controls an AM fungal mycelium was present. Proliferation of fungal hyphae including any associated microbes must therefore lead to the import of microbial communities into both the mycorrhizal and the non-mycorrhizal donor root compartments likewise. For this reason it was possible to overcome the insufficient similarity among the mentioned treatments. The latter is an important shortcoming of earlier experiments that have quantified fungal transfer of N to host plants, as others have criticised (He *et al.* 2003; Smith and Smith 2011; Veresoglou *et al.* 2012).

The present study showed that more N is transferred to host plants via the AM fungal ERM when dead donor roots have formerly been AM-colonised (WT plants) compared with roots that have not been colonised intra-radically (*rmc* mutant plants). Although it can not be excluded that the effect was also driven by a higher hyphae length density in the respective treatment, this observation strongly suggests that a proportion of the transferred N was directly

supplied by fungal structures located within the dead donor roots. Subsequent to the death of the root, the former intra-radical mycelium and vesicles might have served as propagules that germinated, and engaged with the symbiotic ERM part that was associated with the receiver root. Germinating hyphae of different spores belonging to the same isolate of *Glomus* species can anastomose. That allows the fungi to quickly distribute resources around the self-joining, spreading mycelium, as impressively demonstrated earlier (Giovannetti *et al.* 1999; de la Providencia *et al.* 2005). Increased fungal transfer of N captured from colonised dead roots is presumably translatable to other substances such as P, allelochemicals or signalling molecules. Such solutes might be transported either passively in the surface water on hyphae, or actively through the fungal cytoplasm (Barto *et al.* 2011) depending on their physical-chemical character. If so, a re-mobilisation of nutrients and solutes from root-internal AM fungal structures located in dead roots and the following distribution in the ERM would create another potential for inter-specific communication and competition among plants within a community. Proximate transport of solutes out of the rhizosphere of the producing plant and passage through the common mycorrhizal network (CMN) to the receiver plant would limit sorption to soil particles and chemical or microbial decomposition. Possibly, diffusion in a continuous layer of surface water on hyphae would enable substances to be transported more directly and faster via the CMN than via diffusion through discontinuous and tortuous water films in the soil matrix, as suggested earlier (Barto *et al.* 2011). More of the initial amount of substances would remain available at greater distances from the supplier plant.

It has been suggested that a non-random nutrient allocation through CMN's would help neighbouring host plants to limit inter-specific differences in resource acquisition and competitive ability, and by these means increase also species diversity (Grime *et al.* 1987; van der Heijden *et al.* 2003). In case of substances affecting plant growth already in small volumes, the transport via the AM fungal mycelium would be expected to have a significant ecological significance for host plants. For example, AM fungi can transfer disease resistance and induced defence signals between mycorrhizal plants (Song *et al.* 2010), and concomitantly neighbouring non-host species were excluded from such an underground pathway. Resulting, AM fungal networks would be expected to increase the competitiveness of host plants opposite other possibly more dominant non-host species. However, the report on the transport of root-originated compounds (Barto *et al.* 2011) together with the observation of fungal N transfer from decaying roots in the present study indicate that AM fungi may have a hitherto overlooked but considerable function in re-location processes of substances from dying roots

and therewith in inter-plant competition and communication. More studies on this topic would be needed in future.

6.2 The impact of soil disruption on AM fungal development and functioning

In view of the ubiquitous presence of AM fungi it appears important to understand the effects of agricultural practices on their development. Tillage is performed to incorporate manure and plant residues into soil, it produces an even seedbed for later planting and it is a tool for weed control and therefore lessens the incidence of pests and diseases. There are two main types, conventional (usually using a mouldboard plough) and conservation tillage systems. Conservation tillage, including specific practices such as no-till, ridge-tillage and reduced tillage (Kabir 2005), have been implemented increasingly worldwide to improve soil structure and to reduce soil erosion and surface runoff of nutrients as usually occurring in conventional tillage systems (Triplett and Dick 2008). There has been remarkable interest how the AM symbiosis is affected by tillage, as it physically disrupts the soil containing AM fungi. Tillage was shown to decrease hyphal viability (Kabir *et al.* 1997), decrease the root colonisation of AM fungi on new host plants (Evans and Miller 1988; Jasper et al. 1989; Jasper et al. 1991) and reduce the uptake of P and biomass accumulation of mycorrhizal plants (McGonigle *et al.* 1990; McGonigle *et al.* 1999). In contrast, it was also reported that possible effects due to the disruption treatment were absent (McGonigle and Miller 2000; Duan *et al.* 2011). The inconsistency of the results might mirror the complexity due to physical-chemical changes that follow tillage. This may include the change of the distribution of nutrients in the profile, especially P (Dick 1983), and also the distribution of AM fungal spores in the soil (Smith 1978). Former experiments in pot experiments conducted severe mechanical disruption but neglected to study the effect of reduced tillage practices where soil layers are broken up and loosened but not mixed. This might be important under the aspect that reducing tillage could (besides other advantages) minimise AM fungal hyphae destruction. Moreover it has not been studied to what extent the spatial distribution of the external mycelium in the bulk soil is related to a fungal species specific susceptibility to disruption with regard to infection potential and fungal contribution to following host plant growth.

6.2.1 The effects of tillage systems on the infectivity of AM fungi

In the present study soil disturbance was conducted by loosening the soil and avoiding a vertical dislocation of soil layers during the tillage process (as achieved by e.g. chisel

cultivation). Consequently, the distribution of soil nutrients as well as the distribution and densities of spores were maintained, whereas only the hyphal network was disconnected. When mechanically disrupted by these means, a pre-established AM fungal external mycelium was not significantly affected with respect to its infection potential and growth promotion of a following plant. The *Glomus* isolates compared to fungi in the field soil inoculum had the highest extent of spatial spread in the bulk soil and it is likely that those fungi could better compensate for the disruption due to a faster hyphae growth. This accords with the findings of McGonigle *et al.* (2003) and Duan *et al.* (2011) that faster growing *Glomus* isolates (there *G. intraradices* and *G. mosseae*) were less susceptible to disruption than *Gigaspora* species, which had a much slower hyphal spread at the time of disturbance. It is important to note that the outcome of the symbiosis (i.e., fungal contribution to plant P nutrition) is largely influenced not only by the hyphae length density in the soil but also by the fungal specific capacity for nutrient transfer to the root via the external mycelium (McGonigle *et al.* 2003). The results showed that plants colonised by AM fungi from field soil inoculum with low hyphal density in soil chambers distant from the host plant root thus contributed clearly to plant P supply compared with plants colonised by *Glomus* species that intensively spread into the distant bulk soil. Concluding, given the experimental soil conditions of the present study, a highly beneficial AM symbiosis may not only depend on a hyphal translocation of P over large distances. More likely, depending on the AM fungal species, an efficient P supply can also be achieved when taken up from zones close to the host roots, as shown earlier with studies tracing radioactive labelled P (Smith *et al.* 2000). Furthermore, a high fungal specific capacity for hyphal nutrient transfer might compensate a low hyphal density in soils (see also McGonigle *et al.* 2003).
Remarkably, the extent of AM fungi to promote the growth of their host was similar in both the symbiosis with the first plant (maize) as well as with the following plant (sweet potato; see Chapter 4). Once the external mycelium has been established in the bulk soil, the functional capacity seemed to persist from one plant generation to the next, and the effect even occurred with different host plant species following one after another.

There are two main aspects involved in the effect of conventional tillage that are possibly responsible for the deterioration of the outcome of AM fungal symbioses. Firstly, conventional mouldboard ploughing can be followed by remarkable losses by surface runoff of nutrients such as P compared with non-turning chisel-plough tillage (Blevins *et al.* 1990). In cases where mouldboard ploughing in field experiments reduced the uptake of P in early developmental stages of mycorrhizal plants (McGonigle *et al.* 1990; McGonigle *et al.* 1999) it could not be

excluded that the effect might have been induced by other side factors such as altered nutrient distribution or mineralisation rates in the soil due to tillage. However, in the present study a possible nutrient loss effect was unlikely, since the disruption treatment in the experiment avoided a vertical disarrangement of the soil (see Chapter 4).

Secondly, another side effect in conventionally tilled fields that has been discussed in literature is the reduction of the number of infective AM fungal propagules in the rooting zone when tilled to more than 15 cm depth (Kabir *et al.* 1998). This is possibly a result of a dilution of top soil layers when overlaid by the soil of deeper layers (Smith 1978) since top soil layers usually contain a higher proportion of fungal spores (Smith 1978; Oehl *et al.* 2005). Accordingly, others have assumed that a high propagule density is able to compensate for the negative effect of tillage (Jasper et al. 1991; McGonigle and Miller 2000). However, the present results indicate that reduced tillage may be classified as a neutral agricultural practice with regard to AM fungal symbiotic functioning when fungi possess a sufficient reservoir of spores and / or a fast hyphae development. Furthermore, as shown with the increased N transfer via a symbiotic ERM, N resources contained in root residues are captured by a disrupted hyphal network of *G. intraradices* after its re-establishment (see Chapter 3). An increased N transfer after disruption may indicate that reduced tillage together with remaining plant residues in agro-ecosystems may be beneficial for crop plant growth and also contribute to the fungal capture of nutrients. Concluding, the implementation of reduced tillage systems (that are loosening but not mixing the soil) is unlikely followed by direct disadvantages for AM fungal development. A similar harmless effect has recently been reported for AM fungal P transfer after disruption (Duan *et al.* 2011).

6.2.2 The colonisation of a root by a detached ERM is followed by early plant growth promotion

Under conditions of minimal soil disruption AM fungi were able to re-colonise a following plant within a short time period, as shown for the sweet potato plants colonised by the extra-radical mycelium of all used AM fungal inocula within four weeks, resulting into a remarkable increase of host plant uptake of P and biomass increment. In case AM fungal-friendly management methods are implemented in an agricultural field (as for example reduced tillage in combination with low fertiliser input), AM fungi may substantially contribute to the growth of crop plants especially under abiotic conditions unsuitable for the development of young plants. Such conditions might include low soil temperatures (e.g. in the spring season), especially accompanied by the accumulation of plant residues in reduced tillage systems

compared with conventional tillage systems (Kladivko *et al.* 1986). Such factors can slow down optimal root development and therewith also limit the P nutrition of the seedling. Furthermore, a limited P uptake capacity of marginally developed root systems of young plants may be compensated by the supply of P via the fungal mycelium. In this study, sweet potato plants, obviously highly responsive to AM fungal colonisation, benefited in very early growth phases from their symbiotic partner. Accordingly, Bressan *et al.* (2000) showed that *in vitro* inoculation of somatic embryos of sweet potato with *Glomus etunicatum* improved embryo survival and plantlet formation. Studying sequentially harvested mycorrhizal sweet potato plants, O'Keefe and Sylvia (1993) demonstrated that significantly improved yield was related to early enhanced tissue P concentration due to AM fungal colonisation. The authors also observed a seasonal change in plant P status and suggested that sweet potato plants may have a strategy to store P by the re-allocation of P from the shoots to the roots. Drought stress resistance of plants can be improved by mycorrhizal colonisation (Subramanian *et al.* 1995; Neumann *et al.* 2009), especially during the time of main growth or storage-root formation of sweet potato plants (O'Keefe and Sylvia 1993). It can be concluded that, despite a physical soil disruption followed by agricultural practices similar to reduced tillage, AM fungi are able to colonise fast and to improve P supply in the early phases of plant development. Therewith, the symbiosis may sustainably improve the following crop yield of plant species that are very responsive to mycorrhizal colonisation.

6.3 AM fungal sporulation in dead roots is strategic

6.3.1 The preferred proliferation of ERM towards organic matter and the attraction of AM fungal sporulation by root fragments

The present research provides new data on quantities and patterns of AM fungal sporulation within dead plant roots (see Chapter 5). The results were well supported, as they were confirmed in three independent experiments conducted in different experimental set-ups. Here, uniquely, it was shown that remarkable high numbers of spores can be observed in root fragments. Hyphal proliferation was observed in both the empty, air-filled gaps in soil as well as inside of root fragments. In contrast, spores were deposited in and on the surface of root fragments. That is concert with other reports, demonstrating a preference of AM fungi to proliferate into soil patches enriched with plant residues (Joner and Jakobsen 1995; Hodge et al. 2001; Hodge and Fitter 2010), and higher sporulation quantities have been observed inside empty seed cavities than inside inert glass capillaries (Rydlova *et al.* 2004). This indicates that

AM fungi are possibly attracted by root fragments due to the function as a protective shelter in combination with an increased availability of nutrients from the organic matter. Sporulation into dead roots and other hollow bodies has been consistently observed using different isolates of *G. mosseae* (see Chapter 5) and with diverse *Glomus* isolates (*G. claroideum* and *G. intraradices*) originating from different field sites (Rydlova *et al.* 2004). This sporulation habit might not be restricted to a small group of *Glomus* species but might be characteristic for the majority of AM fungal species having a strong disposition to produce spores on the ERM.

Like above-ground litter fall, below-ground detritus, such as dead roots, supports microbial life that is involved in the mineralisation of the detritus. The growth of the ERM in fungal compartments can be enhanced by the application of yeast (Ravnskov *et al.* 1999a), peat extract (Ma *et al.* 2006), or inorganic nitrogen (Bago *et al.* 2004; Ma *et al.* 2006). AM fungi usually show a clear physical proximity to other rhizosphere microorganisms, such as nitrogen fixing and plant growth-promoting bacteria (Gerdemann and Trappe 1974; Ho 1988; Bianciotto *et al.* 2001; reviewed by Artursson *et al.* 2006), and improved growth and establishment of ERM in presence of certain bacteria has been reported (Xavier and Germida 2003). Research that focussed on direct effects of bacteria on AM fungal sporulation still is scarce and is restricted to the observation of new spore production on mycelia that is fed by germinated resting spores in axenic media (e.g. Tobar *et al.* 1996). However, a close association of AM fungi and bacteria that are involved in the decomposition of root fragments might enhance the availability of nutrients (such as P and N) for AM fungi. The trophy towards organic compounds such as root fragments may mirror a high capacity of AM fungi to selectively migrate towards nutrient sources derived from decomposed organic matter, as a strategy for an effective nutrient exploration. Underlining this, a preferential proliferation into patches with organic matter compared with proliferation towards a new, uncolonised host plant was demonstrated (Hodge *et al.* 2001). The authors speculated that the fungus gets a greater benefit from the patch than from the additional C supply by the host, and AM fungi might therefore obtain a growth benefit from organic matter in soil (Hodge and Fitter 2010). Accordingly, an increased host plant root colonisation occurred when AM fungi had access to a separate fungal compartment containing organic matter, and the ERM took up considerable amounts of isotope-labelled N from these patches (Leigh *et al.* 2009). It seems assumable that spore production within root fragments indicates another strategy of AM fungi to (1) improve their survival by using roots as a physical shelter (e.g. hiding from mesofaunal predators), and (2) raise their competitiveness as a symbiont when storing their resources directly in the nutrient-rich soil patches that are not accessible to competing plant roots. As a consequence, this behaviour probably improves

viability and infectivity of AM fungal mycelium resting in soil during non-symbiotic time periods.

6.3.2 Root fragments as a low-weight carrier material for future of AM fungal inoculum production?

The volume of AM fungal inoculum traded worldwide is increasing considerably since the past years (Grotkass *et al.* 2005). Still the most often used carrier materials are based on solid substrates, such as expanded clay and sand, characterised by a high weight and volume. Moreover, the production of pure spores *in vitro* is too costly (Ijdo *et al.* 2011). Here, remarkably high numbers of spores were obtained per unit dry weight of root fragments compared to the colonised host plant roots (see Chapter 5). There probably is a high potential to use trap roots as a suitable target material for spore accumulation. The present work suggests suitable methods to extract spores from the root fragments, and opens up a new perspective on the development of non-sterile inocula, almost free from carrier material. Low-weight AM inocula would reduce the effort required for transport and application, and would clearly improve the acceptance of AM inoculum among potential costumers (Feldmann 2008; Carolin Schneider 2011, personal communication). This may also allow an expansion into new fields of application in plant production and re-vegetation activities. Further investigations that contribute to our knowledge on AM fungal spore accumulation in suitable carrier materials are needed.

7 Summary / Zusammenfassung

7.1 Summary

Occurring in the majority of natural ecosystems existing worldwide, the arbuscular mycorrhizal symbiosis has an impact on most land plant species. The symbiosis is based on the reciprocal exchange of nutrients taken up from the soil by the fungus and transferred to the plant, and plant derived carbohydrates transferred to the fungus. Hyphae networks of arbuscular mycorrhizal (AM) fungi represent pathways for nutrient flux between neighboured plants and also are important resources for new root colonisation. Commonly used agricultural practices often create conditions that may be harmful to the development and diversity of AM fungi. In view of ecological and economical problems going along with intensive plant production systems, more and more sustainable culture systems are implemented. There, AM fungi may play a more significant role for plant development. To achieve an adequate handling of the AM symbiosis in plant production, it is important to understand the impact of common agricultural practices.

The present study contributes to the understanding of physiological aspects of the AM symbiosis, focussing on AM fungal hyphae spread patterns in soil and plant residues contained within as well as nutrient acquisition from dead roots of host and non-host plants. The impact of mechanical soil disturbance on AM fungal development and nutrient transfer potential was also studied. To achieve that the following hypotheses were formulated:

Hypothesis 1

Nitrogen (N) can be absorbed by the extra-radical mycelium (ERM) of the AM fungi from decaying plant roots (donor plant) and delivered to a living plant (receiver plant). Thereby uptake and transfer of N from dead roots will be higher in the presence of a mycorrhizal network than without. (Chapter 3)

Major-Findings

As hypothesized, mycorrhizal receiver plants gained higher quantities of fungal mediated N from donor plants that had been colonised. This increase may have been due to a higher hyphae length density in the soil adjacent to the colonised roots. The results indicate a possible re-mobilisation of N reserves from fungal structures located within the root and a subsequent export to the receiver plant through hyphal networks. The amount of N transferred was low compared to the direct uptake by the plant root. The results confirm the suggestion made in earlier studies that in soils with a low N availability AM fungal N transfer alone cannot meet the N demands of a fast growing plant being N deficient. However, a considerable portion of the N contained in the dead root of the donor plant was transported by the fungus to the receiver plant. If, and under which circumstances AM fungi by absorbing nutrients may reduce N losses through leakage has to be investigated in further studies.

Hypothesis 2

When soil containing an established mycorrhizal network is mechanically disturbed, AM fungal N transfer to a colonised host plant will be significantly reduced. (Chapter 3)

Major-Findings

In contrast to the hypothesis the N transfer to a receiver plant was increased as a consequence of soil disturbance. The fragmentation process probably lead to elevated N losses from root fragments and thereby increased the nutrient availability in the soil readily taken up and transferred by the fungus. As long as the mycelium was symbiotically associated with a host plant the fungus might have a high capacity to re-establish the network after fragmentation and again function as a nutrient transport vessel. A soil fragmenting technique in combination with the incorporation of plant residues may aid a fast assimilation of mobile, inorganic N into the mycelium. This result is contrary to earlier studies where intensive forms of soil disturbance showed negative impacts on the functioning of the AM symbiosis.

Hypothesis 3

When fungal colonisation of plants is established exclusively by the ERM, AM fungal isolates with a higher extent of ERM proliferation in the soil volume prior to mycelium excision will have a higher inoculum potential and growth promoting effect on the subsequent crop. (Chapter 4)

Major-Findings

After excision from a host plant, AM fungal isolates with a higher extent of ERM spread in soil also showed a higher root colonisation rate and contributed to growth of a subsequent plant. Also, AM fungi were able to partly compensate for a slow development of their ERM at the beginning of the symbiosis, possibly by means of a high fungal capacity for nutrient uptake and transfer, as also suggested in earlier reports. Under conditions of low P availability, mycorrhiza-responsive plants may benefit enormously from the colonisation in early phases of growth. AM fungal isolate-specific patterns of vertical root colonisation and horizontal spread of the ERM in soil were consistent in association with both, the initial host (maize) as well as with the successor host plant (sweet potato). This indicates that AM fungi maintain specific growth patterns, irrespective of the host plant species.

Hypothesis 4

The mechanical fragmentation of detached ERM, induced by soil disturbance, reduces AM fungal inoculum potential and consequently reduces fungal contribution to P uptake and growth of the next plant. (Chapter 4)

Major-Findings

There was no evidence that soil disturbance affected the fungal contribution to P uptake or growth of mycorrhizal host plants, when the soil profile was maintained. The tested AM fungal inocula had a high inoculum potential, irrespective of the spatial distribution of the ERM before the disruption. The results suggest that established and fast growing mycelia are not negatively affected by management practices that are loosening and fragmenting the soil without turning it upside down.

Hypothesis 5

Spore development within dead roots will not depend on whether the root originated from a host or a non-host plant species, but rather will increase with root diameters. (Chapter 5)

Major-Findings

The presence of dead roots in soil clearly stimulated hyphal proliferation as well as sporulation on the surface and inside root fragments, irrespective of whether roots originated from a host or a non-host species. In accordance with the outlined hypothesis, the average number of spores per unit root length was higher in root fragments with larger diameter. In comparison with the surrounding soil or air gaps contained within, AM fungal sporulation occurred preferably in dead roots. This observation may be justified by the fact that AM fungi usually prefer to

proliferate into soil partition with elevated nutrient availability. The organic matter from decaying roots release nutrients available to the fungus (directly or indirectly after microbial mineralisation). Since decaying plant roots are widespread in vegetated soils they may serve the fungus as important physical shelter to protect fungal propagules before the establishment of a new symbiosis. Spore aggregation within root fragments may represent a potential technique to obtain AM fungal spores in a low-weight carrier material. This would meet the present demand for the development of AM inoculum products, easy to transport and allowing effective application.

When cultivated in C-loess already in an early developmental stage mycorrhizal sweet potato plants took up more than double the amount of P than non-mycorrhizal plants. The speed with which some of the AM fungal isolates contributed to growth of the next plant after the infection which can partly be explained by the fast spread of mycelium into the soil, known for some AM fungi. Despite a low degree of colonisation in the bulk soil and in roots, the AM fungi showed a high potential to contribute to P uptake of the next crop in an early time of the symbiosis. In condition of low P availability the AM symbiosis may significantly improve the growth of plants which have a relatively low capability to forage for P.

The advantages of the AM symbiosis in plant production can be maximised by implementing suitable culture systems. One suitable method may be to apply conservation tillage combined with reduced fertilisation. Therewith, agricultural practices that do not change the vertical soil profile but are rather fragmenting the soil structure obviously do not have a relevant impact on AM fungal growth or infection potential. Nutrients that derive from plant residues in soil likely play a significant role in low input culture systems. The results showed that AM fungi have a targeted growth towards decaying roots to deposit nutrient reserves therein in the form of spore material. The fungi may absorb nutrients released from the present organic material (such as from decaying roots) and distribute the resources within the hyphal network. Where N is mainly in an immobile form, for example as organic N or ammonium it can become scarcely available to plants. Via the profusely branched mycelium the AM fungus may reach such N sources that are less accessible even for fine plant roots. The fungus can compete with other soil microorganisms for the recently mineralised nutrients (such as N). Even if only part of the assimilated N is transferred to the host plant via the mycelium, the most might be kept in fungal tissues located in top soil layers and may become available for the pants in a long term.

A complete extraction of the ERM from the soil for AM fungal studies bears a considerable operating expense and might explain the predominant use of soil-less media for studies on AM fungal physiology. With the development of techniques that allow the extraction of almost intact ERM it became possible to directly examine effects on morphology and physiology of AM fungi in soils. For the better understanding of cultivation processes and their consequences for the AM symbiosis in plant production future studies should focus on benefits of AM fungal colonisation in early stages of host plant growth in different soil types. More information should be obtained on targeted acquisition of nutrients from organic material by the external mycelium and its closely associated microorganisms.

7.2 Zusammenfassung

Die arbuskuläre Mykorrhizasymbiose ist in einem Großteil aller weltweit verbreiteten Ökosysteme verbreitet und nimmt Einfluss auf das Wachstum einer Mehrzahl aller Landpflanzenspezies. Die Symbiose beruht auf dem wechselseitigen Transfer von aus dem Boden aufgenommenen Nährstoffen vom Pilz zur besiedelten Wirtspflanze und pflanzeneigener Kohlenhydrate von der Pflanze zum Pilz. Hyphennetzwerke der arbuskulären Mykorrhiza- (AM) pilze stellen Transportwege für den Nährstoffffluss zwischen benachbarten Wirtspflanzen dar und sind eine wichtige Ressource für die Besiedelung von Pflanzen. Die in der Landwirtschaft angewandten Kulturmaßnahmen induzieren oft Bedingungen, die die Entwicklung der AM Pilze und deren Diversität beeinträchtigen. Angesichts der ökologischen und ökonomischen Probleme, die mit der intensiven Pflanzenproduktion einhergehen, werden zunehmend nachhaltigere Kultursysteme eingeführt, in denen die AM Pilze einen höheren Stellenwert bei der Pflanzenentwicklung haben. Aufgrund der Empfindlichkeit, mit der AM Pilzen in anthropogen genutzten Böden reagieren, ist es wichtig, die Einflüsse von Kulturmaßnahmen besser zu verstehen und damit zu einem sicheren Umgang mit der Symbiose in der Pflanzenproduktion zu gelangen.

Diese Studie leistet einen Beitrag für ein besseres Verständnis der physiologischen Aspekte der Mykorrhizasymbiose, im Besonderen unter dem Aspekt der Ausbreitungsmuster von Hyphennetzwerken im Boden und in darin enthaltenen Pflanzenteilen sowie der pilzeigenen Nährstoff-Akquirierung aus abgestorbenen Wurzeln von Wirten bzw. Nicht-Wirten. Diesbezüglich wurde auch der Einfluss mechanischer Bodenbearbeitung auf die Entwicklung und Nährstofftransferleistung der AM Pilze untersucht. Zu diesem Ziel wurden die folgenden Hypothesen bearbeitet:

Hypothese 1

Stickstoff (N) wird durch das extra-radikuläre Myzel (ERM) der AM Pilze aus abgestorbenen Pflanzenwurzeln aufgenommen und an eine Empfängerpflanze geliefert. Dabei sind die Aufnahme und der resultierende Transfer von N aus einer toten Wurzel höher in Anwesenheit einer mykorrhizierten Wurzel verglichen mit einer nicht-mykorrhizierten Wurzel. (Kapitel 3)

Ergebnisse

Entsprechend der Hypothese erhielt die Empfängerpflanze über das Hyphennetzwerk mehr N aus einer Wurzel, die vor dem Absterben mykorrhiziert war. Dieser Unterschied kann durch eine höhere Hyphenlängen-Dichte im Boden in unmittelbarer Nähe zur besiedelten Wurzel

begründet sein. Des Weiteren deuten die Ergebnisse auch auf eine Re-Mobilisierung von N Reserven aus Pilzstrukturen des Wurzelinnern und einem anschließenden Export durch das Hyphennetzwerk zur Empfängerpflanze hin. Die transferierte Menge N war im Verhältnis zur Aufnahme durch die Pflanzenwurzel gering. Die Ergebnisse bestätigen Vermutungen aus früheren Studien, dass in Böden mit geringer N Verfügbarkeit der Transport durch die Mykorrhiza nicht signifikant zur Versorgung einer schnell wachsenden Pflanze mit hohem Stickstoffdefizit beiträgt. Verglichen mit in den abgestorbenen Wurzeln der Quellpflanze vorhandenem N Mengen wurde jedoch ein beachtlicher Anteil durch den Pilz zur Empfängerpflanze transportiert. Ob und unter welchen Bedingungen AM Pilze durch die Nährstoffabsorption möglicherweise N Verluste durch Auswaschung verringern können, bleibt in künftigen Studien zu untersuchen.

Hypothese 2

Durch mechanische Bodenstörung, bei der im Boden befindliche tote Wurzeln und das externe Myzel des AM Pilzes zerstückelt werden, wird der Transfer von N durch AM Pilze zu einer mykorrhizierten Empfängerpflanze signifikant reduziert. (Kapitel 3)

Ergebnisse

Entgegen der Hypothese ist der N Transfer zur Empfängerpflanze durch die Bodenbearbeitung erhöht worden. Der Zerkleinerungsprozess führte wahrscheinlich zu gesteigerten N Verlusten aus den Wurzelfragmenten und damit auch zu vermehrter Nährstoffverfügbarkeit. Die Nährstoffe konnten dann vom AM Pilz aufgenommen und transportiert werden. Solange das Myzel in einer symbiotischen Verbindung mit einer Wirtspflanze ist, hat das Myzel des hier verwendeten AM Pilzes scheinbar eine ausgeprägte Fähigkeit, sich nach der Fragmentierung neu zu etablieren und wiederholt als Transportgefäß zu fungieren. Eine lockernde und zerkleinernde, aber nicht wendende Bodenbearbeitungstechnik in Verbindung mit der Einarbeitung von Pflanzenrückständen dürfte dementsprechend die schnelle Assimilierung von mobilem, anorganischem N in das AM Pilzmyzel begünstigen. Dieses Ergebnis steht anderen Studien entgegen, in denen durch intensive Formen der Bodenbearbeitung negative Effekte auf die AM Symbiose verursacht wurden.

Hypothese 3

Bei der Neubesiedelung einer Wirtspflanze ausschließlich mittels des externen Myzels haben solche AM Pilz-Isolate das größere Infektionspotential und den höheren Beitrag zum

Wachstum einer nachfolgenden Wirtspflanze, welche die größere räumliche Ausbreitung des ERM im Boden aufweisen. (Kapitel 4)

Ergebnisse

Gemäß der Eingangshypothese wiesen die Pilze mit dem höheren Grad an ERM Ausbreitung im Boden nach der Abtrennung von einer Wirtspflanze auch die größeren Wurzelbesiedelungsrate und Beiträge zum Wachstum einer nachfolgenden Pflanze auf. Darüber hinaus konnten Pilz-Isolate zu einem beachtlichen Teil ihre langsame ERM Entwicklung zu Beginn der Symbiose kompensieren, vermutlich durch eine hohe spezifische Kapazität für Nährstoffaufnahme und –transport, wie auch vorangegangene Studien postulierten. Unter Bedingungen geringer P Verfügbarkeit können mykotrophe Pflanzen bereits in frühen Wachstumsstadien deutlich von einer AM Symbiose profitieren. AM Pilz Isolat spezifische Muster bei der vertikalen Wurzelbesiedelung und der ERM Ausbreitung im Boden wurden sowohl in der Vorgängerpflanze (Mais) als auch in der nachfolgenden Wirtspflanze (Süßkartoffel) gefunden. Pilzart-spezifische Wachstumsmuster werden wahrscheinlich unabhängig von der gegenwärtigen Pflanzenart vom AM Pilz beibehalten.

Hypothese 4

Die Fragmentierung des ERM durch mechanische Bodenstörung führt zu einem reduzierten Infektionspotential und resultierend auch zu einem verminderten Beitrag des AM Pilzes zu P Aufnahme und Wachstum der nachfolgenden Wirtspflanze. (Kapitel 4)

Ergebnisse

Unter Beibehaltung der vertikalen Anordnung der Bodenschichten hatte die Bodenbearbeitung, entgegen der aufgestellten Hypothese, keinen Einfluss auf Wachstum oder P Aufnahme der besiedelten Wirtspflanze. Die untersuchten Pilz-Inokula wiesen ein hohes Infektionspotential auf, unabhängig vom Ausbreitungsgrad des ERM im Boden vor der Störung. Aufgrund der Ergebnisse ist zu vermuten, dass ein im Boden etabliertes und schnell wachsendes Myzel bei der erneuten Besiedelung von Wirtspflanzen durch lockernde und zerkleinernde Bodenbearbeitung unbeeinträchtigt bleibt.

Hypothese 5

Die Sporenmengen in toten Wurzeln sind unabhängig davon, ob die Wurzel von einer Wirt- oder Nicht-Wirtpflanze stammt, jedoch nimmt die Sporenmengen mit größer werdendem Wurzeldurchmesser zu (Kapitel 5)

Ergebnisse

Die Gegenwart toter Wurzeln im Boden stimulierte deutlich die Ausbreitung von Hyphen und die Sporenbildung auf und in den Wurzelfragmenten, unabhängig davon ob letztere von Wirt- oder Nicht-Wirtpflanzen stammten. Dabei war, entsprechend der Hypothese, die Sporenmenge je Wurzellängeneinheit am höchsten in den Fragmenten mit dem größten Durchmesser. Gegenüber dem umgebenden Boden oder darin enthaltenen Lufteinschlüssen war die Sporenbildung in Wurzelfragmenten deutlich bevorzugt. Diese Beobachtung ist wahrscheinlich darin begründet, dass sich AM Pilze gezielt in Bodenarealen mit erhöhtem Nährstoffangebot aufhalten, hier in Form von organischem Material aus Wurzelfragmenten, welche für den Pilz verfügbare Nährstoffe freisetzen (direkt oder indirekt nach mikrobiellem Abbau). Abgestorbene Pflanzenwurzeln, wie sie in natürlichen Böden vorhanden sind, dienen den Pilzen möglicherweise auch als physikalischer Schutzraum zur Überdauerung des Myzels vor der Etablierung einer neuen Symbiose. Die Aggregation in Wurzelfragmenten stellt eine potentielle Methode zur Gewinnung von AM Pilzsporen in einem sehr leichten Trägermaterial dar. Letzteres würde einen wichtigen Beitrag zum bestehenden Bedarf für die Entwicklung von leicht transportablen und effizient anwendbaren Inokulumprodukten leisten.

Bereits in einer frühen Wachstumsphase nahmen in C-Loess gewachsene, AM besiedelte Süßkartoffelpflanzen etwa die doppelte Menge P auf als unbesiedelte Pflanzen. Die Schnelligkeit, mit der einige Pilzisolate zum verbesserten Pflanzenwachstum beitrugen, war zum Großteil davon abhängig, wie stark die Ausbreitung des ERM im Boden war. Zusätzlich haben AM Pilze eine hohe Kapazität, trotz einer anfänglich nur geringfügig ausgeprägten Besiedelung des Bodens und der Pflanzenwurzel, schon in frühen Stadien der Symbiose schnell zur P Aufnahme der Pflanze beizutragen. Unter Bedingungen geringer P Verfügbarkeit kann die AM Symbiose verstärkt die Entwicklung insbesondere solcher Pflanzen verbessern, welche über eine besonders geringe Fähigkeit zur P Aufnahme verfügen.

Die Nutzung der Vorteile der AM Symbiosen in der Pflanzenproduktion setzt die Implementierung geeigneter Anbausysteme voraus. Eine hierfür geeignete Methode stellt möglicherweise die konservierende Bodenbearbeitung in Verbindung mit reduzierter Düngung dar. Dabei nehmen nicht-wendende, sondern lockernde Kulturtechniken offensichtlich keinen relevanten Einfluss auf Wachstum und Inokulumpotential der AM Pilze. Die aus Pflanzenrückständen in den Boden gelangenden Nährstoffe spielen eine wichtige Rolle in wenig gedüngten Kultursystemen. Die Ergebnisse zeigten, dass AM Pilze unter anderem gezielt abgestorbene Wurzeln zur Deponierung von Reservestoffen besiedeln und aus

vorhandenem organischem Material (so auch aus absterbenden Wurzeln) freigesetzte Nährstoffe in ihr Hyphennetzwerk aufnehmen und darin verteilen. Wo N in immobiler Form dominiert, beispielsweise als organisches N oder Ammonium, kann es für Pflanzen schwer verfügbar werden. Durch sein intensiv verzweigtes Myzel kann der Pilz sich zu derartigen, für feine Pflanzenwurzeln möglicherweise schwer erreichbare, N Quellen gezielt Zugang verschaffen. Dabei könnte der Pilz mit anderen Mikroorganismen um die gerade mineralisierten Nährstoffe (so auch N) konkurrieren. Auch wenn das im Hyphennetzwerk assimilierte N nur zu einem Teil an die Wirtspflanze transferiert wird, so wird es dennoch im Pilzgewebe in den oberen Bodenschichten festgehalten und bleibt damit langfristig für die Pflanze verfügbar.

Eine vollständige Extraktion des ERM aus dem Boden, die eine genaue Untersuchung der AM Pilze erlaubt, ist aufwändig und mag ein Grund dafür sein, dass in bisherigen Studien über die AM Physiologie hauptsächlich bodenlose Medien verwendet wurden. Mit der Entwicklung von Techniken, die die Extraktion von nahezu intaktem ERM erlauben, ist es möglich, tatsächliche Effekte auf die morphologische und physiologische AM Pilzentwicklung im Boden gezielt zu untersuchen. Zum besseren Verständnis über Kultivierungsprozesse und deren Folgen für die AM Symbiose im Pflanzenbau sollte sich die zukünftige Forschung auf die Vorteile einer Mykorrhizabesiedelung in frühen Wachstumsstadien von Wirtspflanzen in unterschiedlichen Bodenarten fokussieren. Es bedarf auch eines besseren Verständnisses über die Gewinnung von Nährstoffen aus organischem Material durch das externe Myzel und mit ihm eng assoziierter Mikroorganismen.

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