Università degli Studi di Palermo

Dipartimento di Agronomia Ambientale e Territoriale

Dottorato di Ricerca in *Agro-Ecosistemi Mediterranei*

S.S.D. – AGR02

Effects of arbuscular mycorrhizal symbiosis on nitrogen dynamics in Mediterranean agro-ecosystems

Dottorando
dott. *Sergio Saia*

Co-Tutor
dr. *Jose Manuel García Garrido*

Tutor
prof. *Dario Giambalvo*

Coordinatore del dottorato
prof.ssa *Adriana Bonanno*

Ciclo XXI

Ricchezza.
Questo è il sentimento che mi accompagna alla fine del lavoro svolto per redigere la tesi di dottorato. Tutte le persone che ho incontrato, con le quali ho lavorato, scherzato, amato, pianto o semplicemente passeggiato hanno contribuito tanto quanto me alla produzione di questa tesi. E tutte mi hanno arricchito, incommensurabilmente arricchito.

Ringrazio Dario e Pepe, oltre che maestri, sono stati anche amici, per avermi dato questa preziosa opportunità e per avermi guidato, corretto e richiamato, pur senza perdere il tatto, ogni qual volta ce ne sia stato bisogno.

Ringrazio Paolo, e Inma S ed Emilio per l'aiuto inestimabile.

Ringrazio Maribel, Nuria, Julia, Mariangeles, Rosita, Bea, Gaetano A, Horst, Inma G, Juan Antonio, Alessandra, Totò e Giuseppe per i consigli, e anche per le cervezas!

Ringrazio Rafa, Rosario, Merche y Alvaro, mis Amigos.
Ringrazio Mar e Rosa, persone speciali.
Ringrazio (in un rigoroso disordine): Vilma, Jose S y Ana, , Ely, Ciro, Jose M, Belen, Isa, Mario, Iván, Iratxe, Miguel Ángel, Manu, Marcello, Marta, Sabine, Jesus, Celia, Benedetta, Marco, Rodolfo, Benedetto y Jean (e chissà quanti ne dimentico…) per i sorrisi, per l'amicizia, per le tapas y las cañas, perchè siamo stati bene insieme.

Ringrazio Vincenzo, Totò, Angelo, Nino, Franco, Francesco e quanti con amicizia e dedizione hanno contribuito alla realizzazione del mio esperimento di campo presso l'azienda Pietranera (S. Stefano di Quisquina, AG - Italia).

Ringrazio i tecnici della Estación Experimental Del Zaidín (Granada – Spagna), senza i quali non avrei potuto, da solo, realizzare la prova in ambiente controllato e saluto con affetto Pedro.

Ringrazio Mamma e Papà, e non serve che ne accenni il motivo.

Ringrazio la mia ragazza, Angela, che in questi tre anni ha *imperterritamente* superato tutte le prove di sopportazione che le ho propinato, in primis stare con me.

Ringrazio, scusando la mia mente fallace, tutti coloro che dimentico in questa lista, la quale sarebbe troppo lunga se citassi tutti i loro nomi.

Grazie a tutti, grazie per avermi arricchito.

Sergio.

TABLE OF CONTENTS

1 INTRODUCTION

Agriculture changes. Thanks to a growing ecological sensibility, international agricultural research is focusing towards sustainable production strategies. These strategies primarily foster to reduce energy input in agro-ecosystems through an optimization of the turnover of the elements and through an improvement of biological control of stresses. So, the sum of this studies leans on the application of the sustainable development in agriculture (Altieri, 1994), which meet to the social expectations of food quality and environmental protection. In sustainable systems, soil microflora and soil fauna are pivotal to control soil borne diseases, to determine species fitness and production quality due to their roles in the biogeochemical cycle of elements. For such matters, scientific research shows growing interest to delve into biodiversity, dynamic and significance of microbial populations in soil and into the mechanisms that rule their biological activity (Werner, 1998; Kennedy, 1998; Bowen & Rovira, 1999).

In the immense soil microbial survey, mycorrhizal fungi and N_2 fixing bacteria (NFB) play a major role as symbionts of cultivated crops. Relating their matter of importance and distribution, NFB-legumes symbiosis has been extensively studied regarding its physiology and benefits to plants (Vance, 2001; Lum & Hirsch, 2003). Mycorrhizas (greek: μύκης (mykes) = fungus; ρίζα (rhiza) = roots, after Frank, 1885) have the same importance and a distribution as widespread as NFB. Mycorrhizas are symbioses between plants (macro- or phyto-biont) and fungi (micro- or myco-bionts). It seems that mycorrhizas appeared on earth about 460 million years ago (Redecker *et al.*, 2000) and helped plants during the early colonization of lands (Pirozynski & Malloch, 1975; Simon *et al.*, 1993; Brundrett, 2002). Thanks to its ancestral occurrence, this association can be found in the 80% of vegetal species (Smith & Read, 1997) and it's responsible of major nutrient and water plant uptake in most of the environments (Harley & Smith, 1983; Trappe, 1987; Allen, 1996), especially in the harshest for plant survival or production (Mosse *et al.*, 1981).

The capability of being infected by mycorrhizas vary with the plant species. Usually legumes are likely to be infected by AM fungi. Some plant species seems to be completely dependent by mycorrhizas for their nutrient uptake and others do not form mycorrhizal associations. The non-mycorrhizal-host traits of some species (manly in the families *Juncaceae, Cruciferae*, *Chenopodiaceae*, *Cyperaceae* and *Caryophyllaceae*) (Wang & Qiu, 2006) might be a derived trait and it might be the outcome of specialization regarding, e.g., the plant habitat (Fitter & Moyersoen, 1996; Strack *et al.*, 2003).

1.1 CLASSIFICATION AND DISTRIBUTION OF MYCORRHIZAS

Mycorrhizal symbioses can be divided in 7 groups basing on histological (Tab. 1-1) and genetic traits. The most frequent and non-specific ones are between Arbuscular Mycorrhizal Fungi (AMF) or Ectomycorrhizal fungi (ECM) and plants. The others kinds of mycorrhizal symbioses have a certain specificity between some fungi *taxa* and plant families. ECM are distinguished by a dense mycelium sheaths around the roots and particular intercellular hyphal invasion of the plant root cortex forming the so called *Hartig net*. From both of the structures, an intricate hyphal web spreads in the surrounding soil (Bolan, 1991). AMF are polynucleate haploid organisms with an obligate symbiotic status. They are characterized for an active penetration into the root cortex and for the formation of arbuscules into the root cells. Arbuscules are tree stem-like shaped organs which set an extended surface area for nutrient exchanges between the fungus and the cell cytoplasm (Bonfante & Perotto, 1992 and 1995).

Tab. 1-1 - Table summarising key differences between mycorrhizal association types (modified after Smith & Read, 1997).

Type	AMF	ECM	Ectendo-	Arbutoid	Mono-tropoid	Ericoid	Orchid
Septate hyphae	- (+)	±	±	+	+	+	+
Hyphae in cells	+	-	+	+	+	+	+
Hyphal coils	±	-	-	-	-	+	+
Arbuscules	+	-	-	-	-	-	-
Mantle	-	+ (-)	+ (-)	+	+	-	-
Hartig net	-	+	+	+	+	-	-
Vesicles	±	-	-	-	-	-	-
Vegetal taxa symbionts	Vascular plants	Gymnosperms & Angiosperms		Ericales	Mono-tropaceae	Ericales	Orchida-ceae
Chlorophyll	+	+	+	±	-	+	±
Fungi	Glomerales	Most Basid-, but some Ascomycetes				Asco-(Basid-)	Basid-

Notes: AMF=Arbuscular Mycorrhizal Fungi; ECM=Ectomycorrhizal Fungi;
Ectendo-=Ectoendoid Mycorrhizal Fungi; - = absent; + = present; (+)= sometimes present;
(-)= sometimes absent; ± = present or absent; Basid- = Basidiomycetes; Asco- = Ascomycetes.

The genetic organization of AMF consists in several haploid nuclei, up to some hundreds, present within one fungal spore (Viera & Glenn, 1990; Hosny *et al.*, 1998). Questions have been risen about the significance of such nuclei population, about their probable genetic redundancy and the negative effects associated with the lack of sexuality (e.g. slow genetic adaptation and accumulation of deleterious mutations) (Judson & Normack, 1996; Butlin, 2002; Gandolfi *et al.*, 2003). Sanders (2002a and 2002b) hypothesized that nuclei could be differentiated in different groups due to the natural mutations and that mutations are conserved during generations thanks to the

multinucleate nature of the AM cell. In addition, this diversity could have played an evolutionary role (Sanders, 2002a). More genetic differentiation could also have occurred through nuclear drift and anastomoses (Giovannetti *et al.*, 2001) that provided random assortment of nuclei (Bever & Morton, 1999; Sanders, 2002b) among different individuals and through to an uneven distribution of different nuclei from parent to newly formed cells during mitosis (Sanders, 2002a).

Presently, all AMF species are members of the phylum *Glomeromycota* (Fig. 1-1) which seems to have a monophyletic origin. It currently includes 4 orders and 13 families phylogenetically sound for both genetic and morphological traits (Schüβler *et al.*, 2001; Walker *et al.*, 2004; Oehl & Sieverding, 2004). *Glomeromycota* numbers more or less 200 species formally described on the basis of spore morphological traits. Everyway, some authors suppose the existence of many unidentified species due to their incapacity or impossibility to sporulate (Bever *et al.*, 1996; Vandenkoornhuyse *et al.*, 2002; Redecker *et al.*, 2003; Wirsel, 2004). The phylogeny of AM fungi is in continuous change. Up to April 2009, 19 genera of AMF can be distinguished (Tab. 1-2). An extensive and up-to-date description of genera and species of AM fungi can be found at the website 'http://www.lrz-muenchen.de/~schuessler/amphylo/'. It is important to pinpoint that the genus *Glomus* (Fig. 1-1b), subdivided in three clades (Groups Aa and Ab as one single genus and Group B as another one) is not completely characterized at the moment and still considered as *morphotaxon*. The phylogenetic position of family *Entrophosporaceae* is also unclear and not presented in Fig. 1-1b and the genus *Appendicispora* and the family *Appendicisporales* are synonymic to *Ambispora* and *Ambisporaceae,* respectively (Walker, 2008). The family *Gigasporaceae* have been recently split in 4 families: *Gigasporaceae*; *Scutellosporaceae*; *Racocetraceae*; and *Dentiscutataceae*. Finally, it's important to report that the genus *Geosiphon* (with the species mycorrhiza-like fungus *G. pyriformis*) is the only taxon forming symbiosis with cyanobacteria of the species *Nostoc punctiforme* (Kluge *et al.,* 2002). A detailed description of genera can be found in Redecker & Raab (2006) who stated that "environmental studies using phylogenetic methods for molecular identification have recovered an amazing diversity of unknown phylotypes, suggesting considerable cryptic species diversity". Everyway, many changes may occur to this classification in the near future, especially for the lower *taxa* for the high genetic variability of AMF, even internal to the species and to the *nuclei* (Sanders *et al.*, 1995; Kuhn *et al.*, 2001; Pawlowska & Taylor, 2004).

AM fungi can be found in several environments (Strack *et al.*, 2003) such as deserts (Corkidi & Rincón, 1997; Dalpé *et al.*, 2000; Titus *et al.*, 2002), rain forests (Brundrett *et al.*, 1999; Guadarrama & Álvarez-Sánchez, 1999; Siqueira & Saggin-Junior, 2001; Zhao *et al.*, 2001; Gaur & Adholeya, 2002), aquatic environments (Khan, 1993), ecosystems with saline (Carvalho *et al.*, 2001; Sengupta & Chaudhuri, 2002) and sodic or gipsic soils (Landwehr *et al.*, 2002). The relatively low number of Artic and Antarctic environments colonized by AMF is probably due to the lack of inoculum carriers rather then to other causes (Allen, 1996).

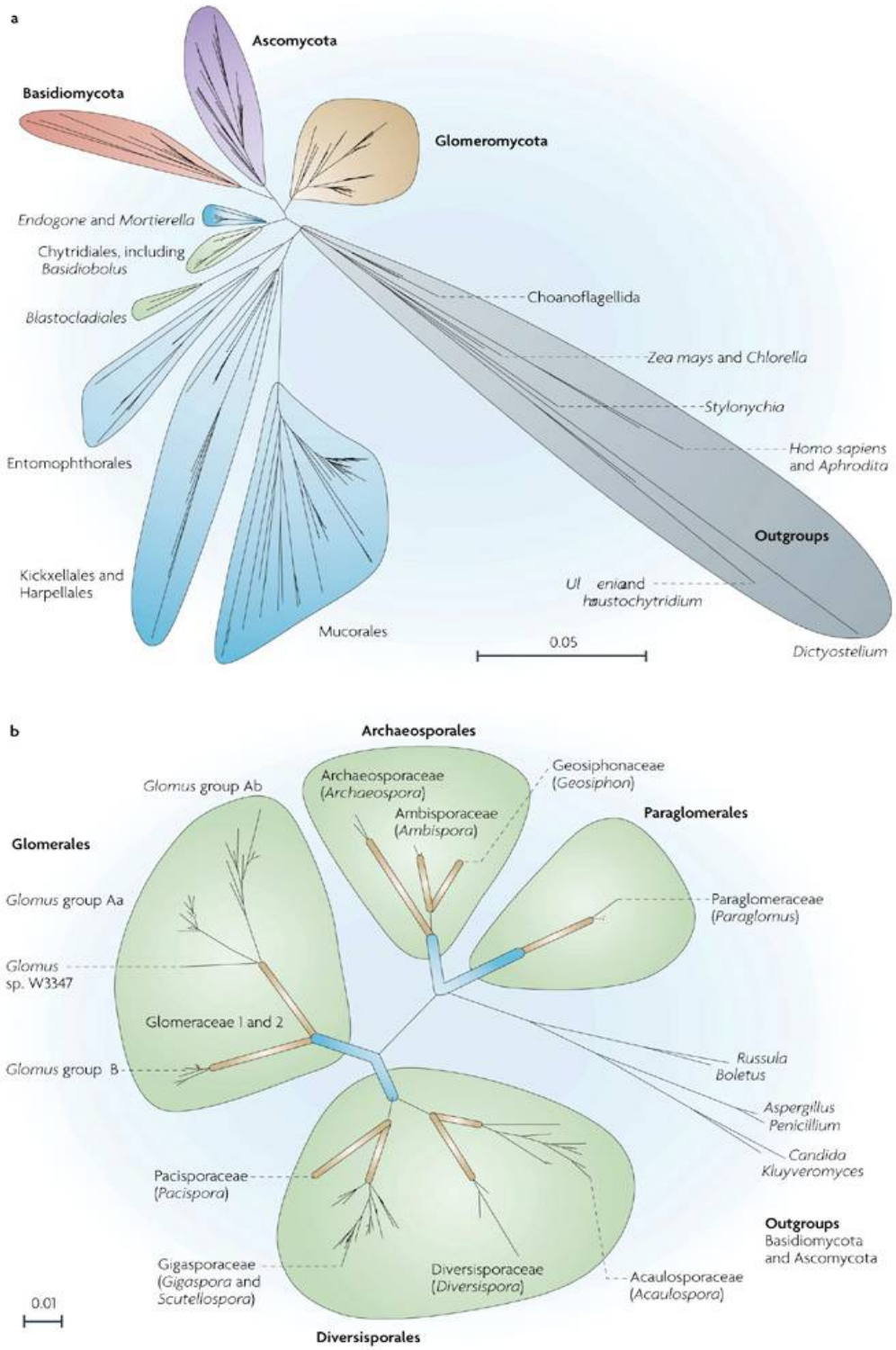


Fig. 1-1 – a) Phylum Glomeromycota positioning in the clade tree of Fungi Reign; b) clade tree of the family of the phylum Glomeromicota (a and b extracted by Schüßler et al., 2001 and modified by Parniske, 2008)

Tab. 1-2 – Classification of AM fungi (extracted by Schüßler's database available at http://www.lrz-muenchen.de/~schuessler/amphylo/)

Orders (4)	**Families (13)**	**Genera (19)**	**No. species (214)**
Glomerales	*Glomeraceae*	*Glomus*	105
Diversisporales	*Gigasporaceae*	*Gigaspora*	9
	Scutellosporaceae	*Scutellospora*	10
	Racocetraceae	*Racocetra & Cetraspora*	9 & 5
	Dentiscutataceae	*Dentiscutata & Fuscutata & Quatunica*	7 & 4 & 1
	Acaulosporaceae	*Acaulospora & Kuklospora*	34 & 2
	Entrophosporaceae	*Entrophospora**	2
	Pacisporaceae	*Pacispora*	7
	Diversisporaceae	*Diversispora* & Otospora**	4 &1
Paraglomerales	*Paraglomeraceae*	*Paraglomus*	3
Archaeosporales	*Geosiphonaceae*	*Geosiphon*	1
	Ambisporaceae	*Ambispora*	8
	Archaeosporaceae	*Archaeospora & Intraspora*	1 & 1

* unclear phylogenetic affiliation

1.2 GENETIC AND HISTOLOGICAL TRAITS OF THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

Arbuscular mycorrhizal (AM) fungi are obligate symbionts of plants. Without its host, AM fungi have a relatively short growth (20-30 days) (Bonfante & Perrotto, 1995) after which modifications in fungal morphology stop hyphal growth. It's still unclear why AMF do not grow saprotrophically in absence of a living host, though it has been demonstrated that *Gigaspora margarita* is capable of DNA replication during spore germination and that "possess the machinery needed for DNA replication even in the absence of the host plant" (Bianciotto & Bonfante, 1993; Bonfante & Perrotto, 1995).

When a fungal spore and/or a fungal hypha comes nearby of living host roots, development of mycelium starts with a reciprocal signaling pathway (Paszkowski, 2006). Roots synthesize and release chemicals, mainly flavonoids (Lum & Hirsch, 2003) and strigolactones (Akiyama *et al.*, 2005), leading to intensified elongation and branching of AM hyphae (Tamasloukht *et al.,* 2003). By his own way, as soon as hypha touches the root, it starts forming an *appressorium* and releasing exo- and endoglucanases, cellulases, xyloglucanases and pectolytic enzymes including polygalacturonase (García-Romera *et al.,* 1991; García-Garrido *et al.,* 1992a, 1992b and 1996; Rejon-Palomares *et al.,* 1996), which improve fungal penetration through plant cell walls. The plant facilitates the penetration of the fungus opening an epidermal cleft in the anticlinal cell walls of two adjacent epidermal cells (Parniske, 2004; Demchenko *et al.*, 2004). Then fungal hyphae are allowed to pass intracellularly through an exodermal cell and an adjacent cell from the outermost cortical layer (Hause & Fester, 2005). Genre *et al.* (2005) also showed that *Medicago truncatula* rhizodermal cells form a pre-penetration *apparatus*, a hollow column composed of microtubules, microfilaments and endoplasmic *reticulum cisternae*, that guides the invading hypha through the cell *lumen*. It's also notable that such a *apparatus* is formed before fungal invagination of the plant cell, so before that fungus enters the root cells apoplast. Moreover, the pre-penetration *apparatus* is not induced in plant mutants in which *appressoria* formation is allowed but who are unable to form a complete symbiosis. After having entered the first cell layer, the hyphae pass through the outer layers of living cells, surrounded by a plant perifungal membrane that is histologically continuous with the plasma membrane. This step is maybe the bottleneck of the complete establishment of the AM symbiosis (Parniske, 2004). In *Lotus japonicus* mutant defective for the synthesis of the perifungal membrane, the penetration of the hypha into the outer layers of the plant cell lead to a death of both the plant cell and the hyphal tip (Bonfante *et al.,* 2000).

When the fungus reaches the inner cortex layer, it starts growing and branching throughout the apoplast under the control of the plant (Ivashuta *et al.*, 2005; Paszkowski *et al.*, 2006). During this process, some hyphal branches penetrate inner cortical cells without forming any *appressoria* and, by repeated dichotomous branching, initiate the synthesis of a characteristic tree-like structure, the arbuscule. It is not known what triggers the fungal entrance into the cell (Paszkowski, 2006), maybe a radial sugar gradient between the vascular tissue and the outer cell layers is involved in induction of the arbuscules formation (Blee & Anderson, 1998). Arbuscules surely are the key

organs of the mycorrhiza because are responsible for nutrient exchange between the symbionts. During the arbuscule formation and its life-span, the plant cell undergoes hard morphological and physiological changes. In details, plasmalemma starts to invaginate, the vacuole becomes fragmented and a periarbuscular membrane is synthesized in *continuum* with the plasma membrane (Harrison, 1999), amyloplasts disappeare and the number of organelles such as Golgi bodies and plastids increases (Bonfante & Perotto, 1992 and 1995) and form a network covering the arbuscule (Fester *et al.*, 2001). In this network, plastids are connected to each other by tubular structures called 'stromules' (Köhler & Hanson, 2000). At the same time, the plant cell *nucleus* increases in size owing to unfolding of its chromatin, though maintaining its ploidy (Berta *et al.*, 1990b), and it moves to a central position, surrounded by plastids forming "octopus-," "millipede"-like or ring-shaped structures (Strack *et al.,* 2003) whose presence indicates an intensified metabolism. Once the arbuscule and the peri-arbuscular membrane are completely formed, the plant and the fungus start a reciprocal and active exchange of nutrients. Many transporters and other enzymatic complexes have been identified onto the peri-arbuscular membrane, mainly P transporters (Paszkowski *et al.*, 2002; Harrison *et al.*, 2002; Glassop *et al.*, 2005; Maeda *et al* ., 2006) and ATPases (Gianinazzi-Pearson *et al.*, 1995). In the arbuscules containing cell, particular mycorrhizal induced chitinases are similarly expressed and regulated (Bonanomi *et al.*, 2001). The arbuscule senesces and collapses after some days of activity (4–10 days, Sanders *et al.*, 1977) and the fungal structures are then completely degraded by the plant cell. The plant cell recovers its original morphology (Jacquelinet-Jeanmougin *et al.,* 1987) and can allow a second fungal penetration and arbuscule formation (Alexander *et al.*, 1988 and 1989; Hause & Fester, 2005). Salzer *et al.* (1999) and Walter *et al.* (2000) suggested that arbuscule senescence is somehow dependent by plant responses to intracellular colonization. Moreover, the collapse could be caused by endogenous fungal signaling or coordinated signalling cross-talk (Paszkowski, 2006).

In late phases of the symbiosis, all AM fungi form intra- or inter-cellular vesicles, except for the ones of the genera *Scutellospora* and *Gigaspora*. Vesicles are lipid-rich storage organs (Smith & Read, 1997). Finally, the life cycle of the AM fungi finish and start again with the formation of new extraradical mycelia and spores. Another colonization process is then began by the new spores or by the hyphae in soil (Smith & Read, 1997). The overall extent of AM colonisation is controlled by the plant (Solaiman *et al.,* 2000; Nishimura *et al.,* 2002), but the expression of the related operones is not completely understood (Staehelin *et al.,* 2001). Everyway, the genetic determinants that control the colonization and its efficiency are of great agricultural potential (Parniske, 2004).

1.3 DIRECT INTERACTIONS BETWEEN AMF AND BACTERIA

AM fungi can interact with soil bacteria both directly, *via* a trophic and physiological dialogue, and indirectly, by modifying the environment in which bacteria live (Barea *et al.*, 2002; Johansson *et al.*, 2004). In this section, I will focus on direct relations between AM fungi and bacteria. Two kinds of AMF-bacteria interaction can be described: (1) between AM extraradical hiphae and bacteria living in the soil and (2) between AM fungi of the family *Gigasporaceae* and bacteria passing their entire life cycle into the cytoplasm of the AM fungus cells, thus establishing a symbiosis between fungus and bacterium.

Many bacteria live in the rhizosphere and attach to AM hyphae (Bianciotto *et al.*, 1996a). It is not clear if the attachment of some bacterial strain to AM hyphae is governed by a chemical and/or molecular dialogue between AM fungus and bacterium, but is has been demonstrated that some bacterial strains specifically respond to some AM fungi (Andrade *et al.*, 1997; Artursson *et al.*, 2005) suggesting a certain degree of specificity (Artursson & Jansson, 2003; Toljander *et al.*, 2006). Results of both Andrade *et al.* (1997) and Artursson *et al.* (2005) suggest that AM fungi are more commonly associated to Gram-positive and γ-proteobacteria than Gram-negative bacteria. Boddey *et al.* (1991) supposed that mycorrhizal extra radical mycelium (ERM) act as vehicle for spreading of bacteria in soil. The attachment of bacteria to AM hyphae seems to follow a two-step process (Vande Broek & Vanderleyden, 1995). In the first phase, a labile binding, probably an electrostatic attraction, is established between the two kinds of microbes (Artursson *et al.,* 2006). In the second step, cellulose fibrils or other bacterial polymers may be involved in the formation of a more stabile binding between AM fungi and bacteria (Bianciotto *et al.,* 2001). The attachment of bacteria on AM hyphae is of great agronomic significance. Indeed, a close contact between bacterial cells and root structures is an important feature for many bacterial effects on plant. Moreover, it is a prerequisite for an effective nodule formation in N_2 fixing symbioses. Regard to AM symbiosis, a close contact of bacterial and fungal cells could be important in determining AM growth and activity. Upon that matter, Azcón (1987) and Linderman (1997) showed that plant growth promoting rhizobacteria (PGPR), a group of microbes whose activity can result in plant benefits, have a stimulatory effect on growth of AM fungi. Moreover, Sanchez *et al.* (2004) found a common gene expression in *M. truncatula* in response to a fluorescent *Pseudomonas* and an AM fungus.

The knowledge about the symbiotic life of some bacteria into AM fungi is still controversial. As already mentioned, some AM fungi from the family *Gigasporaceae* can harbour bacteria living symbiotically into all structures of the AM life cycle, except for arbuscules (Bianciotto *et al.,* 1996b). These authors demonstrated that AM endosymbiotic bacteria are able to complete their whole life cycles within the AM cell and that they are Gram-negative and rod-shaped. AM endosymbiotic bacteria were formerly associated to the genus *Burkholderia*, but recent findings assign it to the species *Candidatus* (*Ca.*) *Glomeribacter gigasporarum* (Bianciotto *et al.*, 2003). Different authors attempted to cultivate such bacteria on cell-free media with no results (Bianciotto *et al.*, 2004; Jargeat *et al.*, 2004). However, they found that these bacteria were vertically transmitted by generations of AM fungi trough vegetative spores. The

non-cultivability and the relatively little genome size of *Ca. G. gigasporarum* typical of all symbiotic bacteria gives clues about its obligate symbiotic status. Moreover, the coenocytic nature of the AM mycelium surely facilitates the migration of AM endosymbiotic bacteria into the AM fungus (Artursson *et al.,* 2006). This hypothesis is supported by the demonstration of an active bacterial proliferation into the AM fungal mycelium (Bianciotto *et al.*, 2004).

No information is available about the physiological role of AM endocellular bacteria. By comparing genomic library developed from *G. margarita* spores, some authors supposed that some genes of endosymbionts of AM fungi are involved in the phosphate transport, in chemotaxis and in coat formation of bacterial endospores (Ruiz-Lozano & Bonfante, 2000; Minerdi *et al.*, 2002). In particular, Minerdi *et al.* (2001) found a DNA region containing putative nif genes in the genoma of *Ca. Glomeribacter gigasporarum*, so it could also be possible that these bacteria are involved in the N_2 fixation from atmospheric sources and contribute to N nutrition in the AM fungus. However, no reports are available about the possibility of N_2 fixation in AM fungi.

The process of cyclical infection of AM fungi by bacteria has not been described. However *Geosiphon pyriforme*, a mycorrhiza-like fungus, was found to harbor a cyanobacteria through an endocytotic process (Schüßler & Kluge, 2001) so it is presumable that it also occurs in AM fungi.

1.3.1 DIRECT RELATIONS BETWEEN AMF AND RHIZOBIA

The symbiosis between legumes and AMF displays several similarities to the NFB-legumes symbiosis by a genetic and eco-physiological point of view (Gollotte *et al.*, 2002). Both symbioses share common steps (briefly: attraction, recognition, entrance, establishment, development of a specialized interface and coexistence with host plant) (Lum & Hirsch, 2003). Provorov *et al.* (2002) showed that a lot of mutants lacking for the ability of establishing the N_2 fixing symbiosis (referred as 'Nod-') are also unable to establish the mycorrhizal one (referred as 'myc-'). Despite the obvious differences between the two symbioses and the uniqueness of each of them, many plant genes are expressed with apparent similar functions in both symbiotic relations. These common features induced LaRue & Weeden (1994) and Provorov *et al.* (2002) to suppose that NFB-legume symbiosis evolved from a set of pre-adaptations during co-evolution of plant with AMF.

During early stages of both symbioses, a molecular and chemical dialogue occurs between AM fungi or NFB with each of their respective host by means of flavonoids in plant exudates (Recourt *et al.*, 1992; Harrison & Dixon 1993, 1994) which take part in the recognition of partners and development of associations. In the N_2 fixing symbiosis, the release of plant exudates induces the production of bacterial Nod factors, molecules that dictate the relations between symbionts (Djordjevic *et al.*, 1997). Plant exudates also induce germination and growth of AM hyphae and hyphal branching when the fungus approaches the plant root. These effects are host-specific and occur only at the presence of host exudates as also shown by Elias & Safir (1987), Gianinazzi-Pearson *et al.* (1989) and Giovannetti *et al.* (1993). As already mentioned, plant flavonoids in roots

exudates exert a stimulus on AM infection. Xie *et al.* (1995) showed that the application of exogenous Nod factor also stimulates AM colonization and that this stimulus was correlated with an enhancement of specific plant flavonoids. It has been shown how this stimulus is not only host dependant, but also AMF genus-specific (Chabot *et al.*, 1992).

The attachment and penetration of AMF and NFB into plant roots is a multi-step procedure involving host participation. The first step of AMF infection is the formation of an appressorium. Even if chitin, the major component of fungal tissues, is know to be an elicitor of plant defence reactions, the appressorium contact with epidermal root cell doesn't cause the typical defence reactions from plants, which triggers particular mechanisms in order to allow AMF but no other fungi to enter the root (García-Garrido & Ocampo, 2002). Anyway, AMF entrance into the plant is more dependant by the fungus rather than the plant. With respect to N_2 fixing symbiosis, molecules produced by NFB during the early stages of the infection are related to fungal chitin and it suggests a plant ability to recognize different form of chitins and similar compounds hence regulating its own defenses the penetrations of symbionts. Once entered the roots cells, both AMF and NBF form particular exchange structures, named respectively "arbuscule" and "bacteroid's membrane". Plant always form an interface between root cell and symbiotic structures. The periarbuscular membrane (between plant cell and AMF) carries xyloglucans, nonesterified polygalaturonans, arabinogalactans and hydroxyproline-rich glycoproteins and similar components can be found in bacteroid compartments (Perotto *et al.*, 1990). Finally, the use of Nod- and/or Myc- legume mutants in research has contributed to an enhanced understanding of the signaling process between host plant and micro-symbiont (NFB and/or AMF) (Gollotte *et al.*, 2002), although further studies are needed to a better understanding of the molecular dialogue between symbionts.

1.4 CONTRIBUTION OF AM SYMBIOSIS TO PLANT GROWTH AND NUTRIENT UPTAKE

AM symbiosis plays a key role in promoting plant growth and nutrient uptake, especially P (van der Heijden & Sanders, 2002; Garg *et al.*, 2006; Hoeksema *et al.*, 2010). Such support mainly occurs in growth-limiting environments (Smith & Read, 1997) or in crops where it isn't possible to use mineral fertilizers (Scullion *et al.*, 1998). Many reports show that AM symbiosis improves growth in drought-stressed conditions (Augé, 2001) and that its effects on drought tolerance can either be mediated by the higher P inflow (Kwapata *et al.*, 1985; Osonubi *et al.,* 1991) or occur irrespective of a higher P uptake in AM than non AM plants (Augé *et al.*, 1994; Schellenbaum *et al.*, 1999). Indeed, AM fungi have side effects on soil hydraulic properties (Augé *et al.*, 2004) and soil structure (Rillig & Mummey, 2006) and these effects help plants to cope with water deficit. In field crops, several reports show that AM fungi inoculation improves plant dry matter (DM) production for economically important *taxa* of plants such as legumes (Fitter, 1985; Barea *et al.,* 1987; Kristek *et al.*, 2005) and grasses (Al-Karaki *et al.,* 2004). Generally AM symbiosis enhance root biomass thanks to the improved mineral nutrition, but sometimes it induced depressive effects on root DM (Koide, 1985; Fitter, 1977) and root length (Berta *et al.*, 1990a). The AM symbiosis influences many aspects of root physiology such as plant rooting and root morphology,

nutrient acquisition rates and root reactions against stresses (Atkinson *et al.*, 1994; Kapulnik & Douds, 2000; Berta *et al.*, 2002). It has been sometimes reported that AM may have a negative effect on plant growth (Ryan & Angus, 2003; Li *et al.*, 2008) but it has been found only for some plant species grown in pot in symbiosis with single AM strains.

The effects of the AM symbiosis on nutrient uptake are clearly visible when nutrient availability for plants is scarce due to low nutrient concentration in soil or to low nutrient diffusibility (George, 2000). It is the case of nutrients such as P, Zn and Cu and others minerals (Barea *et al*, 2005), though AM symbiosis is also efficient in enhancing uptake of N (Jin *et al.*, 2005; Govindarajulu *et al.*, 2005). The enhanced nutrient uptake by AM plants in comparison to non-AM plants mainly depends on a better scavenging of soil area thanks to (1) the little dimensions of AM hiphae, which can access nutrient sources normally unavailable to plant root and root hairs (Chen *et al.*, 2005; Jakobsen *et al.*, 2005); and to (2) the possibility for AM fungi to absorb nutrients in forms unavailable for plants, such as organic N or organic P (Jayachandran *et al.*, 1992; Tarafdar & Marschner, 1994; Koide & Kabir, 2000; Hodge *et al.* 2001). It has been shown that AM hyphae have an average diameter of 3 to 7 μm while the diameter of the finest root hair is 5 to 20 μm (Wulfsohn & Nyengaard, 1999; Bago, 2000; Dodd *et al.*, 2000) AM hyphal length densities may be up to hundreds-fold greater than root length densities. This features allow AM fungi to better explore the soil volume and so raise the probability that ions with low diffusion coefficient are absorbed (Miller *et al.*, 1995). Moreover, AM hyphae are more effective than roots to absorb and utilize nutrients (O'Keefe & Sylvia, 1992; Cui & Caldwell, 1996).

The area of soil in which AM symbiosis spread and establish its activity it's also called mycorrhizosphere (Barea *et al.*, 2002). In this area AM influences bacterial activity (Azcón-Aguilar & Barea, 1992; Bianciotto *et al.*, 2001; Bianciotto & Bonfante, 2002) and bacterial populations (Mansfeld-Giese *et al.*, 2002; Marschner & Timonen, 2005); it contributes to the formation of water-stable aggregates (Miller & Jastrow, 2000; Jeffries *et al.*, 2002); it contributes to the reduction of stresses by pollutants (Gianinazzi *et al.,* 2002; Gianinazzi e Schüepp, 1994; Bethlenfalvay e Linderman, 1992) and it plays a role in the defense of plants against biotic stresses (Rabie, 1998; Werner *et al.*, 2002). The sum of these effects is important in sustaining plant growth. The AM contribution on the suppressed plant yield loss due to diseases are related to a number of suggested mechanisms as: improved nutrition (Declerck *et al.*, 2002), competition for colonization sites (Fitter & Garbaye, 1994), production of AM anti-microbial compounds (Benhamou *et al.*, 1994), and priming of plant immune system (Pozo *et al.*, 2002; Pozo & Azcón-Aguilar, 2007).

In order to perform its activities into the mycorrhizosphere and plant roots, the AM symbiosis has a very high carbon demand and act as an important carbon sink for the plant: AM fungi can drain up to the 20% of the total carbon fixed by the plant (Bago *et al.*, 2000; Johnson *et al.*, 2002), irrespective of plant P or N status (Wright *et al.*, 1998a and 1998b). If we take into account that AM plants often have a higher DM production than non-AM plants, it means but that the improvement of plant photosynthesis by AMF is higher than the AM carbon demand, as results by many authors suggest (Harris *et al.*, 1985; Ames e Bethlenfalvay; 1987). Plant regulate the amount of carbon transferred to the AM fungus. This regulation relies on the net

transfer of P, and probably also N, by the AM fungus to the plant (Fitter, 2006; Javot *et al.*, 2007). Sometimes AM symbiosis can even suppress plant P transporters that are expressed under P deficient conditions, as demonstrated with molecular tools by Liu *et al.* (1998) and Burleigh & Harrison (1999). This increases plant reliance on AM symbiosis for P uptake so improving the transfer of C from plant to fungus and could be an important issue for the survival of the AM fungi.

1.4.1 EFFECTS ON P UPTAKE

Phosphorus is the most limiting element after N for plant growth and production (Vance *et al.*, 2000) and over 80% of P in soil is in unavailable forms (organic or insoluble) to plants (Holford, 1997; Schachtman *et al.*, 1988). Inorganic P, such as other nutrients, has a very low soil diffusibility and the high plant demand and uptake frequently generate a P depletion zone around of the root surface (Trenbath, 1976; Jungk & Claassen, 1989). In order to face its needs, plant have evolved several strategies to obtain P when it is not available (Vance *et al.* 2003; Hammond *et al.* 2004; Raghothama, 2005) among which AM symbiosis is one of the most important (Lum & Hirsch, 2003).

The importance of AM symbiosis to absorb P from soil is related to the ability of AM hyphae to scavenge soil for P reserves normally unavailable to plants (Miyasaka & Habte, 2001) thanks to four features: (1) AM extraradical mycelium (ERM) is able to explore a higher soil volume than plant roots; (2) AM hyphae have a very small diameter if compared to both plant root and plant root-hair, thus increasing P absorbing surface; (3) AM fungi form polyphosphates in its cells, thus lowering internal P concentration and generating a high P gradient in AM cells respect to soil matrix; and (4) AM fungi produce organic acids and phosphatases which catalyze the release of P from organic complexes (Bucking & Shachar-Hill, 2005). Mycorrhizal P uptake pathway is regulated by molecular mechanisms which imply the expression of mycorrhizal P transporters to load P from soil into AM hiphae (Harrison & van Buuren, 1995), its active translocation into the intraradical mycelium and delivery to the host root by means of specific P transporters (Paszkowski *et al.* 2002; Glassop *et al.* 2005).

Reports on higher plant P uptake of AM plants than non mycorrhizal plants are too much to be all reported (McGonigle & Fitter, 1988; Crush, 1995; Ning & Cumming, 2001; Cavagnaro *et al.*, 2003; Gazey *et al.*, 2004; van der Heijden *et al.*, 2006; Schweiger *et al.*, 2007). The most of pot studies involving AM fungi shows a higher P uptake, even if it not always related to plant growth responses (Smith *et al.*, 2003, 2004). Koide & Kabir (2000) showed that, in monoxenic conditions, mycorrhizal ERM was able to access to an organic P sources and transfer it to plant. On the other side, in non-mycorrhizal roots, P availability for plant is exclusively related to the presence and length of the root hairs (Marschner & Dell, 1994). The efficiency of P absorption by AM fungi varies with the AM fungus species (Jakobsen *et al.*, 1992). In field conditions, AM fungi are normally present and studies about their importance in plant P uptake are not easy to perform because of the problems encountered in suppressing natural AM infection (Pedersen & Sylvia, 1997). However, many authors have attempted to perform field experiments, mainly focusing on the benefits of an enhanced

AM infection, and results seem interesting. Schweiger & Jakobsen (1998) directly measured P uptake by AM symbiosis in field grown wheat and found that the symbiosis is responsible for the most of P absorbed by plants thus confirming the results by Liu *et al.* (1998) and Burleigh & Harrison (1999). Black & Tinker (1977) found a higher total P uptake in mycorrhizal potatoes than non-mycorrhizal control, due to the higher DM yield because no differences on P concentration due to AM symbiosis were observed. Rangeley *et al.* (1982) had similar result in field grown white clover, while Hayman & Mosse (1979) found both a higher P concentration and higher total DM yield in AM clover than non AM control, above all in low-P fertilized soil. In pea, Kristek *et al.* (2005) found higher P concentration and P yield in AM than non-AM plants, especially in drought-stressed conditions. Finally, Al-Karaki *et al.* (2004) found that AM inoculation was capable of increasing P concentration and uptake in field grown wheat both in well-watered and drought-stressed conditions. It has sometimes been reported a null effect of AM symbiosis on P uptake in field grown soybean (Ganry, 1982), but it probably occurred because of the low soil P concentration (4.2 ppm measured with the Truog method) that impaired plant growth.

1.4.2 EFFECTS ON N UPTAKE

AM symbiosis could take up N from soil (Miller & Cramer, 2004) and further translocate it to the plant (Chalot *et al.*, 2006). It has been shown that AM fungi respond to different N sources or N availability (Breuninger *et al.*, 2004; Cappellazzo *et al.,* 2007). However, few studies are available on AM effect on plant N status and the impact of N absorbed by AMF on plant N balance is still unclear. AM symbiosis is active in the absorption of N from inorganic sources (Ames *et al.*, 1983; Johansen *et al.*, 1993). In pot, using ^{15}N-labelled nitrate as fertilizer, Tobar *et al.* (1994) shown that AM symbiosis considerably contributed to N uptake by lettuce both in drought-stressed and in well-watered conditions. The authors concluded that AM symbiosis is active in the uptake of nitrate from soil. However, AM symbiosis is better suited to take up N when it is in N-NH_4^+ form as shown by Hawkins *et al.* (2000).

Recent findings shows that AM fungi possess the machinery for a direct N uptake from organic sources (Cappellazzo *et al.,* 2008). After an experiment in monoxenic conditions, Hawkins *et al.* (2000) showed that the presence of the AM fungus was able to absorb N from organic sources and transfer it to the host plant. They also showed that the amount of N adsorbed by the AM fungus from organic sources depends on the organic N form and on the AM fungus strain. The results from Hawkins *et al.* (2000) confirm the ones achieved by Cliquet *et al.* (1997) who showed that presence of AM symbiosis is responsible for an enhanced N acquisition from soil as both nitrate and amino-acids in ryegrass grown in a microcosm system. Such results accord with early and recent evidences about the ability of AM fungi to promote organic nitrogen decomposition and its further uptake (Hodge *et al.*, 2001; Whiteside *et al.*, 2009), but these effects have been studied only in microcosm systems and are not confirmed by pot and field studies. In addition, the mechanisms implied in the AM stimulus of organic N mineralization are still uncertain. In particular it is not clear if AM fungi promote the direct uptake of organic nitrogen, or if they stimulate the activity of N-mineralizer bacteria and promote the uptake of mineralized N.

Many legumes access to the atmospheric N source thanks the N_2 fixing symbiosis and release great amount of N in soil as both dead tissues and exudates. When a legume is intercropped with a non N_2 fixing species, N derived from the atmosphere (Ndfa) can become available to the non-fixing plants *via* a N transfer (Simard *et al.*, 2002; Høgh-Jensen, 2006). The AM symbiosis play an important role in the phenomenon of the N transfer between companion crops. First, AM fungi can mediate direct N transfer between a N donor and a N receiver plant (He *et al.,* 2003) by directly linking the root systems of companion crops. Secondly, AM fungi can absorb root N rich exudates and further transfer it to the host plants (Kapulnik & Douds, 2000; Paynel *et al.*, 2001). The amount of N transferred by AM hyphae is variable. Results from pot experiments suggest that AM symbiosis may facilitate interplant N transfer between legumes and grasses (Hamel *et al.,* 1991; Frey & Schüepp, 1992 and 1993), while others obtained no AM effect on interplant N transfer (Rogers *et al.,* 2001). In field, very few reports are available about AM effects on N transfer and results are still inconclusive.

1.4.2.1 Effects on symbiotic N_2 fixation

Symbiotic N_2 fixation is fundamental in the biogeochemical cycle of N. It represents the major N input for legumes in almost all environments and it is responsible for the most of available N to all plant communities. AM symbiosis can enhance legume symbiotic N_2 fixation (Barea *et al.*, 2005; Antunes *et al.*, 2006a; Chalk *et al.*, 2006) and receive in turn N as some report seem to suggest (Scheublin & van der Heijden, 2006).

The interactions between AMF- and NFB-legumes symbioses can occur at both colonization and nutritional level. The nutritional aspect is obviously related to the enhanced nutrition of P, Zn, Cu and other immobile nutrients supplied by the AM fungi and important for the NFB-legumes symbiosis (Clark & Zeto, 2000; Vance, 2001). Li *et al.* (1991) found that Cu concentration and quantity in *Trifolium repens* was enhanced by AM symbiosis due to AM hyphal uptake. As a matter of fact, P is fundamental for symbiotic N_2 fixation. In non limiting conditions, legume nodules have a 2-3 fold P-dependency than roots in which are formed (Mosse, 1986; Almeida *et al.*, 2000; O'Hara, 2001; Cuttle *et al.*, 2003), especially in the earlier stage of their development (Smith *et al.*, 1979; Asimi *et al.*, 1980; Drevon e Hartwig 1997; Tang *et al.,* 2001). P deficiency reduces the number and mass of nodules and the activity of N_2-ase; it increases oxigen permeability of bacteroids so causing an oxidative damage to the N_2 fixing enzymatic machinery (Hunt & Layzell, 1993; Ribet & Drevon 1995; Vadez Rodier *et al.*, 1996). AM symbiosis can also directly affect nodules or bacteroids thus establishing a tripartite symbiosis between AM fungi, N_2 fixing bacteria and legumes. It is not known how the AM symbiosis directly affects the NFB-legume symbiosis. However it has been shown that AM fungi reduce the oxidative damage to nodules (Ruiz-Lozano *et al.*, 2001a) and plant (Goicoechea *et al.*, 1998; Porcel *et al.*, 2003).

AM symbiosis seems to cooperate with NFB-legume symbiosis also in alleviating water or salinity stress for the host plant (Azcón *et al.,* 1988; Azcón & El-Atrash, 1997). The stimulus of AM symbiosis to N_2 fixation and to legume growth is dependant on the combination of rhizobial strain and AM fungus (Rao *et al.*, 1986). In a *ad hoc*

experiment, Azcón *et al* (1991) showed that inoculation of alfalfa grown in pot with different AM fungi and rhizobial strains resulted in different plant growth, %Ndfa (the percentage of total Ndfa above total plant N content) and N and P uptake. Similar results were found by Xavier & Germida (2002), who showed that lentil response (in terms of growth, harvest index, N and P content) to inoculation with different AM fungi or *R. legumnosarum* strains varied both with the *Rhizobium* strain and with the AM fungus. In field conditions, very reports are available on the effects of AM fungi on N_2 fixation by legumes. In soybean, Ganry *et al.* (1985) achieved higher Ndfa in AM than non AM crop when it was grown with a soluble phosphate fertilizer in a low P containing soil, but the same authors reported that AM inoculation didn't produce any growth or Ndfa benefit when no P fertilizer was added.

The tripartite symbiosis has a very high carbon cost, as also shown by several authors (Pang & Paul, 1980; Kucey & Paul, 1982; Harris *et al.*, 1985). Generally, legumes inoculated with effective strains of AM fungi and N_2 fixing bacteria have a higher photosynthesis rate than non-infected plants and this compensate for the C cost of the microsymbionts. In forage legumes subjected to mowing, photosynthesis rates are reduced and the AM symbiosis could compete with the NFB for carbon in the early regrowth stage. The response of both symbioses to above-ground plant removal is similar, but AM symbiosis seems to suffer less than nodules after plant clipping: nodules lose weight more rapidly than roots (Bayne *et al.,* 1984), especially during the first 15 days after clipping (Vance *et al.*, 1979), while AM fungal biomass is reduced less than root biomass (Bayne *et al.,* 1984; Allsopp, 1998) and AM root colonization can even increase (Eom *et al.*, 2001; Pietikäinen *et al.*, 2009). In this phase, the competition for carbon between AM fungi and NFB can negatively affect the N_2 fixation rates of the N_2 fixing symbiosis. Using field-grown *Hedysarum coronarium* subjected to repeated harvests, Barea *et al.* (1987) obtained a higher amount of Ndfa in the AM inoculated than the not inoculated crop and stated that a effective AM fungi-legume combination could improve legume growth, N uptake and Ndfa as just as a P fertilization. In a similar experiment, Shivaram *et al.* (1988) found a higher amount of Ndfa in the AM than non-AM inoculated *Macroptilium atropurpureum*. However both authors (Barea *et al.,* 1987; Shivaram *et al.*, 1988) found no effects of AM inoculation on the %Ndfa indicating that the higher amount of Ndfa in AM inoculated than not inoculated crops depended solely on the promotion of plant growth and not by a stimulus of the N_2 fixation activity.

1.5 EFFECTS OF AM SYMBIOSIS ON SOIL BACTERIAL ACTIVITY AND COMMUNITIES

The net of AM extraradical hyphae represent an ecological niche for bacteria. Bacteria living in the mycorrhizosphere are affected by AM fungal activity both directly, by its effects on soil matrix, and by indirectly influencing plant physiology and its relative effects on rhizosphere. Mycorrhizas also affect the community composition of soil bacteria (Marschner *et al.*, 2001; Artursson *et al.*, 2005) by different mechanisms: (1) modifying mycorhizosphere pH (Bago & Azcón-Aguilar, 1997), (2) affecting activity of free enzymes (Tarafdar & Marschner, 1994), (3) competing for inorganic nutrients (Christensen & Jakobsen, 1993), (4) decreasing the amount of root

exudation (Graham *et al.,* 1981; Dixon *et al.*, 1989) and (5) modifying root exudates compositions (Po & Cumming, 1997; Marschner *et al.*, 1997). In addition, AM fungi release exudates which either promote or impair bacterial development and activity (Toljander *et al.*, 2007). One of the most important AM exudates is surely glomalin (Rillig *et al.*, 2002), a glycoprotein whose concentration is related to water stability of soil aggregates (Wright & Upadhyaya, 1998). The net of AM extraradical hiphae itself contribute to soil aggregates stability by enwrapping soil particles (Rillig & Mummey, 2006). Because microorganisms are mainly present in soil aggregates, AM activity in soil can have positive effects on soil microbial population (Andrade *et al.*, 1998). Moreover, it seems that bacteria can use senescent structures of AM fungi as *pabulum*, as also suggested by the enhanced chitinolitic activity in rhizosphere of AM plants (Abdel-Fatah & Mohamedin, 2000).

Usually AM fungi decrease the growth rate of bacteria (Christensen & Jacobsen, 1993) or induce them in a state of starvation (Marschner & Crowley, 1996; Marschner *et al.*, 1997), though AM fungi may induce an increase in certain functional group of bacteria as actinomicetes and phosphate-solubilizing bacteria (Secilia & Bagyaraj 1987; Kothari *et al.,* 1991; Posta *et al.*, 1994; Toro *et al.*, 1997). With respect to N cycle, Amora-Lazcano *et al.* (1998) found that AM symbiosis increased the population density of ammonia oxidizers, while reducing those of ammonifier and nitrifier bacteria.

1.6 AIMS OF THE WORK

The present thesis aimed to obtain information about the contribution of the AM symbiosis in alleviating the effect of abiotic stresses on crops grown in a typical Mediterranean environment and to test if AM symbiosis affects symbiotic N_2 fixation and organic N mineralization activities and finally plant N uptake.

In particular, two experiments were carried out. The first trial was performed under field conditions and had the specific aim to determine the effect of AM symbiosis on forage yield, quality, and biological N_2 fixation of berseem (*Trifolium alexandrinum* L.) grown under both well-watered and drought-stressed conditions.

The second trial was performed in pots to test the hypothesis that plant N uptake would be increased by the direct or indirect effects of AM fungi on OM decomposition process. In order to achieve this objective, it has been studied the effect of plant inoculation with AM fungi on soil enzymatic activity and microbial community, as well as the effects of AM symbiosis on plant N capture from different sources of organic matter.

2 MATERIALS AND METHODS

2.1 EXPERIMENT 1

Several pot studies have shown the beneficial effects of AM symbiosis on plants grown in water-stressed conditions; even if in other research the effects were absent or negative (Augé, 2001). However, limited information is available on the effects of AM symbiosis on crop drought tolerance under field conditions. In many Mediterranean areas, spring rainfall is scarce, which stresses plants and limits crop productivity. In such environments, AM symbiosis could play an important role in alleviating the effects of drought on crop yield and quality. The aim of this field experiment was to determine the effect of AM symbiosis on growth of berseem (*Trifolium alexandrinum* L.) grown under both well-watered and drought-stressed conditions. This experiment was funded by the *progetto SI.CO.BIO.S*, Regione Siciliana, Italia.

2.1.1 SITE DESCRIPTION

The experiment was carried out in 2007–2008 at the Pietranera field station, in Sicily (Italy) in a Mediterranean area (37°33'N – 13°30'E, 170 m a.s.l.) on a Vertic Haploxerept soil on which wheat was previously grown. The topsoil (0–40 cm) characteristics were: 38% clay, 25% silt, and 37% sand; pH 8.4; 1.27% organic matter; and 0.85‰ total N. The experimental site has a semi-arid climate (as defined by Emberger, 1955). Long-term mean annual rainfall is ~550 mm, mostly during the autumn–winter period (74%), and with a lesser amount during spring (18%). The mean minimum and maximum temperatures are 10.0 °C and 23.4 °C, respectively. Natural AM spore population in the native field involved the genera *Glomus* and *Acaulospora*. Overall AM spore density was 5 spores 10 g^{-1} air-dried soil. AM hyphal mass was not detected.

2.1.2 TREATMENT ESTABLISHMENT AND CROP MANAGEMENT

There were two treatments: (1) soil moisture regime: rainfed (DS) or well-watered (WW); (2) crop mycorrhization: AM inoculation (+AM) or AM suppression (–AM). Minimal rainfall occurred during the spring (55% below long-term average), resulting in drought-stressed conditions for crops in the rainfed treatments. So, well-watered crops received sprinkle irrigation (20 mm at 116 days after sowing [DAS] and 70 mm at 123 DAS). Suppression of mycorrhizal symbiosis was achieved by spraying plots with systemic fungicides as a drench. In order to suppress spore germination, *Captan* (20 mg a.i. m^{-2}), *Carbendazim* (20 mg a.i. m^{-2}), and *Benomyl* (20 mg a.i. m^{-2}) were used at sowing. During the growth of the crops, *Benomyl*, *Fenpropimorph*, and *Carbendazim* were applied once per month at a rate of 10, 5, and 5 mg a.i. m^{-2}, respectively in order to reduce AM hyphal growth and AM symbiotic activity. These fungicides are capable

of suppressing AM symbiosis without affecting plant growth (Dodd & Jeffries, 1989; Sukarno *et al.,* 1993; Udaiyan *et al.*, 1995; Kjøller & Rosendahl, 2000; Schweiger *et al.*, 2001). Mycorrhizal sub plots received and equal amount of water. AM inoculation involved the application of a commercial AM inoculum at a rate of 12 g per kg of seed following the manufacturer's recommendations. The inoculum consisted in pure spores of *Glomus intraradices* Schenk and Smith (1000 spores g^{-1}) and *Glomus mosseae* (T.H. Nicolson and Gerd.) Gerd. and Trappe (1000 spores g^{-1}). The experimental design was a split plot design replicated 4 times with water regime as the main plot and mycorrhization as the sub-plot. Sub plot was 72 m^2. The experimental design included all factorial combinations.

Soil was ploughed at 30 cm depth on summer and arrowed on autumn to control weeds and prepare suitable seedbed conditions. Such tillage practices presumably reduced the amount of natural AM inoculum, especially the AM hyphal mass (Kabir, 2005). Seeds (2.85 g 1000 $seed^{-1}$) of berseem (*T.alexandrinum* cv Lilibeo) were sown by hand at the 3rd of January 2008 in rows 25 cm apart. Sowing density was 1200 viable seeds m^{-2}. Weeds were removed by hand.

Plots were cut at 5 cm stubble height at 76 (first cut) and 116 (second cut) DAS. After the second cut, berseem regrowth was measured cutting different microplots (0.6 m^2 wide) at 7, 14, 21, and 28 days after the second cut (DAC).

The ^{15}N isotope dilution technique was used to estimate N_2 fixation by berseem, using annual ryegrass (*Lolium multiflorum* var. *westerwoldicum* cv. Elunaria) was used as the reference crop (ryegrass received the same treatments as berseem; sowing density was 1200 viable seeds m^{-2}). The ^{15}N-labelled fertilizer ($[NH_4]_2SO_4$ with an isotopic composition of 10 atom% ^{15}N) was applied at 116 DAS (second cut) following the application procedure described by Høgh-Jensen & Schjørring (1994). Briefly, ^{15}N-labelled fertilizer was applied as drench at a rate of 8 kg N ha^{-1} to two microplots in the middle of each sub plot. The area of ^{15}N-fertilized microplots was 2.25 m^2. The rest of the plot outside this area received an equal amount of non-labelled ammonium sulfate.

2.1.3 PLANT HARVEST AND ANALISYS

Epigeic and hypogeic sample areas of berseem and ryegrass were harvested and analyzed for root AM infection and N concentration as shown in Tab. 2-1.

Tab. 2-1 – Table summarizing date of sampling and analysis on berseem (B) and ryegrass (R). DAS and DAC for 'days after sowing' and 'days after the second cut', respectively.

DAS	DAC	Description	% AM colonization	Biomass	N and ^{15}N concentration
55	-		B+R		
76	-	first cut		B+R*	
116	0	second cut		B+R*	
123	7	regrowth		B	
130	14	regrowth	B+R	B+R	B+R
137	21	regrowth		B	
144	28	regrowth	B+R	B+R	B+R

* *Ryegrass was harvested above cutting height, only.*

At each cut, the sample areas were harvested both above and below ground by removing the top 20 cm of soil. Plants were counted and separated into taproots, removed leaves, and stems and heads. At first cut (76 DAS) and second cut (116 DAS), residual leaves and stems (below cutting height) were also separated. The fresh weight of each sample was determined, and the leaf area of the leaves (separately for residual and removed at the first and second cut) was immediately measured on a 10 g subsample. Each subsample was oven dried and weighed. Similar measurements were done for ryegrass.

Biomass samples of both berseem and ryegrass, collected at 14 and 28 DAC, were analyzed for total N and ^{15}N enrichment (δ^{15}N), using an elemental analyzer – isotope ratio mass spectrometry (EA-IRMS, Carlo Erba NA1500).

At 7, 14, 21, and 28 DAC, ~1 kg soil was taken from two soil layers (0-20 cm and 20-40 cm depth), weighted and dried at 105° until constant weight. Soil dry weights were recorded and soil moisture content was measured by gravimetric method.

At 55 DAS and at 14 and 28 DAC, five plants with their roots were collected in each treatment by careful excavation the top 20 cm of soil and taken to laboratory.

Lateral roots were excised (root tips were discarded), rinsed free of soil and washed in distilled water, cut in pieces of 1 cm length and mixed. A representative sample of roots (more or less 2 g) per sample area was cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue using the method described by Phillips & Hayman (1970). Root colonization by AM fungi was further measured according to Giovannetti & Mossee (1980).

2.1.4 CALCULATIONS AND STATISTICAL ANALISYS

Data on ^{15}N enrichment of biomass were used to calculate the percentage of clover nitrogen derived from symbiontic N_2 fixation (%Ndfa) according to Fried and Middleboe (1977):

$$\%Ndfa = (1 - \frac{Atom\%^{15} N_{berseem}}{Atom\%^{15} N_{ryegrass}}) \times 100$$

where Atom%$^{15}N_{berseem}$ represents the Atom%^{15}N excess of berseem tissue, and Atom%$^{15}N_{ryegrass}$ represents Atom%^{15}N excess of ryegrass tissue. The ^{15}N-natural abundance of the atmosphere (0.3663%^{15}N) was used for calculating the Atom % ^{15}N excess of both crops. The amount of N fixed by berseem clover was estimated as:

$$N\ fixed = Total\ clover N \times \frac{\%Ndfa}{100}$$

Analysis of variance (procedure ANOVA, SAS Institute, 2004) was performed separately per cut according to the experimental design. All measured variables were assumed to be normally distributed. All variables corresponding to proportions were arcsine transformed before analysis to assure a better fit with the Gaussian law distribution. Treatment means were compared using Tukey's test (P values) at 5% probability level.

2.2 EXPERIMENT 2

Where mineral fertilizers can't be used, N availability for plant depends on net N mineralization (Nadelhoffer *et al.,* 1985) and plants compete for N with soil microorganisms, such as soilborne fungi and bacteria. The effect of AM symbiosis on mineralization of organic nitrogen is still unclear. Early studies have shown that AM fungi can improve plant N from organic matter (e.g. Hodge, 2003), but no information is available about its mechanism.

The aim of this experiment was to determine the effects of AM symbiosis on the process of organic N mineralization, on plant N capture and on soil bacterial community. This experiment was funded by the *fondo Ex-60% 2007* "Ruolo della simbiosi micorrizica sull'efficienza di utilizzazione dell'azoto in differenti genotipi di frumento", Università degli Studi di Palermo, Italy; and by the *proyecto AGL2008-00742/AGR,* Ministerio de Ciencia e Innovación (MICINN), Spain.

2.2.1 GROWTH CONDITIONS, EXPERIMENTAL DESIGN AND TRATMENT ESTABLISHMENT

The experiment was conducted at Experimental Station of the Zaidín (CSIC) (Granada, Spain) during the winter of 2008 in a conditioned glasshouse, with 25/19 °C day/night temperature, a photoperiod of 16 h. Additional light at a photosynthetic photon flux density of 460 μmol m^{-2} s^{-1} was provided if necessary.

A complete randomized factorial design replicated 4 times with two factors was adopted. Treatments were (1) AM inoculation: *Glomus mosseae* (T.H. Nicolson and Gerd.) Gerd. and Trappe isolate BEG 12; and not inoculated control; (2) addition of organic matter (OM): soil amended with ^{15}N-enriched biomass of maize (root or leaves); not amended soil. An identical set of pots with OM treatments and no plant was made in order to evaluate the effects of adding OM on N mineralization processes and bacterial community. For the mycorrhizal treatments, inoculum was obtained from a thoroughly homogenized rhizosphere sample coming from a open-pot culture of *Sorghum bicolor* L. and consisted in soil, spores and mycelia. AM inoculum was added to the appropriate pots at a rate of 1 g inoculum per pot. For the OM treatments: ^{15}N-enriched organic matter was prepared by a previous maize cultivation on ^{15}M enriched soil. Maize was harvested at before anthesis and separated in roots, stems and leaves. Fine roots (under ~1 mm diameter) and leaves were used as organic amendant. Roots and leaves were cut in ~1 mm and ~1 mm^2 pieces, respectively. AM colonization of maize root was 17%. N concentrations (in parenthesis, the corresponding ^{15}N abundance [APC] are given) of maize tissues was 1.90% (4.78% Atom percent PC) and 1.56 (3.94% APC) for leaves and roots, respectively. Organic biomass was oven dried at 80 °C for 1 day before adding to mixture.

Each pot (10 cm diameter, 11 cm height, 72 in total) was filled with 600 g of a quarts sand:soil mixture (2:1). Soil properties were 37% sand, 43% silt, 20% clay; 1.8% organic matter, pH 8.1 (soil:water 1:1); 0.12 mS cm^{-1} saturated E.C. (25°C); 1.05‰ , 6.2 ppm and 132 ppm of N, P (as P_2O_5) and K (as: K_2O), respectively; 10.06% total Ca; 99

ppm soluble Ca and 16 ppm Mg. Before mixing soil with sand, soil and AM inoculum bacterial microflora were extracted by suspending 500 g soil or 500 g inoculum in 1.5 l distilled water. After shaking and decanting, the suspension was filtered (11 μm mesh) in order to discard natural AM fungi. After shaking and decanting, the suspension was filtered (11 μm mesh) in order to discard natural AM fungi. Both soil and sand were 2 mm sieved and autoclaved (121ºC for 20 min). ^{15}N enriched maize biomass was added at a rate of 4.6 g dry OM per one kg mixture and both OM amended and not amended mixtures were steam sterilized at 95°C for 1 h during three consecutive days in order to completely impair biological (both fungal and bacterial) activity. In order to normalize the differences in the starting microbial community, each pot received 30 ml soil suspension filtrate and 30 ml AM inoculum suspension filtrate before starting the experiment (Koide & Li, 1989; van der Heijden *et al.*, 1998). OM added with AM inoculum was negligible if compared to the quantity added for the OM treatment.

Seeds of wheat (cv. Simeto) were surface sterilized and germinated on wet filter paper in Petri dishes for 3 days. Five seedling were transplanted per each pot and thinned to 3 plants four days after transplanting. During the experiment, each pot received 5 ml modified Hoagland's solution (Hoagland & Arnon, 1950) and 50 ml tap water once per 5 and 3 days, respectively. Modified Hoagland's solution used in the experiment was lacking P and had 10% N strength, only.

One third of the pots (4 pots per each treatments combination) were harvested at 7 weeks after transplanting (WAT), while the others two were harvested at 9 and 13 WAT, respectively. Due to a technical problem of the greenhouse, air temperature reached a maximum daily temperature of 35°C during the lest week of the experiment and it resulted in a thermal stress for plants that slightly reduced plant yield. During this week, soil temperature was maintained by giving pot 50 ml water once a day. At each harvest, a sample of soil per pot and total plant biomass were taken. Soil samples were saved at -80°C for further analyses.

2.2.2 PLANT ANALYSIS

Plant biomass was immediately separated into roots, leaves+stems and heads and fresh weights were recorded. Roots were rinsed free of soil, cut into 1-cm fragments and thoroughly mixed. Representative root samples were taken for determination of root AM colonization and fungal alkaline phosphatase (ALP) and succinate dehydrogenase (SDH). Shoots, heads and remaining roots were oven dried at 80 °C for 24 h and dry weight was taken. For measuring AM root colonization, root samples was cleared with 10% (w/v) KOH and stained with 0.05% (v/v) trypan blue using the method described by Phillips & Hayman (1970). For measuring ALP and SDH, root samples were previously cleared (as described by Vierheilig *et al.*, 2005) for 2 h in a solution containing 0.05 M Tris/citric acid (pH 9.2), 0.05% sorbitol, 15 units ml^{-1} cellulase and 15 units ml^{-1} pectinase (both enzymes were from *Aspergillus niger*). Roots samples were subsequently rinsed in distilled water and placed in the approriate staining for ALP and SDH as described in Tisserant *et al.* (1993) and Smith & Gianinazzi-Pearson (1990), respectively. Measures of root AM colonization, ALP and SDH fungal activities were made by observing root pieces under the microscope and counting 250-300 total

intersections by the grid intersect method (Giovanetti & Mossee, 1980). Above ground and root plant biomasses were analyzed for total N and ^{15}N enrichment, using an elemental analyzer – isotope ratio mass spectrometry (EA-IRMS, Carlo Erba NA1500).

2.2.3 SOIL ENZYMATIC ACTIVITY, DNA EXTRACTION AND PCR-DGGE ANALYSIS

Soil enzymatic activities were measured for both wheat-cultivated and uncultivated pots. Four enzymatic activity were measured: dehydrogenase activity (according with García *et al.*, 1997) as an index of microbial activity; casein protease (also referred as 'casein hydrolizing activity' or 'caseinase', measured according with Ladd & Butler, 1972) as measure of protein hydrolysis to mono- and dipeptides; BAA-protease (according with Nannipieri *et al.*, 1980; and with Tabatabai, 1994) and urease (according with Kandeler & Gerber, 1988) as measure of amino-acids deamination.

The total DNA was extracted from 250 mg of soil samples collected at the last sampling date (13 WAT) and from the bacterial inoculum (4 extractions per each pot) by the bead-beating method, following the manufacturers' instructions MoBio UltraClean Soil DNA Isolation kit (MoBio Laboratories Inc., Solana Beach, CA, USA) with a few modifications, including the repetition of the second step (Inhibitor Removal Solution) to remove trace concentrations of PCR inhibitors. The DNA samples from each treatments were mixed and checked for concentration and quality using the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware; USA). PCR was performed with the 16S rRNA universal bacterial primers (TIB®MOLBIOL, Berlin, Germany) F341, R907 and GC-F341 (P3) to amplify the V3–V5 hypervariable regions of 16S rRNA genes. Primer P3 contains the same sequence as F341 but with an additional 40-nucleotide GC-rich sequence (GC clamp) at its 5′ end (Yu & Morrison, 2004). The PCR program was initiated by a hot start of 5 min at 94 °C; after 9 min of initial denaturation at 95 °C, a touchdown thermal profile protocol was used, and the annealing temperature was decreased by 1 °C per cycle from 65 °C to 55 °C; then 20 additional cycles at 55 °C were performed. Amplification was carried out with 1 min of denaturation at 94 °C, 1 min of primer annealing, and 1.5 min of primer extension at 72 °C, followed by 10 min of final primer extension. The total reaction mixture of the first PCR consisted of 25 µl with the following ingredients: approx. 1 ng of extracted DNA, 1 µM primer F341, 1 µM primer R907, 0.2mM dNTPs, 5 U Taq polymerase (Bioline GmbH, Germany), 1X PCR buffer (included in the) and 1.5 mM $MgCl_2$, and sterile Milli-Q water to a final volume of 25 µl. The second amplification was performed by using 1 µl of the products of the first reaction as template. In this amplification, primers P3 and R907 were used under the same conditions as described above. PCR products were analyzed by electrophoresis in 2% agarose gels stained with GelRed™.

PCR-DGGE was performed to evaluate the effect of treatments on bacterial community of soil. DGGE analyses were conducted 4 times, using 5, 7, 10 and 15 µl of the latter PCR product, respectively, loaded into a 45–60% urea–formamide–polyacrylamide gradient gel. An INGENYphorU System (Ingeny International BV, The Netherlands) was run at 100 V for 16 h at 59 °C to separate the fragments. Gels were silver stained according to Radojkovica & Kusic (2000) and scanned. Band patterns

from the clearest gel were further analyzed. Band patterns in different DGGE lanes were compared with the UVImap Analysis software (UVItec Limited, Cambridge, UK). The lanes were normalized to contain the same amount of total signal after background subtraction and the gel images were straightened and aligned to give a densitometric curve. Bands were assigned and matched automatically and then checked manually. Band positions were converted to RF values between 0 and 1.

2.2.4 CALCULATIONS AND STATISTICAL ANALISYS

According to Allen *et al.* (2004), the N Recovery Fraction (%N_{REC}; i.e. the N deriving from OM above N applied with OM) of wheat was calculated as follows:

$$N_{REC} = N_t \times \frac{^{15}N_a - ^{15}N_b}{^{15}N_c - ^{15}N_b} \quad ; \quad \%\, N_{REC} = \frac{N_{REC}}{f} \times 100$$

where N_t is the total N content (g pot^{-1}) in wheat; $^{15}N_a$, $^{15}N_b$, and $^{15}N_c$ are the ^{15}N isotopic concentration of wheat grown with the organic amendant (either maize leaves or roots), without the organic amendant, and of the organic amendant (either maize leaves or roots), respectively. Finally, f is the total N of the organic amendant (either maize leaves or roots).

Profile similarity by DGGE lanes was calculated by determining species evenness (E) and abundance (A) as follows:

$$E = n / n_{max} \quad ; \quad A = n / n_{tot}$$

where n is the number of lines from a given band; n_{max} is the number of band from the line with most number of bands; and n_{tot} is the total number of line detected in all DGGE lines (i.e. the sum of all common and uncommon bands).

Nei and Li's similarity coefficients (Nei & Li, 1979) was also calculated for the total number of lane patterns from the DGGE gel and the similarity coefficients calculated were then used to construct a dendrogram using the unweighted pair-group method with arithmetical averages (UPGMA).

DGGE banding data were used to estimate three diversity indices by treating each band as an individual operational taxonomic unit (OTU). For these analyses, each band was presumed to represent the ability of that bacterial species to be amplified (Ibekwe & Grieve, 2004). Each banding pattern was used as a a semi-quantitative measure of bacterial diversity (Dilly *et al.,* 2004). The Shannon index of general diversity H′ (Shannon & Weaver, 1963) and the reciprocal Simpson index of dominance D_2 (Simpson, 1949) were calculated from the number of bands present and the relative intensities of the bands in each lane. The similarity coefficients calculated were then used to construct a dendrogram using the unweighted pair-group method with arithmetical averages (UPGMA). Estimates of the number of clusters (soil bacterial communities) were achieved using pseudo F and t^2 statistics (Milligan & Cooper, 1985). The relevance of the cluster tree was evaluated by computing the cophenetic

correlation coefficient (routine COPH of the NTSYS-pc package). The closer the cophenetic coefficient to 1, the more relevantly the cluster tree reflects the structure of the data. Finally, matrices based on and DGGE banding pattern and enzymatic activities distances were compared calculating the Mantel's test statistic Z (Mantel, 1967), and significance was determined using 1000 permutations (routine MXCOMP of the NTSYS-pc package; Rohlf, 1998).

Moreover, Shannon index of equitability E_H was calculated by dividing H′ by lnS. The intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon H′ and Simpson D_2 indexes were calculated from the following equations:

$$H' = -\sum(Pi * \log Pi); \qquad D_2 = 1/\sum Pi^2$$

where $Pi = n_i/N$; n_i is the height of peak and N is the sum of all peak heights in the curve.

DGGE bands can be considered as the ability of that bacterial taxonomic unit to be amplified (Ibekwe & Grieve, 2004) and Dilly *et al.* (2004) proposed DGGE banding pattern as a semi-quantitative measure of bacterial diversity. To test if the similarities observed within and between samples were greater or less than those expected by chance, band matching data were stored as a binary matrix and analyzed using Raup and Crick's probability-based index of similarity S_{RC} (Raup & Crick, 1979). The S_{RC} is the probability that the randomized similarity would be greater than or equal to the observed similarity, and S_{RC} values above 0.95 or below 0.05 signify similarities and differences, respectively, which are not random assortments of the same species (bands or OTUs) (Rowan *et al.*, 2003). S_{RC} was calculated using the PAST (Palaeontological statistics, version 1.97) program (Hammer *et al.*, 2001).

Data on plant production, quality, root AM infection and activity and on soil enzymatic activities were subjected to analysis of variance (ANOVA) according to the experimental design. Data of enzymatic activity from unplanted and wheat-cultivated pots analyzed separately. All measured variables were assumed to be normally distributed. All variables corresponding to proportions were arcsine transformed before analysis to assure a better fit with the Gaussian law distribution. Treatment means were compared using Tukey's test (P values) at 5% probability level.

3 RESULTS AND DISCUSSION

3.1 EXPERIMENT 1

Total rainfall during the growing season was 13% lower than the long term average (551 mm). During spring, rainfalls were markedly lower (–56%) than normal and it caused drought stressing conditions for the crop. Average, minimum and maximum temperatures were similar to long-term averages (Fig. 3-1).

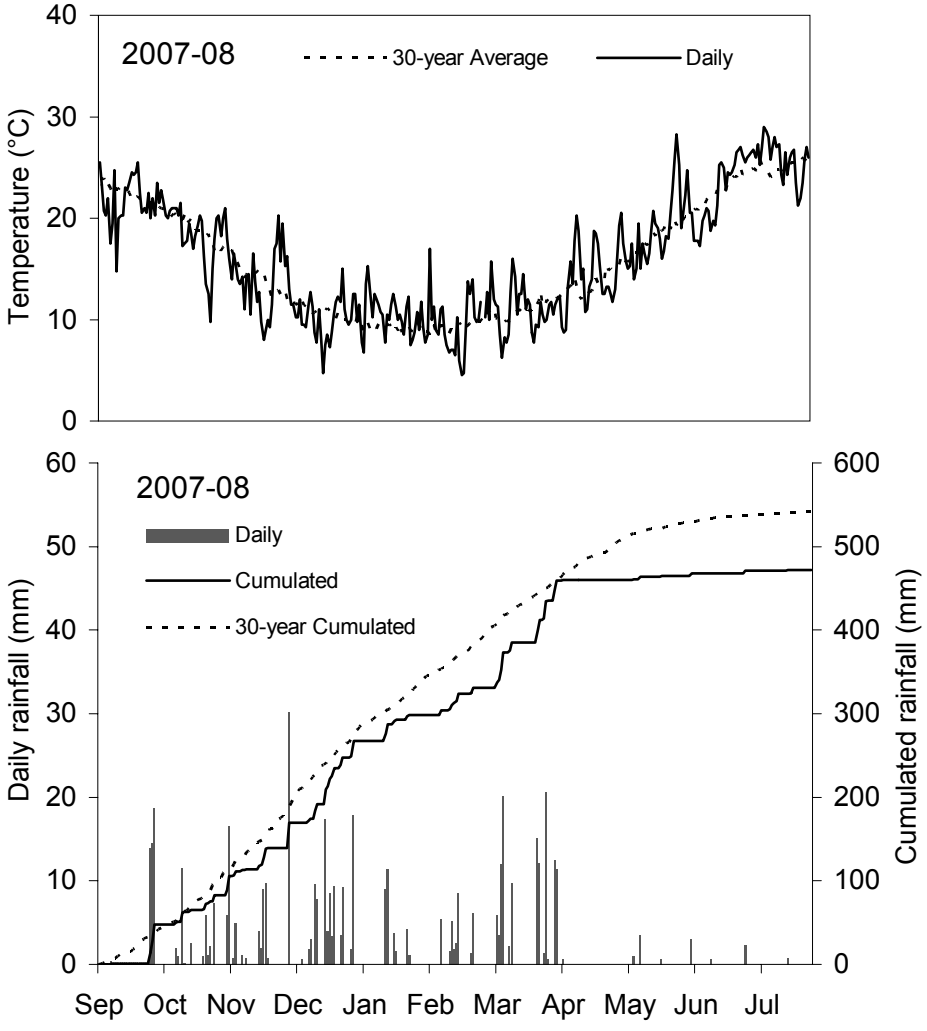


Fig. 3-1 – Daily average air temperature and daily and cumulated rainfall during the 2007–2008 growing season at the study area in Pietranera field station (37°33'N – 13°30'E, 170 m a.s.l.), Sicily, Italy.

3.1.1 BIOMASS PRODUCTION AND ROOT COLONIZATION BY AM FUNGI AT THE FIRST AND SECOND CUT

At 55 DAS, mycorrhizal infection in +AM treatments was 32.4% and 23.6% for berseem and ryegrass, respectively. The fungicides applied negatively affected the root infection by AM fungi. Root AM colonization in –AM treatments was 7.0% in berseem and 1.7% in ryegrass. These results accord with reports from pot and field studies by several authors (Dodd e Jeffries, 1989; Sukarno *et al.,* 1993; Udaiyan *et al.*, 1995).

At 76 DAS (first cut), root dry matter (DM) yield, aboveground biomass (both removed and residual), and respective Leaf Area Indices (LAIs) of berseem were significantly higher in the +AM than the –AM treatment, whereas, at 116 DAS (second cut) no significant effect of mycorrhization treatment were observed (Tab. 3-1).

Given C limitation induced by the detopping of berseem, the advantages of the AM symbiosis were probably neutralized by the C cost of the AM fungi. Koide & Elliot (1989) and Kiers & Denison (2008) found that plants should suppress symbiotic association if the benefits are smaller than the C costs. On the other side, Goss & de Varennes (2002) and Ganry *et al.* (1985) argued that AM symbiosis was important in nutrient uptake in the early phase of the crop cycle, when roots are not well developed and still have a low nutrient absorbing ability. This could explain why the effects of AM treatments were significant only at the first cut. In contrast with results from the present study, Barea *et al.* (1987) found no effect of inoculation of AM fungi on *Hedysarum coronarium* plants at the first harvest, but in the subsequent regrowths AM inoculation significantly enhanced dry matter yield comparing with not inoculated plants. AM infection didn't affect ryegrass production and plant traits both at the first and second cut. Kaschuk *et al.* (2010) reported that yield response of legumes to AM infection in field varies with the plant species and range from yield depression to yield improvement.

*Tab. 3-1 – Berseem above ground (removed and residual) and root biomass and ryegrass above ground removed biomass, LAIs and % leaves in mycorrhizal inoculated (+Myc) mycorrhizal depressed (-Myc) crops at 76 (first cut) and 116 (second cut) DAC. Values are means for 8 replicates. Significance of treatments is given: ns, non significant; *, $p < 0.05$; **, $p < 0.01$.*

		First cut			Second cut		
		- Myc	+ Myc		- Myc	+ Myc	
Berseem (*Trifolium alexandrinum*)							
Removed above ground biomass	g DM m^{-2}	113	136	**	393	410	ns
LAI		2.51	2.87	*	4.62	4.97	ns
% leaves		74.7	68.2	**	40.6	41.5	ns
Residual above ground biomass	g DM m^{-2}	46	51	*	155	158	ns
LAI		0.44	0.51	*	1.31	1.33	ns
% leaves		32.2	32.8	ns	28.6	28.5	ns
Root biomass	g DM m^{-2}	35	41	*	36	36	ns
Ryegrass (*Lolium multiflorum*)							
Removed above ground biomass	g DM m^{-2}	45	45	ns	383	391	ns
LAI		1.0	1.0	ns	4.53	4.49	ns
% leaves		100.0	100.0	ns	51.0	46.8	ns

3.1.2 EFFECTS OF AM INOCULATION ON BERSEEM PRODUCTION AND SYMBIOTIC N_2 FIXATION UNDER DIFFERENT WATER REGIMES

After the second cut, the effects of AM symbiosis was studied on berseem clover grown in the field under both drought and well-watered conditions as described in the section 2.1.2.

As expected, soil moisture contents, measured at 7, 14, 21, and 28 days after the second cut (layers 0-20 cm and 20-40 cm) were always significantly higher in the well-watered than the drought-stressed conditions (Fig. 3-2). Soil moisture contents weren't affected by AM treatment and interaction between water regime and AM treatment has never been found.

At 14 DAC, AM infection was on average 8.8% and 37.7% in the –AM and +AM treatments, respectively, and no effect of the soil moisture regime treatment was observed. At 28 DAC, root AM colonization was very low in the –AM treatments irrespective of soil moisture regime (8.7% on average). In +AM treatments, AM infection was significantly higher in the drought-stressed than well waters treatments (66.0% and 52.4%, respectively). Such result is consistent with findings from pot studies (e.g. Azcón *et al.*, 1988, for alfalfa), while other authors (Meddich *et al.,* 2000) have observed a higher AM infection in well-watered than drought-stressed berseem. However, in the latter experiment, drought was more severe than in our research. I

hypothesize that the effects of drought vary with its intensity: in moderate stress conditions, the plant favours AM infection to take advantage of the symbiosis, while when drought is severe, probably the plant lacks of photosynthates to feed AM fungi and so AM infection is reduced.

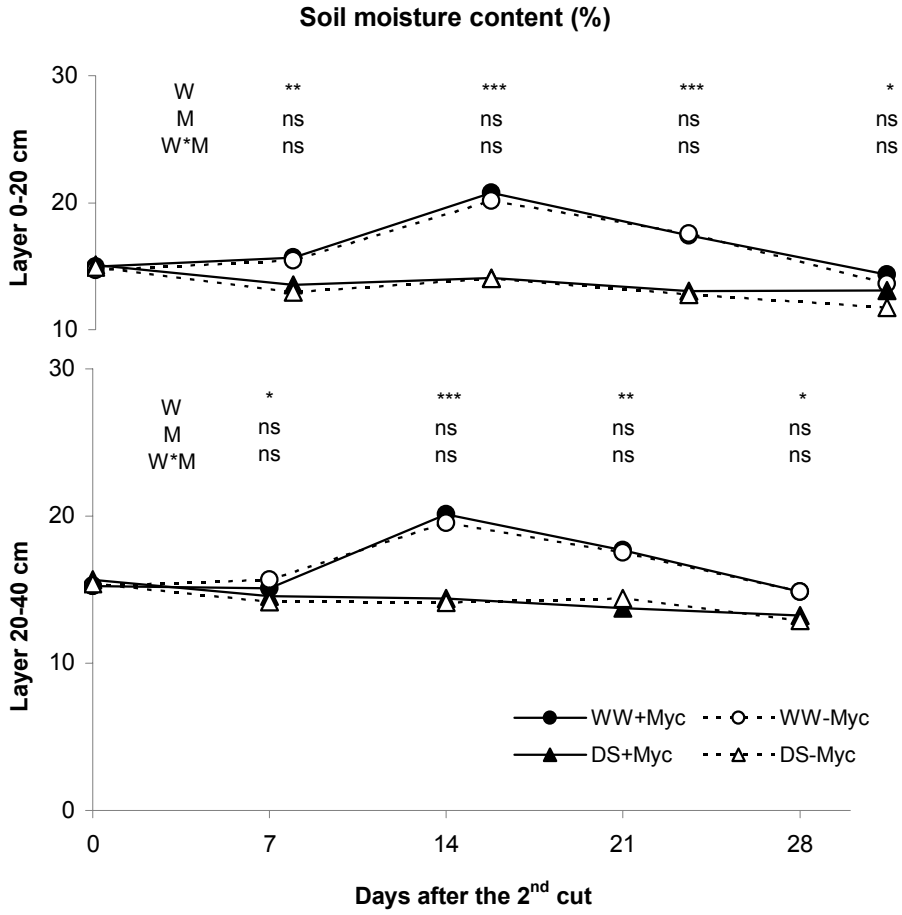


*Fig. 3-2 – Effects of treatments on soil moisture content (0 to 20 cm and 20 to 40 cm depth) during regrowth after the second cut. Values are means for 4 replicates. WW, DS for well-watered and drought-stressed plots, respectively; +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed plots, respectively. Significance of treatments per each cut is given (W, soil moisture treatment; M, crop mycorrhization treatment, W*M, interaction): ns, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.*

The effects of treatments on berseem above ground (AG) biomass during regrowth after the second cut is shown in Fig. 3-3. Since from 7 DAC, berseem biomass was significantly higher under well-watered than drought-stressed conditions. The differences determined by water regime markedly increased at 14 DAC and remained unvaried at 21 and 28 DAC.

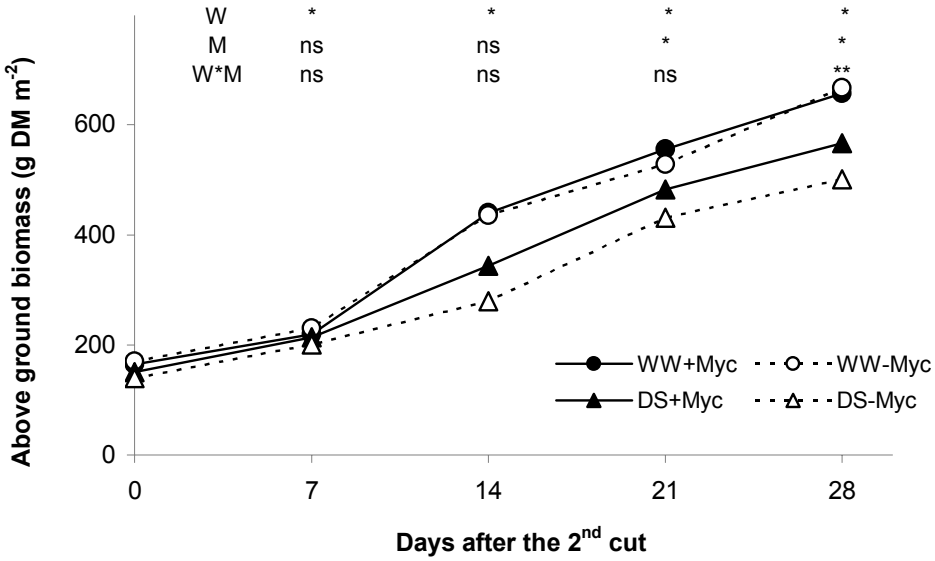


*Fig. 3-3 – Effects of mycorrhization and soil moisture regime on berseem above ground biomass during regrowth after the second cut. Values are means for 4 replicates. WW, DS for well-watered and drought-stressed crops, respectively; +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed crops, respectively. Significance of treatments per each cut is given (W, soil moisture treatment; M, crop mycorrhization treatment, W*M, interaction): ns, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.*

AM symbiosis didn't affect AG biomass at 7 and 14 days after the second cut (DAC) both under weel-watered and drought-stressed conditions. It is probable that the benefits of AM symbiosis during the first regrowth phase were impaired due to a competition for carbon between AM fungi and shoots. Indeed, during this phase, legume regrowth ability is highly dependent on the plant's capacity to mobilize C and N reserves stored in roots and stubble (Ta *et al.,* 1990; Ourry *et al.*, 1994; Volenec *et al.,* 1996; Avice *et al.*, 1997) and on residual leaf area (Meuriot *et al.,* 2004) to rebuild its photosynthetic apparatus and the high sink strenght of the AG parts of the plant could have limited the resources available for the AM fungi, and it didn't produce any growth benefit. This hypothesis is confirmed by the fact that at 21 and 28 DAC, when plants have already rebuilt its photosynthetic apparatus to satisfy both shoots and AM fungi C demands, a higher AG biomass was observed in +AM than –AM treatment.

The advantages of the AM symbiosis were higher in drought-stressed than well-watered conditions, particularly at 28 DAC. This result is consistent with findings of

several pot studies (Azcón *et al.*, 1988; Ruiz-Lozano *et al.*, 1995; Marulanda et al, 2003; Porcel & Ruiz-Lozano, 2004). Several authors reported that the AM contribution to plant drought tolerance are related to drought avoidance mechanisms such as hyphal water uptake (Ruiz-Lozano *et al.*, 1995) and enhanced plant osmotic adjustment (Goicoechea *et al.,* 1998; Kubikova *et al.*, 2001) that favour plant water uptake from soil. Some authors (Ruiz-Lozano *et al.*, 1996; Ruiz-Lozano *et al.*, 2001b) suggested that the advantages of the AM symbiosis in drought stressing conditions are related to a protection effect of AM fungi against oxidative damages generated by drought (Bartels, 2001). However, other authors (Al-Karaki *et al.,* 2004) observed a beneficial effect of an increased AM infection to yield and quality of wheat grown in field both in well-watered and drought-stressed conditions.

Both treatments applied (water regime and mycorrhization) didn't singnificantly affect berseem root biomass in all sampling dates (Fig. 3-4). Other researches (Hawkins & George, 1999; Li *et al.*, 2006) have found no differences in root dry weight due to the presence of the AM symbiosis. The hypogeic:epigeic biomass ratio was higher in plants grown in drought than well-watered conditions, and it wasn't affected by the AM treatment (Fig. 3-5).

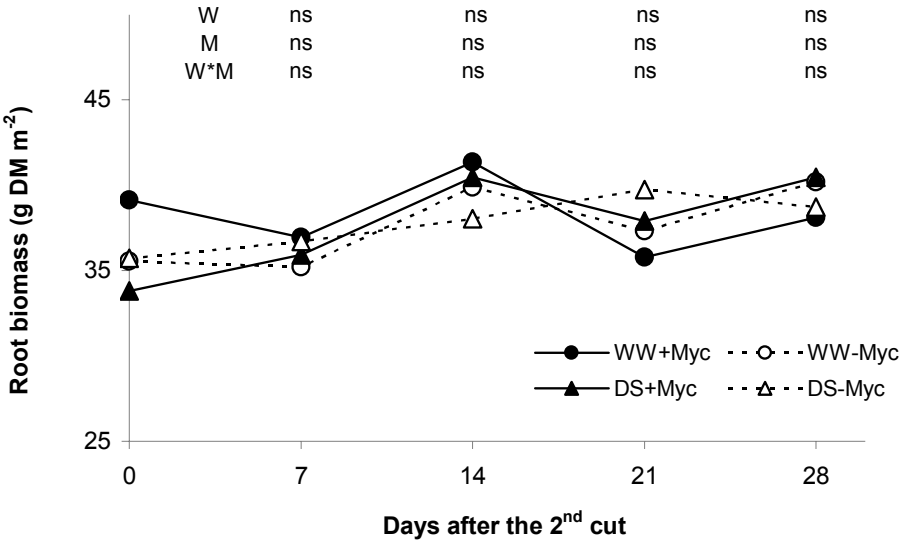


*Fig. 3-4 – Effects of mycorrhization and soil moisture regime on berseem root during regrowth after the second cut. Values are means for 4 replicates. WW, DS for well-watered and drought-stressed crops, respectively; +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed crops, respectively. Significance of treatments per each cut is given (W, soil moisture treatment; M, crop mycorrhization treatment, W*M, interaction): ns, non significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001*

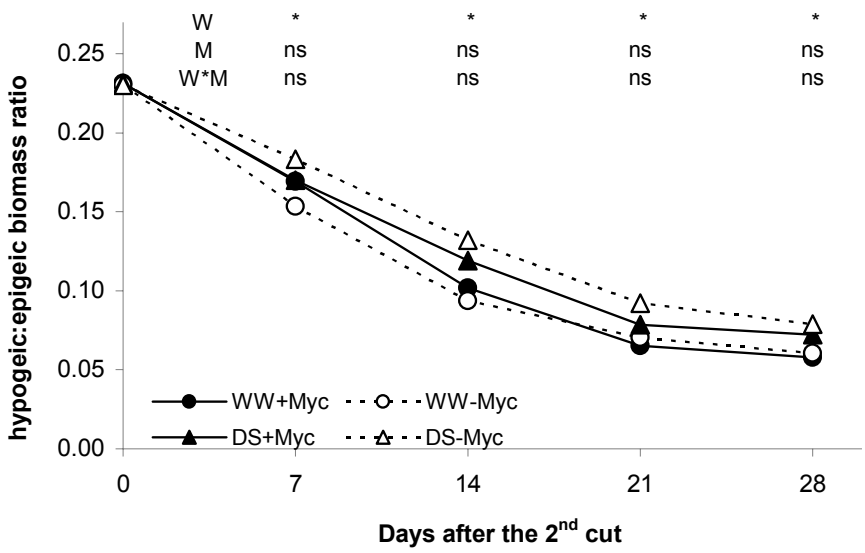


*Fig. 3-5 – Effects of mycorrhization and soil moisture regime on hypogeic:epigeic biomass ratio of berseem during regrowth after the second cut. Values are means for 4 replicates. WW, DS for well-watered and drought-stressed crops, respectively; +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed crops, respectively. Significance of treatments per each cut is given (W, soil moisture treatment; M, crop mycorrhization treatment, W*M, interaction): ns, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.*

A higher leaves, but lower stems and heads percentages were observed in biomass of berseem grown under well-watered than drought-stressed conditions (Fig. 3-6, Fig. 3-7, Fig. 3-8 for leaves, stems and heads, respectively). The differences in stems and heads percentages due to the soil moisture content increased during time. Crop mycorrhization never affected the proportion of the botanical fractions on the epigeic biomass of berseem.

Leaf area index was significantly higher in the well-watered than drought-stressed crop (Fig. 3-9), lhe highest LAI values were observed at 21 days after the second cut (5.42 and 2.88, respectively). For this trait, no significant effects of crop micorrhization were observed, as well.

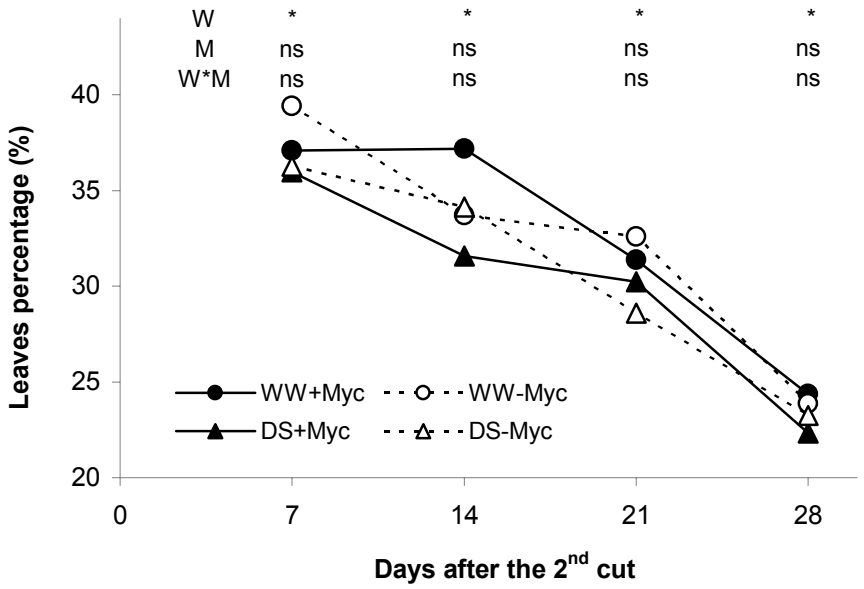


Fig. 3-6 – Effects of mycorrhization and soil moisture regime on leaves percentage of berseem during regrowth after the second cut. Values are means for 4 replicates. See Fig. 3-5 for symbols and abbreviations.

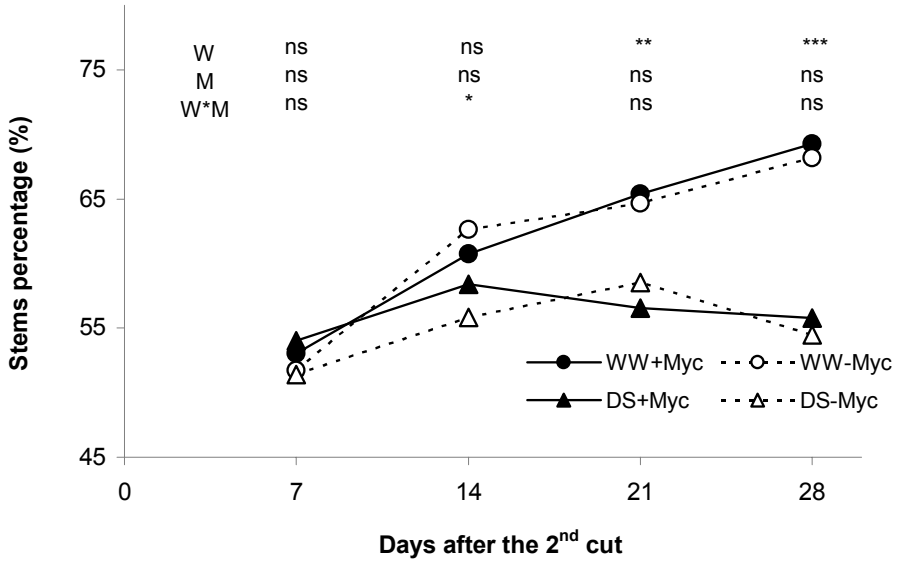


Fig. 3-7 – Effects of mycorrhization and soil moisture regime on leaves percentage of berseem during regrowth after the second cut. Values are means for 4 replicates. See Fig. 3-5 for symbols and abbreviations.

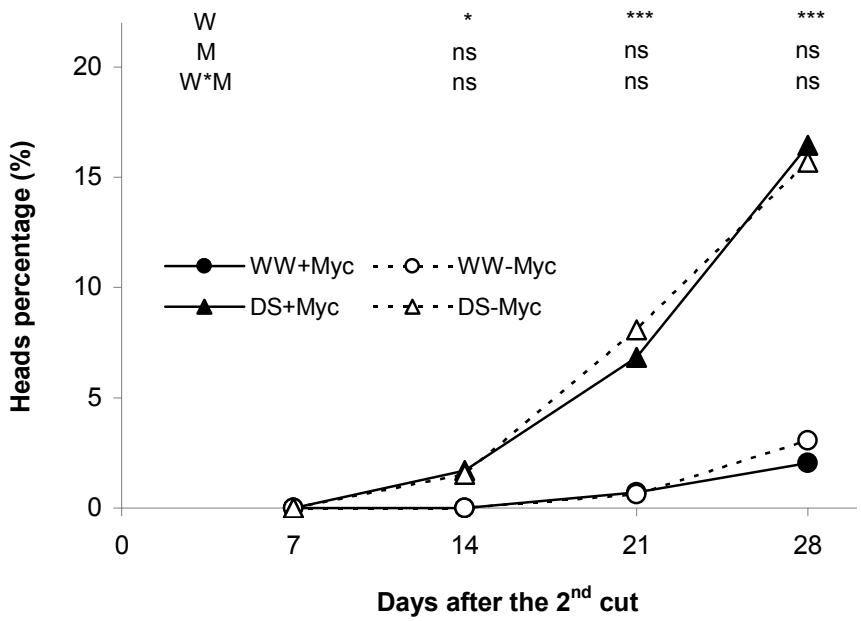


Fig. 3-8 – Effects of mycorrhization and soil moisture regime on heads percentage of berseem during regrowth after the second cut. Values are means for 4 replicates. See Fig. 3-5 for symbols and abbreviations.

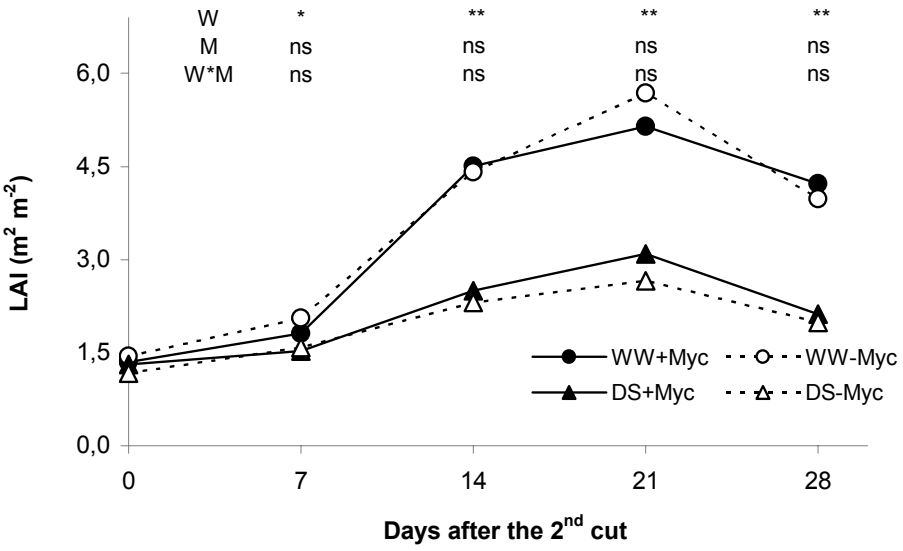


Fig. 3-9 – Effects of mycorrhization and soil moisture regime on LAI of berseem during regrowth after the second cut. Values are means for 4 replicates. See Fig. 3-5 for symbols and abbreviations.

*Tab. 3-2 – N concentration, N uptake and Ndfa of berseem at 14 and 28 days after the second cut in crops grown under well-watered and drought-stressed (rainfed) conditions. +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed crops, respectively. Each value is a mean for 4 replicates. Significance of treatments is given: ns, non significant; *, $p < 0.05$; **, $p < 0.01$.*

		Drought-stressed		Well-watered		W	M	W*M
		- Myc	+ Myc	- Myc	+ Myc			
Measures at 14 days after the 2nd cut								
N concentration								
above ground N	%	3.1	3.1	3.1	3.0	ns	ns	ns
root N	%	1.8	1.8	1.9	1.9	ns	ns	ns
N uptake								
above ground N	g N m^{-2}	9.2	10.6	13.1	13.1	*	ns	ns
root N	g N m^{-2}	0.6	0.7	0.8	0.8	**	ns	ns
Ndfa								
percentage		49.9	49.9	60.7	56.4	*	ns	ns
amount	g N m^{-2}	4.4	5.7	7.8	7.3	**	ns	ns
Measures at 28 days after the 2nd cut								
N concentration								
above ground N	%	2.5	2.4	2.5	2.5	ns	ns	ns
root N	%	1.8	1.8	1.8	1.8	ns	ns	ns
N uptake								
above ground N	g N m^{-2}	12.3	13.7	16.9	16.2	*	ns	*
root N	g N m^{-2}	0.7	0.7	0.7	0.7	ns	ns	ns
Ndfa								
percentage		40.4	52.9	50.3	52.7	*	ns	*
amount	g N m^{-2}	5.2	7.6	8.9	8.9	*	*	*

N concentration in AG and root biomass of berseem and total N uptake didn't vary with crop mycorrhization both at 14 and 28 DAC, except at 28 DAC where AM symbiosis enhanced total N uptake in the drought-stressed crop, only (Tab. 3-2). Such results are consistent with findings by other authors (Hawkins & George, 1999; Aliasgharzad *et al.,* 2006). On the contrary, Azcón & Al-Atrash (1997) showed that AM symbiosis increased N concentration and N uptake in *M. sativa* of almost two- and threefold, respectively, comparing to the non-mycorrhizal control. Soil moisture regimes didn't affect N concentration, but, given its effect on DM yield, it markedly influenced total N uptake at both 14 and 28 DAC.

Ndfa (as both as percentage of total N uptake and the amount of N fixed) was significantly higher in well-watered than in drought-stressed conditions at both 14 and 28 DAC. Several researches showed that symbiotic N_2 fixation of legumes is highly sensitive to soil water deficiency (as reviewed by Zahran, 1999). This occurs because nodule activity is more sensitive to drought than shoot and root metabolism (Albrecth *et al.*, 1994). Results from the present research seem to confirm this assumption; in fact the reduction of symbiotic N_2 fixation caused by drought was higher than DM yield.

At 14 DAC, no effect of crop mycorrhization was observed on N_2 fixation, but at 28 DAC, a higher Ndfa was observed for +AM than –AM in the drought-stressed (rainfed) conditions, only. Many authors stated that N_2 fixation can be improved by AM symbiosis, resulting in a higher amount of N fixed and in a higher proportional dependence of the legume on atmospheric N_2 (Schoeneberger *et al.*, 1989; Olesniewicz & Thomas, 1999; Goss & de Varennes, 2002; Antunes *et al.*, 2006b) and in many cases such results were attributed to the benefits of AM fungi on improved P acquisition (Barea & Azcón, 1983).

However very few data exist on the interactive effects of AM symbiosis and environmental factors on the performance of the tripartite symbiosis and particularly on the amount of symbiotic N_2 fixation. Porcel *et al.* (2003) found that N_2ase activity increased more in AM than non-AM soybeans in a controlled environment and that this increase was more evident under drought-stressed conditions. Ruiz-Lozano *et al.* (2001a) found that AM symbiosis was able to reduce nodule senescence under drought stress conditions and attributed this effect to a protection against oxidative damages which have detrimental effects on N_2ase activity.

3.1.3 BIOMASS PRODUCTION, QUALITY AND ROOT AM INFECTION OF RYEGRASS UNDER DIFFERENT SOIL MOISTURE REGIMES

Data about biomass production, quality and root AM infection of annual ryegrass at 14 and 28 DAC are given in Tab. 3-3.

Fungicide applied strongly depressed AM infection in both sampling moments. Root AM colonization of +AM treatments was significantly lower under well-watered than drought-stressed condition at both 14 and 28 DAC as already observed in berseem. Similar results were found by Ruiz-Lozano *et al.* (1995) for lettuce infected by *G. mosseae* and by Manske *et al.* (1995) in wheat. However, other authors found depressive (Al-Karaki *et al.*, 2004) or null effects (Ruiz-Lozano *et al.*, 1996) of drought stress on root AM colonization in non leguminous crops. As for berseem, here I hypothesize that the effects of drought vary with its intensity. In addition, it seems that the effect of water regime on root AM colonization of non-leguminous species seems to vary with the AM fungus strain: for example, Ruiz-Lozano *et al.* (1995) found a higher AM colonization in drought-stressed than well-watered lettuce inoculated by *G. mosseae*, but not with other AM fungi. In the present research, it is probable that plant stimulated AM infection in a higher extent under drought-stressed than well-watered conditions in order to overcome the stressing effect of water deficiency. However, plant growth was surely impaired by the lack of N fertilization irrespective of the water regime and mycorrhization treatments. This is clearly visible considering that plant N concentration at both 14 and 28 DAC was almost a half if comparing with N fertilized ryegrass grown in similar conditions (Giambalvo *et al.,* 2005).

A higher water availability resulted in both higher AG biomass and N uptake at 14 and 28 DAC and it surely depended by the higher transpiration rates under well-watered than drought-stressed conditions, as also shown by Yin *et al.* (2009). No differences on root biomass have been found under the different water regimes.

*Tab. 3-3 – Root AM colonization, above ground and root biomass, LAIs, % of leaves, stems and spikes, hypogeic:epigeic biomass ratio and N concentration and uptake of ryegrass at 14 and 28 days after the second cut in crops grown under well-watered and drought-stressed (rainfed) conditions. +Myc, and –Myc for mycorrhizal inoculated mycorrhizal depressed crops, respectively. Each value is a mean for 4 replicates. Significance of treatments is given: ns, non significant; *, $p < 0.05$; **, $p < 0.01$.*

		Drought-stressed		Well-watered		W	M	W*M
		- Myc	+ Myc	- Myc	+ Myc			
Measures at 14 days after the 2nd cut								
Root AM colonization	%	7.6	35.5	3.0	30.9	*	*	ns
Above ground biomass	g DM m^{-2}	229	230	379	406	***	ns	ns
% leaves	%	15.9	18.8	14.8	19.0	ns	*	ns
% stems	%	17.1	19.0	16.4	17.8	ns	ns	ns
% spikes	%	0.8	0.5	0.3	0.0	ns	ns	ns
LAI		1.30	1.26	2.57	2.80	*	ns	ns
Root biomass	g DM m^{-2}	29	36	33	45	ns	***	ns
Hypogeic:epigeic biomass ratio		0.13	0.16	0.09	0.11	*	*	ns
N concentration								
above ground N	%	1.86	1.85	1.88	1.59	ns	ns	ns
root N	%	0.72	0.76	0.65	0.62	*	ns	ns
N uptake								
above ground N	g N m^{-2}	4.26	4.26	7.13	6.46	**	ns	ns
root N	g N m^{-2}	0.22	0.28	0.21	0.28	ns	**	ns
Measures at 28 days after the 2nd cut								
Root AM colonization	%	11.5	49.2	11.2	37.0	*	*	*
Above ground biomass	g DM m^{-2}	376	348	532	526	*	ns	ns
% leaves	%	20.1	20.0	19.0	18.2	ns	ns	ns
% stems	%	18.7	18.5	17.4	20.6	ns	ns	ns
% spikes	%	14.5	15.6	14.9	15.7	ns	ns	ns
LAI		1.60	1.43	3.15	3.36	*	ns	ns
Root biomass	g DM m^{-2}	39	44	40	45	ns	*	ns
Hypogeic:epigeic biomass ratio		0.11	0.13	0.08	0.09	*	ns	ns
N concentration								
above ground N	%	1.73	1.40	1.66	1.30	ns	*	ns
root N	%	0.83	0.76	0.76	0.61	ns	ns	ns
N uptake								
above ground N	g N m^{-2}	6.50	4.87	8.83	6.84	*	*	ns
root N	g N m^{-2}	0.32	0.33	0.31	0.28	ns	ns	ns

AM symbiosis significantly enhanced root biomass irrespective of the soil moisture regime in both samplings. It's usually believed that AM fungi impair root development by competing for C. However, other authors showed that AM can improve root growth, especially in nutrient limited environments (Behl *et al.*, 2003; Schroeder & Janos, 2005; Li *et al.,* 2006). In the present experiment, the lack of N or P fertilizers limited plant growth. In such conditions, AM fungi could have promoted root branching so resulting in a higher root biomass (Atkinson *et al.,* 1994). The presence of the AM symbiosis had no effects on the above ground biomass at both 14 and 28 DAC. In agreement with the present results, several authors found that AM symbiosis can result in no positive AG growth responses at vegetative stages (Simpson & Daft, 1990; Baon *et al.*, 1992; Graham & Abbott, 2000; Zhu *et al.*, 2001; Ryan & Graham, 2002; Ryan & Angus, 2003; Li *et al.,* 2005 and 2006).

N concentration and uptake at 28 DAC was significantly lower in AM than non AM plants. Also Wallace *et al.* (1982) found a lower N uptake in plants subjected to clipping and attributed this effect to the competition for N and C between roots and AM fungi, especially when plants were grown under limiting N availability. Ganry (1982) found than AM symbiosis reduced the uptake of both fertilizer and soil N in a field experiment, while Hodge (2003) showed that AM fungi lowered N capture by *Plantago lanceolata* and *L. perenne* in monocultures and attributed this effects to the interplant competition for N. However, some reports showed that AM symbiosis promote N uptake by plants grown in N limiting (Azcón *et al.,* 2008) or drought-stressed conditions (Goicoechea *et al.,* 1997), while other authors found no positive effects of AM symbiosis on N uptake of plants grown under low N availability (Hawkins & George, 1999) or after defoliation (Hokka *et al.,* 2004). It seems that the contribution of AM fungi to N uptake becomes negligible when roots and hyphae exploit a common space (Hodge *et al.*, 2000b; Mäder *et al.,* 2000).

3.2 EXPERIMENT 2

3.2.1 RESULTS

Significances of data of wheat production and quality are shown in Tab. 3-4. OM addition significantly affected root AM infection (Fig. 3-10), alkaline phosphatases (ALP, Fig. 3-19) and succinate dehydrogenase (SDH, Fig. 3-12) activities in all samplings. Roots AM colonization was on average 23,2%, 34,9% and 33,3% at 7, 9 and 13 weeks after transplanting (WAT), respectively. ALP activity at the first (7 WAT) and second (9 WAT) sampling date was higher in plants grown in amended soils. At the third sampling (13 WAT), ALP activity of plants grown in root amended soil was lower than plants grown in leaves amended or not amended soils. SDH activity in mycorrhizal roots was enhanced at 7, but depressed 9 and 13 WAT, by the addition of organic matter to soil.

Fig. 3-13 and Fig. 3-14 show wheat epigeic and root biomass, respectively. At the 3rd sampling, root biomass decreased of almost 50% irrespective of treatments due to a thermal stress during the last week of growth, but the effect of AM symbiosis and OM addition to plant growth and N uptake were similar to those observed at the 1st and 2nd cut. AM symbiosis improved above ground plant growth in all sampling, except in wheat grown in root amended pot at the 7 and 9 WAT. Addition of organic matter lowered above ground and root biomass both in AM and non AM treatments. In particular, addition of maize roots to soil consisted in higher depressive effects than addition of maize leaves to soil, especially at the second (7 WAT) and third (9 WAT) samplings. AM symbiosis didn't affect root:epigeic biomass ratio (R/S, Fig. 3-15) On average, R/S (Fig. 3-15) was higher plants grown in not amended that amended soil. An interaction between plant inoculation and OM addition was found. At the first and second sampling dates, R/S of plants grown in root amended and not amended soil was higher in non AM than AM plants, but didn't affect R/S of plants in soil amended with maize leaves. At the third sampling date, AM symbiosis affected R/S in leaves amended soil, only.

No differences in root and shoot N concentration were found among treatments applied (Tab. 3-4). On average, shoot N concentration was 1.36%, 1.13% and 1.23% at the first, second and third sampling date, respectively, while root N concentration was 0.59%, 0.53% and 0.71%, respectively. Differences observed in total N uptake among treatments were similar to those observed in plant growth (data not shown). AM symbiosis in wheat significantly lowered both N recovery fraction (%N_{REC}) (Fig. 3-16) and the percentage of plant N deriving from organic matter in comparison with non mycorrhizal treatments (Fig. 3-17).

*Tab. 3-4 – Significances of treatments (OM, Myc, OM*Myc for organic matter addition, AM inoculation treatments and interaction, respectively) at 7, 9 and 13 weeks after transplanting on root AM colonization and activity, wheat epigeic and root biomass production, N concentration and uptake, N recovery fraction (N_{REC}) and percentage of plant N deriving from OM. ns, non significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.*

			7 weeks after			9 weeks after			13 week after		
		df	Myc 1	OM 2	Myc * OM 2	Myc 1	OM 2	Myc * OM 2	Myc 1	OM 2	Myc * OM 2
AM infection	%		-	***	-	-	***	-	-	ns	-
ALP activity	%		-	***	-	-	***	-	-	***	-
SDH activity	%		-	***	-	-	***	-	-	**	-
Total biomass	g pot^{-1}		**	***	**	ns	***	***	*	***	ns
Epigeic biomass	g pot^{-1}		**	***	*	ns	***	***	*	**	ns
Root biomass	g pot^{-1}		**	***	***	ns	***	***	*	***	***
Root/Epigeic biomass ratio	g g^{-1}		ns	***	**	ns	***	***	ns	**	**
Epigeic N concentriation	%		ns	ns	ns	ns	ns	ns	ns	ns	ns
Root N concentriation	%		ns	ns	ns	ns	ns	ns	ns	ns	ns
Total N uptake	mg pot^{-1}		*	***	ns	ns	***	*	**	*	ns
N uptake in epigeic biomass	mg pot^{-1}		**	**	ns	ns	**	*	**	*	ns
N uptake in root biomass	mg pot^{-1}		ns	***	**	*	***	*	***	***	***
N recovery fraction	%		***	***	***	***	ns	*	***	*	ns
% of plant N deriving from OM	%		***	***	***	***	ns	ns	***	***	*

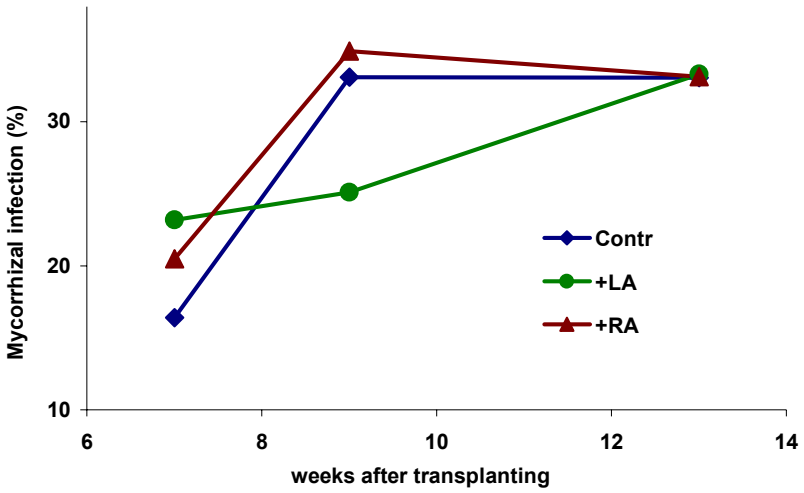


Fig. 3-10 – Percentage of root AM colonization of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) at 7, 9 and 13 weeks after transplanting.

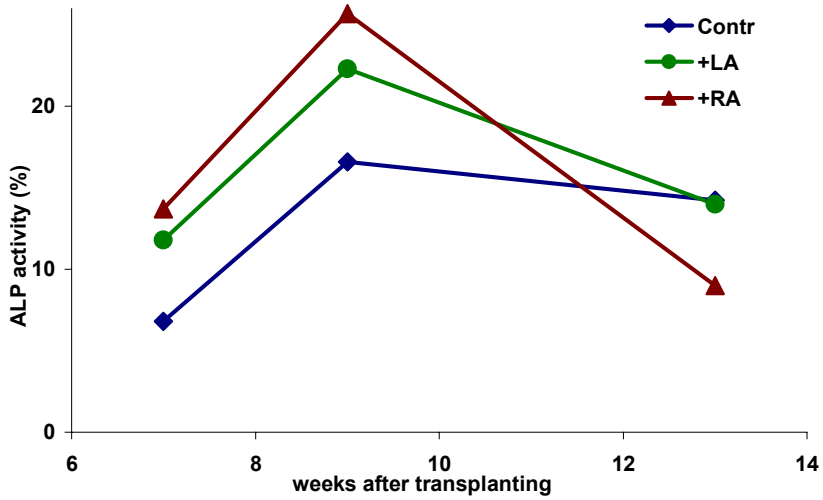


Fig. 3-11 – Root AM colonization percentage after alkaline phosphatase (ALP) staining of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) at 7, 9 and 13 weeks after transplanting.

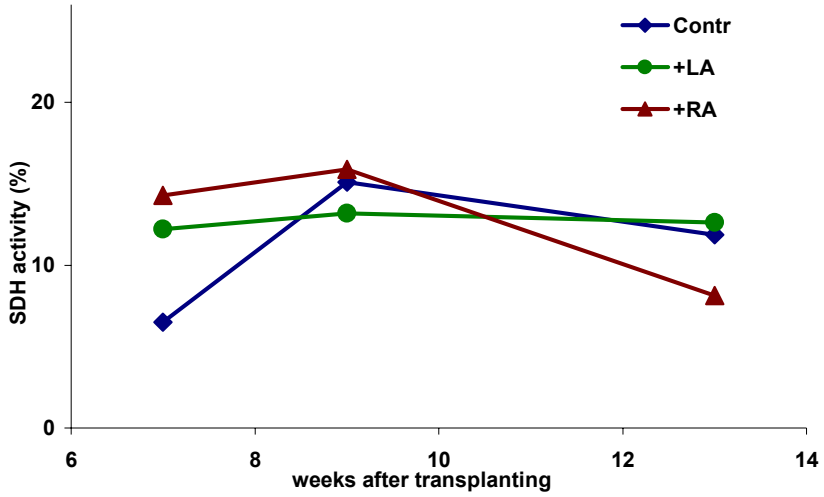


Fig. 3-12 – Root AM colonization percentage after succinate dehydrogenase (SDH) staining of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) at 7, 9 and 13 weeks after transplanting.

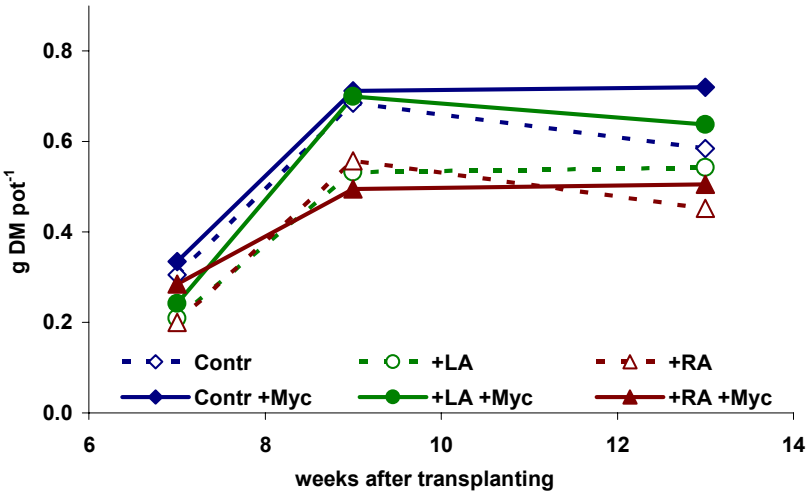


Fig. 3-13 – Epigeic biomass of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) and infected with G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting.

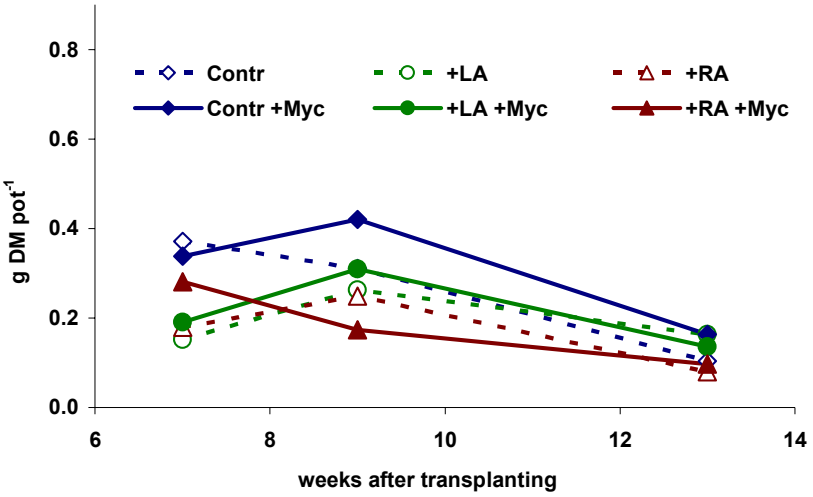


Fig. 3-14 – Root biomass of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) and infected with G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting.

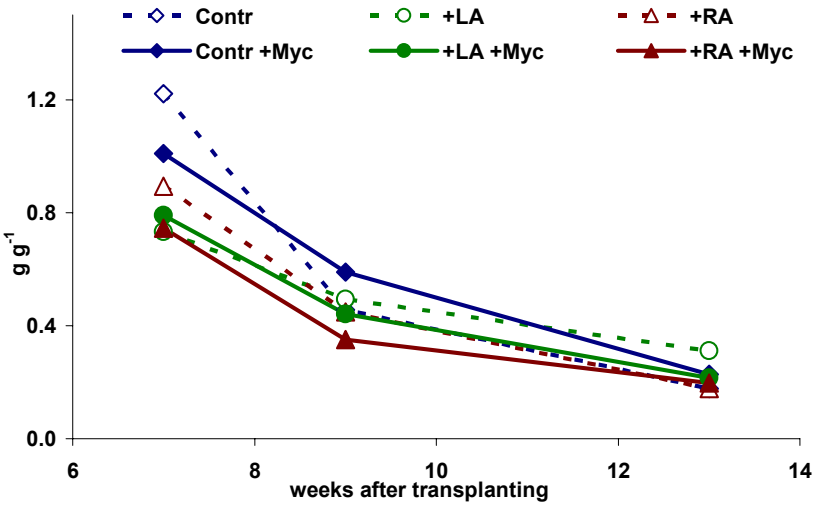


Fig. 3-15 – Root:Epigeic biomass ratio of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) or non amended soil (Contr, blue lines) and infected with G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting.

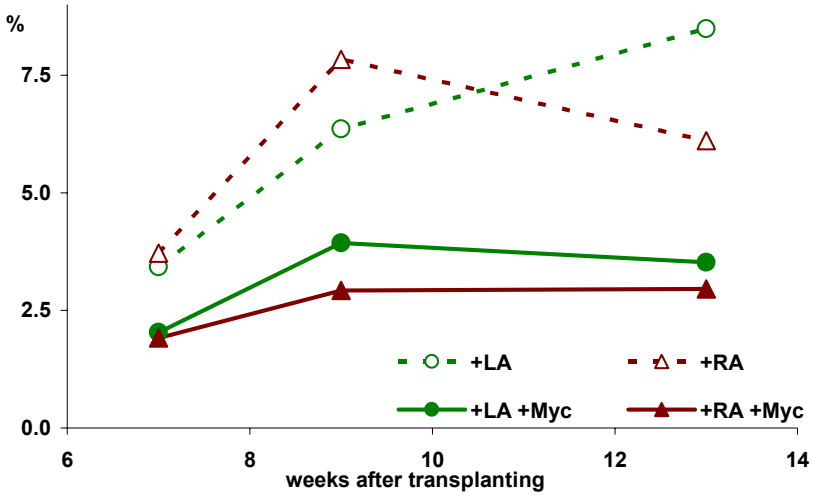


Fig. 3-16 –N recovery fraction (N_{REC}) of wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) and infected with G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting.

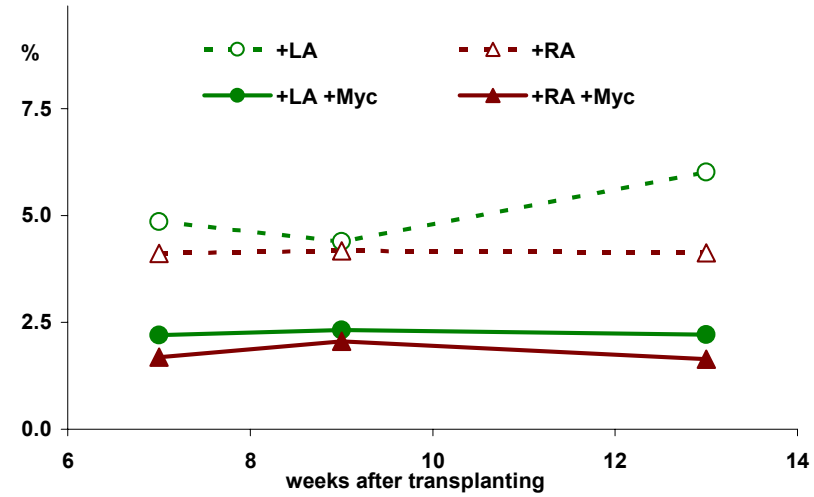


Fig. 3-17 – Percentage of plant N deriving from OM in wheat grown in soil amended with maize leaves (+LA, green lines) or maize roots (+RA, red lines) and infected with G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting.

Significances of data from soil enzymatic activity measurement in both planted and unplanted pots are given in Tab. 3-5. In unplanted soil, addition of maize leaves resulted in a higher enzymatic activity in comparison with no OM addition (Fig. 3-18), but its effects on casein hydrolyzing activity were observed in the first sampling date, only. Addition of maize roots didn't affect casein hydrolizing and deammination activities in unplanted soil, but enhanced dehydrogenase activity in any of the sampling dates.

In planted soil, on average, all enzymatic activities were higher in leaves amended than non amended soil. Addition of maize roots to soil lowered urease and caseinase activities in comparison with non amended soil. The effects of AM symbiosis on soil enzymatic activity varied with OM treatment (Tab. 3-5 and Fig. 3-19). In particular, AM improved dehydrogenase activity at the first sampling in soil amended with maize root, only, and at the second sampling date in soil amended with both maize leaves and root. At the third sampling, soil dehydrogenase activity was higher in all AM than non AM treatments. Caseinase activity was improved at the first but depressed at the third sampling by AM symbiosis, while no difference among treatments were found at the second sampling. This effect is particularly evident in soil amended with maize leaves. AM symbiosis significantly improved BAA-protease in all samplings.

To compare DGGE profiles (Fig. 3-20), Nei & Li's indices were determined and UPGMA was used to create a dendrogram describing pattern similarities (Fig. 3-20a). The cophenetic coefficient calculated between the correlation matrix and the cluster tree was 0.78, indicating that the cluster tree was fit to explicate the difference or similarities between treatments. The dendrogram generated showed that bacterial profiles from non mycorrhizal wheat rhizosphere clustered together with a homology coefficient of about 66% (Fig. 3-21a, red coloured cluster), while bacterial profiles from AM wheat mycorrhizosphere clustered together with a homology coefficient of about 62% (Fig. 3-21a, blue coloured cluster). Bacterial profile by non cultivated non amended pots clustered with profiles from non mycorrhizal rhizosphere, while bacterial profiles from unplanted soil with leaves-amendant clustered with profiles from mycorrhizal rhizospheres. Bacterial profile from unplanted soil amended with maize roots and from the original bacterial inoculum clustered alone.

The structural diversity of the microbial communities was also examined by the Shannon (H′) and Simpson (D_2) diversity indexes (Tab. 3-6). Higher H′ and D_2 were found for bacterial communities of AM than non AM rhizosphere and for bacterial communities of non amended than leaves amended soils. Bacterial communities from root amended soils showed a higher degree of structural similarity to non amended soils than the ones from leaves amended soils. Tab. 3-6 also shows the S_{RC} values obtained from the comparison of soils from all treatments and from the original bacterial inoculum. No significant dissimilarities ($S_{RC} < 0.05$) were found. A similarity greater than that expected by chance ($S_{RC} > 0.95$) was observed in many cases, above all between amended treatments. Raup & Crick probability-based indices of similarity showed that AM treatment determined stronger change in soil bacterial communities when no OM was applied in comparison to those of OM added soils (Tab. 3-6). Fig. 3-20b shows the dendrogram generated by the Raup and Crick cluster analyses. The mantel test Z between matrices based on and DGGE banding pattern (UPGMA)

and enzymatic activities distances was 0.22, meaning little correlation between bacterial community structure and soil enzymatic activity.

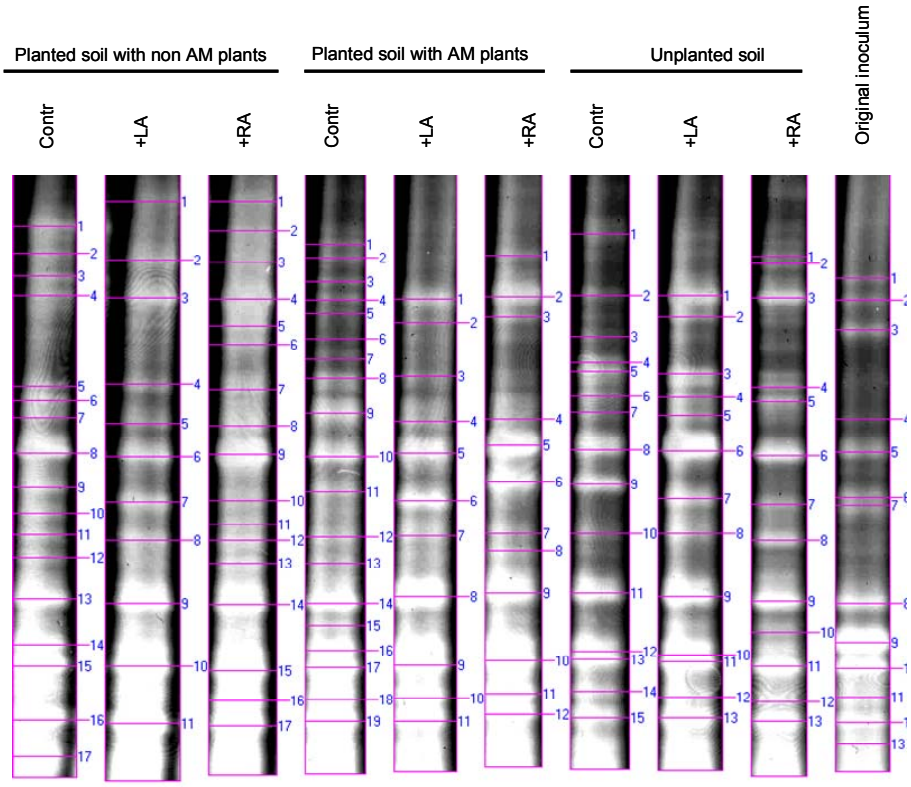


Fig. 3-18 – DGGE profiles from bacterial communities of soils from the applied treatments. Contr, +LA, and +RA for non amended, amended with maize leaves and amended with maize roots, respectively; Original Inoculum for bacterial community from the applied inoculum of microorganisms.

*Tab. 3-5 – Significances of treatments (OM, Myc, OM*Myc for organic matter addition, AM inoculation treatments and interaction, respectively) at 7, 9 and 13 weeks after transplanting on enzymatic activity of soil from planted and unplanted pots. ns, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.*

		Significance at different sampling moments								
		7 weeks after			9 weeks after			13 week after		
		Myc	OM	Myc * OM	Myc	OM	Myc * OM	Myc	OM	Myc * OM
Enzymatic activity in planted pots	df	1	2	2	1	2	2	1	2	2
Deshydrogenase activity	µg INTF g^{-1} h^{-1}	ns	***	***	***	***	***	**	**	ns
Urease activity	µg NH_4^+ g^{-1} h^{-1}	***	***	***	**	ns	ns	***	ns	ns
BAA-protease activity	µg NH_4^+ g^{-1} h^{-1}	***	***	***	***	***	*	***	**	**
Casein hydrolizing activity	µg Tyrosine g^{-1} h^{-1}	***	***	***	ns	ns	ns	***	***	***
Enzymatic activity in unplanted pots										
Deshydrogenase activity	µg INTF g^{-1} h^{-1}	-	***	-	-	***	-	-	***	-
Urease activity	µg NH_4^+ g^{-1} h^{-1}	-	***	-	-	***	-	-	***	-
BAA-protease activity	µg NH_4^+ g^{-1} h^{-1}	-	***	-	-	***	-	-	***	-
Casein hydrolizing activity	µg Tyrosine g^{-1} h^{-1}	-	**	-	-	ns	-	-	ns	-

Fig. 3-19 – (a) Dehydrogenase; (b) Casein hydrolyzing protease; (c) Urease; (d) and BAA- hydrolyzing protease in unplanted soil amended with maize leaves (+LA, dashed green lines with open circles) or maize roots (+RA, dashed red lines with open triangles) or not amended (dashed blue lines with open diamonds) at 7, 9 and 13 weeks. Different unit scales were used in urease and BAA- hydrolyzing protease grafics.

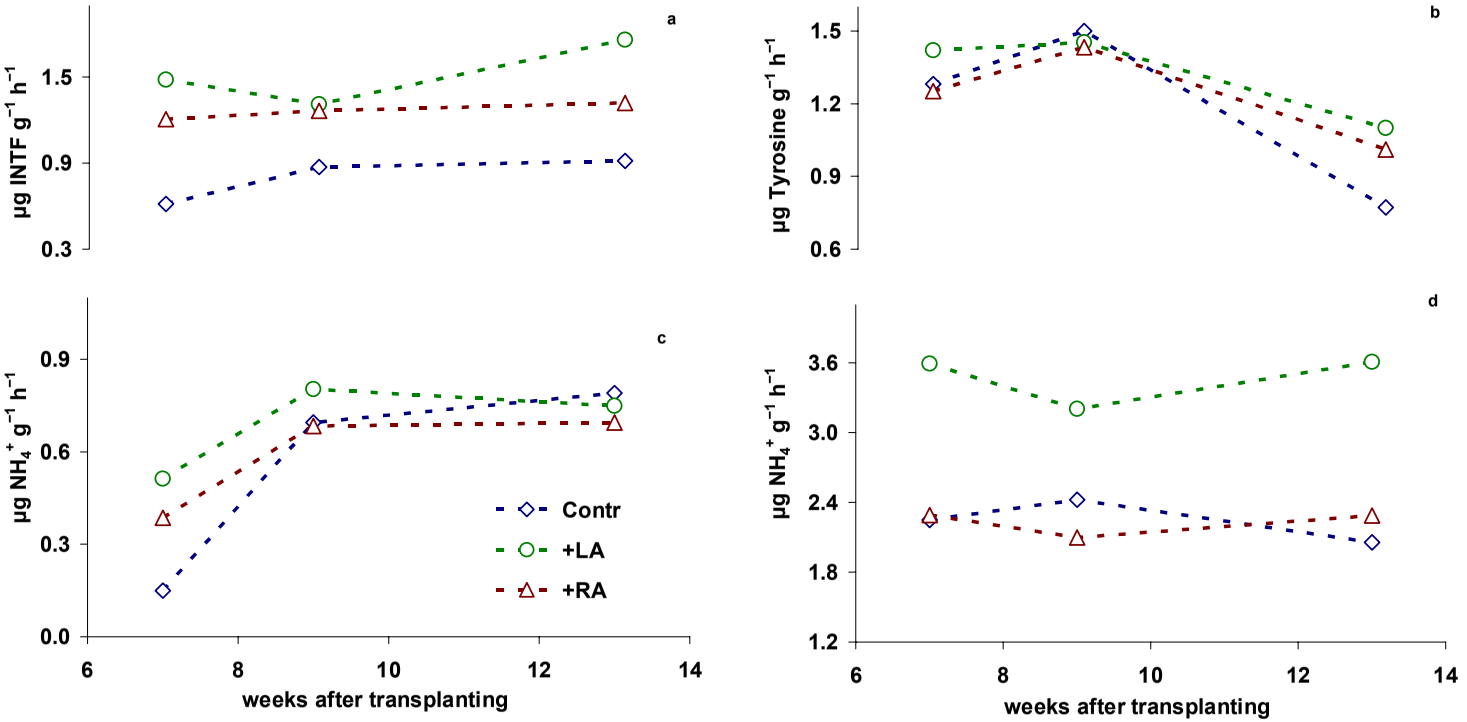
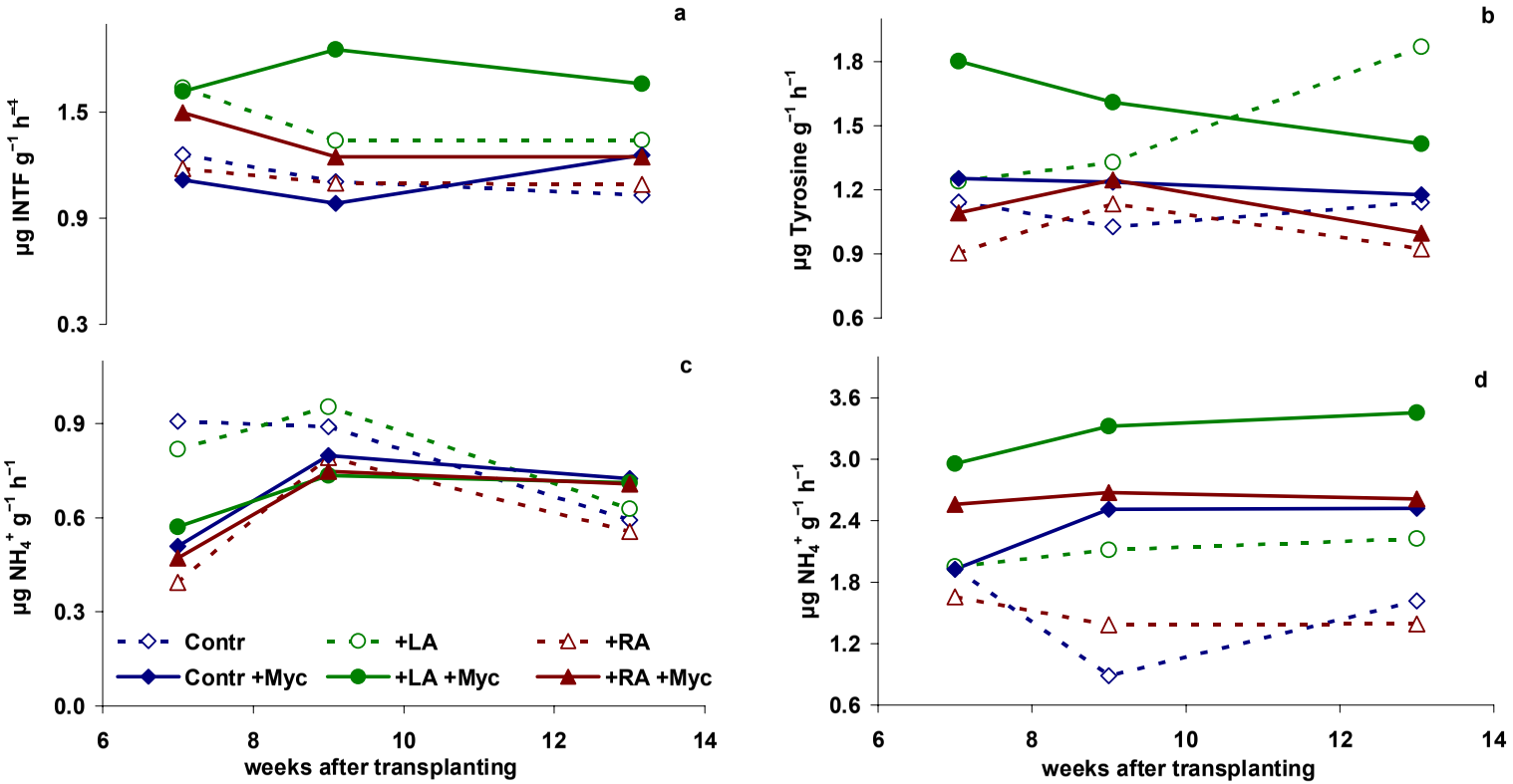


Fig. 3-20 – (a) Dehydrogenase; (b) Casein hydrolyzing protease; (c) Urease; (d) and BAA- hydrolyzing protease in planted soil amended with maize leaves (+LA, green lines with circles) or maize roots (+RA, red lines with triangles) or not amended (blue lines with diamonds) in which wheat plants were grown in symbiosis with the arbuscular mycorrhizal fungus G. mosseae (+Myc, continuous lines with closed symbols) or not (dashed lines with open symbols) at 7, 9 and 13 weeks after transplanting. Different unit scales were used in urease and BAA- hydrolyzing protease grafics.



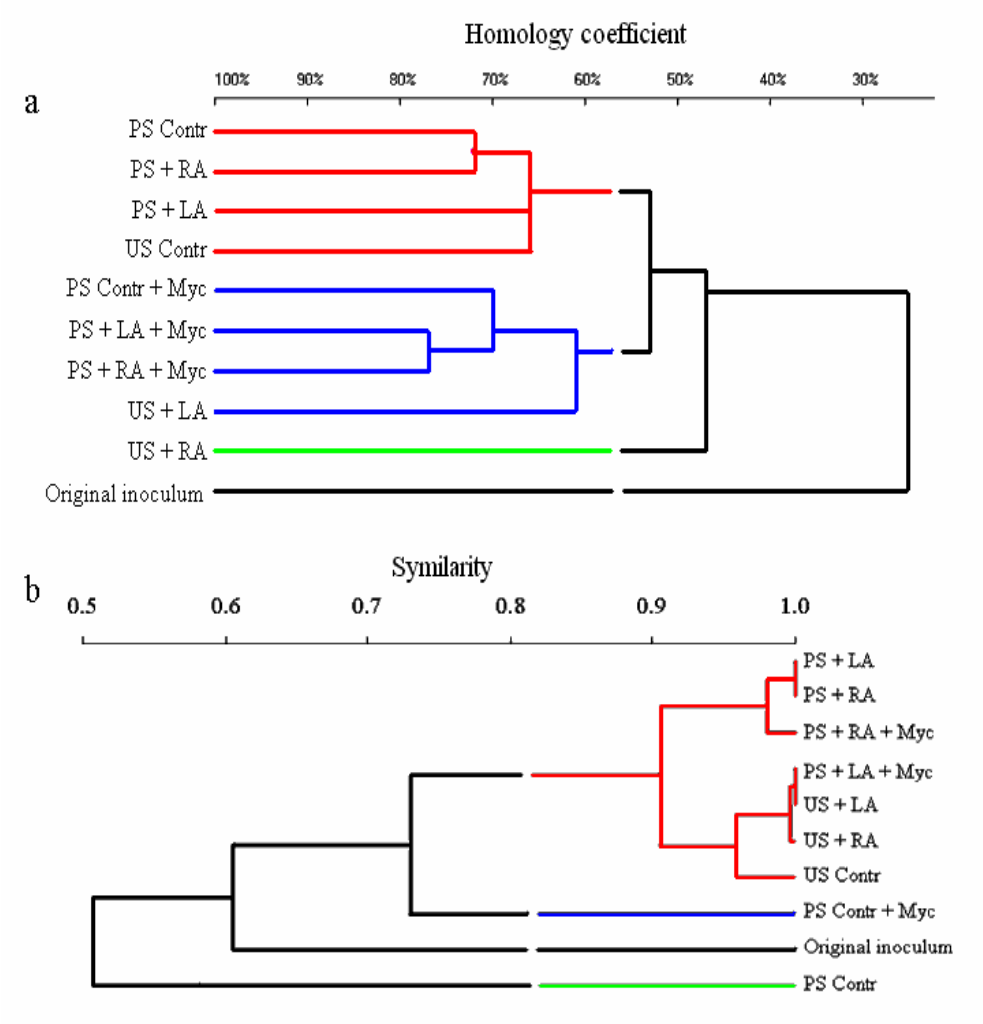


Fig. 3-21 – (a) Nei and Li's similarity coefficients (UPGMA dendrogram, tolerance 1.0%) and (b) Raup and Crick probability-based index of similarity cluster analyses for DGGE profiles from bacterial community structure of soils from the applied treatments. Each cluster with a different colour. PS, and US for planted and unplanted soil, respectively; Contr, +LA, and +RA for non amended, amended with maize leaves and amended with maize roots, respectively; + Myc for soil in which wheat was cultivated in association with AM fungus G. mosseae; Original Inoculum for bacterial community from the applied inoculum of microorganisms.

Tab. 3-6 – Species richness, evenness and abundance, Shannon indices of general diversity and equitability, Simpson index of dominance of the bacterial community of soils from the applied treatments and Raup and Crick probability-based index of similarity between treatments.

		Planted soil with non AM plants			Planted soil with non AM plants			Unplanted soil			Bacterial inoculum
		Control	Leaves amended	Root amended	Control	Leaves amended	Root amended	Control	Leaves amended	Root amended	
Species richness	S	17	11	17	19	11	12	15	13	12	13
Species evenness	$E=n/n_{max}$	0.895	0.579	0.895	1.000	0.579	0.632	0.789	0.684	0.632	0.684
Species abundance	$A=n/N_{tot}$	0.472	0.306	0.472	0.528	0.306	0.333	0.417	0.361	0.333	0.361
Shannon index of general diversity	H′	2.17	1.61	2.21	2.87	1.60	1.99	2.63	2.06	2.13	1.81
Shannon's equitability	$E_H=H'/lnS$	0.77	0.67	0.78	0.97	0.67	0.80	0.97	0.80	0.86	0.71
Simpson index of dominance (reported as reciprocal)	1 / D or D_2	16.20	10.30	16.11	16.69	10.52	11.79	14.34	12.89	11.41	11.30
Raup and Crick's probability-based index of similarity											
Planted soil with non AM plants	Control										
	Leaves amended	0.72									
	Root amended	0.38	**1**								
Planted soil with non AM plants	Control	0.11	0.79	0.58							
	Leaves amended	0.68	**0.99**	**0.97**	0.81						
	Root amended	0.79	**0.97**	**0.99**	**0.97**	**1**					
Unplanted soil	Control	0.61	0.84	0.60	0.85	**0.96**	0.93				
	Leaves amended	0.64	**0.96**	0.87	0.48	**1**	**0.99**	**1**			
	Root amended	0.49	**0.99**	0.80	0.64	**1**	**0.95**	0.92	**1**		
bacterial inoculum		0.15	0.85	0.39	0.17	0.93	0.71	0.30	0.83	0.67	

3.2.2 DISCUSSION

Percentage of root AM colonization of wheat increased until the second sampling (9 week after transplanting) and remained constant until the third one. In spring wheat, Zhu & Smith (2001) observed that AM colonization reached its highest values at the seventh week of growth. On average, root AM infection was similar to that observed by other authors in pot studies (Li *et al.*, 2005 and 2006), but lower than that observed under field condition by Al Karaki *et al.* (2004). Probably, as shown by several authors (Bååth & Hayman, 1984; Koide, 1991; Allsopp & Stock, 1992), in pot the fine root system of wheat is able to completely explore the available soil volume and in such conditions the cost:benefit ratio of AM symbiosis for nutrient uptake is reduced, thereby plants reduce the amount of C fed to the AM fungus and root AM infection decreases. The highest values of ALP and SDH activities were observed at 9 WAT (2nd sampling), in accordance with Zhu *et al.* (2001). ALP is related to the rate of P transfer from AM fungi to plants and can be used as marker for analyzing the AM symbiotic efficiency (Tisserant *et al.*, 1993). Since wheat shows high P requirements during the first 7-9 weeks after emergence (Römer & Schilling 1986) and such requirements are not easy to satisfy by root uptake, it's expectable that during this phase, plants show a certain dependency on AM symbiosis for P absorption (Zhu & Smith, 2001).

Addition of OM exerted a depressive effect on plant growth and N uptake, in accordance with other studies (Seligman *et al.*, 1986; Hodge *et al.*, 1998). Considering that addition of OM increased the soil microbial activity, as data from dehydrogenase activity suggest, and that soil microorganisms compete better than plants for nutrients on a short timescale (Jackson *et al.*, 1989; Kaye & Hart, 1997), it's possible that this depressive effect was due to a higher sequestration of available inorganic N by microorganisms in OM than non amended soil. On the other hand, it has been shown that bacteria decreased N availability for plant uptake when C:N ratio of the added organic residues is higher than 12.5 (Killham, 1994; Hodge *et al.,* 2000a).

On average, plants inoculated with *G. mosseae* (BEG 12) yielded 15,6% more biomass relative to the non-mycorrhizal plants. Other researches have shown that growth response of wheat to AM infection is variable (Al-Karaki & Al-Omoush, 2002; Li *et al.*, 2005 and 2006) in relation to many conditions such as genotypic differences in root morphology, nutrient uptake and symbiotic efficiencies or to different growth conditions, in particular, nutrient availability. Several researches highlighted that AM symbiosis improve plant growth and nutrient uptake especially when plants are grown under nutrient limiting conditions (Azcón & Ocampo, 1981; Vierheilig & Ocampo, 1991; Karagiannidis & Hadjisavva-Zinoviadi, 1998), such as in the present study.

In accordance with other studies (Tobar *et al.*, 1994; Azcón *et al.*, 2001), a higher N uptake was observed in AM than non AM plants. It has been shown that by means of the AM symbiosis, plants can better scavenge the soil volume (Miyasaka & Habte, 2001) so enhancing the possibility to absorb the available N. This effect is particularly important when AM extraradical hyphae explore soil volumes different of those explored by the plant root system (Hodge *et al.*, 2001), such as in field conditions; whereas in pot, the wheat root system *per se* is able to explore the entire soil volume and it implies a reduction of the advantage of AM symbiosis for nutrient uptake. Therefore, as suggested by Hodge *et al.* (2000a), AM symbiosis could have improved N

uptake in host plants by being more effective than the sole roots to compete for inorganic nitrogen with soil microorganisms. An indirect confirmation of this mechanism can be found in the higher proteolytic activity observed in the non AM than AM rhizosphere. Infact, considering that soil proteases are inducible enzymes which are progressively released when organic:inorganic soil N ratio increases (Gill & Modi, 1981; Chrost, 1991), it's conceivable that the higher proteolytic activity in AM than non AM treatment depended by a mycorrhizal depletion of inorganic nitrogen, given that, to my knowledge, there's no evidence regarding the secretion of proteases by AM fungi.

In non AM treatments, at 13 weeks after transplanting, the percentage recovery of ^{15}N from OM ($\%N_{REC}$) was 8.49% and 6.11% when maize leaves or maize roots, respectively, were added to soil. These differences can be due to the different C:N ratio of the added OM (higher in maize roots than leaves). Indeed, the rate of mineralization of the OM strictly depends on the C:N ratio of the substrate being decomposed: the lower its C:N ratio, the higher its mineralization rate. In a 39 days long trial, Hodge *et al.* (1998) observed in different grasses values ranging from 3.2% to 5.0% of the $\%N_{REC}$ from OM when *L. perenne* shoots with a 31:1 C:N ratio were added to soil, whereas in a 49 days long trial, the same authors (Hodge *et al.*, 2000c) found a $\%N_{REC}$ value of 11% when *L. perenne* shoots with a 21:1 C:N ratio were added to soil.

The root infection with AM fungi strongly reduced the $\%N_{REC}$ of the N applied with OM (-50% on average in comparison to non AM control); at the same time, the soil proteolytic activity was higher in AM than non AM treatments, which, in theory, should imply a higher rate of N mineralization and therefore a higher availability of N from organic sources; so it should have improved the $\%N_{REC}$ from OM in AM than non AM treatments. Also Hodge *et al.* (2000b) showed that the AM symbiosis accelerates decomposition of fresh organic matter, without promoting an increase of $\%N_{REC}$ from organic matter. In order to explain the decrese of $\%N_{REC}$ observed in AM than non AM treatments, I hypothesize that microorganisms and AM plants differentially competed for the different sources of N (from soil or OM): AM plants better competed for soil mineral N, whereas bacteria better utilized N from added OM. Other research highlighted that, on a short timescale, microorganisms compete better for N from organic sources (Nadelhoffer *et al.*, 1985; Kaye & Hart, 1997; Hodge *et al.*, 2000a) and so, the reduction of the $\%N_{REC}$ in AM than non AM treatments could be due to a temporal sequestration of N from organic sources by bacteria. An indirect confirmation of this hypothesis came from Hodge (2003), who found, at harvest, a higher percentage of N mineralized from the applied organic matter in soil of AM than non AM treatments.

Addition of OM to soil consisted in a strong change in the enzymatic profile and bacterial community structure of both planted and unplanted soil with a overall increase in both proteolityc and dehydrogenase activity of soil microorganisms. It has been shown, under controlled conditions, that the addition of fresh organic matter to soil trigger into activity many dormant r-strategist microoganisms (De Nobili *et al.*, 2001) and it may exert a priming effect on mineralization activity of stable soil organic matter (Fontaine *et al.*, 2003). In the present experiment, a lower protease activity was observed in soil amended with maize roots than maize leaves. It is likely that these differences somehow depended on the chemical-bromatologic composition of the material being decomposed (Bonmatí *et al.*, 2009).

The addition of organic matter decreased H' and D_2 from the DGGE banding patterns, suggesting a decrease in bacterial diversity. It is possible that the addition of OM increased the competitiveness of the r-strategist bacteria both for preexistent and added nutrients (Andrews &Harris, 1986; Fontaine *et al.*, 2003), with a subsequent decrease of the abundance of the K-strategist bacteria. This decrease was marked so much so the abundance of K-strategist bacteria was under the detection limit of DGGE protocol. A similar result was obtained by Dilly *et al.* (2004).

Plant inoculation with AM fungi determined an increase in H' and D_2 from the DGGE banding patterns, suggesting that bacterial diversity increased in AM treatments than non AM ones. Marschner *et al.* (2001) found a lower bacterial species abundance in rhizosphere of plants inoculated with *G. mosseae* than non mycorrhizal plants, whereas Amora-Lazcano *et al.* (1998) observed an increase in the number of ammonia oxidizers and a decrease in the numbers of ammonifying and denitrifying bacteria due to plant inoculation with AM fungi. Moreover, Mansfeld-Giese *et al.* (2002) observed a selective mycorrhizal influence on bacterial strains. Indeed, AM fungal infection alters root exudation qualitatively and quantitatively (Graham *et al.,* 1981; Dixon *et al.*, 1989; Po & Cumming, 1997; Marschner *et al.*, 1997), which preferentially stimulate ones while depressing other bacterial functional groups, so that it's conceivable that AM influence on bacterial community is mediated by the AM effects on host plant and also by a selective AM influence on rhizosphere populations (Andrade *et al.*, 1998).

The average increase of the enzymatic activity due to the addition of maize leaves to soil was associated to a decreased abundance of the bacterial species, as suggested by the decrease of H' and D_2 indices. On the other side, the higher enzymatic activity (especially BAA- and casein- hydrolyzing activities) due to plant AM inoculation wasn't related to qualitative change in the microbial community. Similar results were achieved by Hodge *et al.* (2001) who found an increase of mineralization activity due to plant AM colonization, but no qualitative changes in microbial community described by phospholipids fatty acid profiling. The latter authors hypothesized that variations in mineralization activity of organic matter were probably a direct result of the presence of the AM fungal hyphae, suggesting that AM fungi could have saprotrophic capabilities, although the mechanisms involved are still unknown.

4 CONCLUSIONS AND FUTURE PROSPECTS

The present research aimed to obtain information about the contribution of the AM symbiosis in alleviating the effect of abiotic stresses to Mediterranean crops and to test if AM symbiosis affects symbiotic N_2 fixation and organic N mineralization activities and finally plant N uptake.

In the field trial, the AM symbiosis improved both plant growth and symbiotic N_2 fixation of berseem grown under drought-stressed, but not under well-watered conditions and it suggests that the AM symbiosis can contribute to crop production in Mediterranean environments where spring rainfall is scarce, which stresses plants and limits crop productivity. Therefore, in such environments, when choosing a management practice (such as tillage, fertilization, etc.) to be applied, it should also be taken into account the aspects concerning the preservation of the natural AM population. Moreover, a valuable agronomical practice could be the inoculation with effective AM fungal strains in order to improve both the natural AM population and the efficiency of the AM symbioses. However, the applicability of this solution needs further studies in order to identify effective combinations of AM strains and plant genotypes and to investigate on the competition between natural and introduced AM fungi in soil.

In the pot trial, the AM symbiosis improved total N uptake in plants and soil N mineralization rates, suggesting that the AM activity is important in improving both plant growth and soil quality. However, AM symbiosis decreased plant N recovery from organic sources, probably due to a different ability between microorganisms and AM plants in utilizing the different sources of N (from soil or OM). The increase of soil enzymatic activity in the AM treatment comparing to non AM counterpart wasn't related to qualitative changes in the microbial community and it suggests a direct effect of the AM fungi on the decomposition rate of OM, possibly through an association with particular functional group of soil microorganisms. This latter aspects need further research because an advancement of knowledge about the role and impact of AM fungi on the decomposition process could surely have practical implication on crop production in Mediterranean environments.

REFERENCES

Abdel-Fatah GM, Mohamedin, AH, 2000. Interactions between a vesicular-arbuscular mycorrhizal fungus (Glomus intraradices) and Streptomyces coelicolor and their effects on sorghum plants grown in soil amended with chitin of brawn scales Biology and Fertility of Soils 32, 401-409

Akiyama K, Matsuzaki K, Hayashi H, 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi ***Nature*** 435, 824–827

Albrecth SL, Bennett JM, Boote KJ, 1994. Relationship of nitrogenase activity to plant water stress in field grown soybeans ***Field crop research*** 8, 61-71

Alexander T, Meier R, Toth R, Weber HC, 1988. Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L and *Avena sativa* L with reference to *Zea mays* L ***New Phytologist*** 110, 363 – 370

Alexander T, Toth R, Meier R, Weber HC, 1989. Dynamics of arbuscule development and degeneration in onion, bean and tomato with reference to vesicular arbuscular mycorrhizae in grasses ***Canadian Journal of Botany*** 67, 2505 – 2513

Alguacil MM, Caravaca F, Roldán A, 2005. Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment ***Biology and Fertility of Soils*** 41, 59–68

Aliasgharzad N, Neyshabouri MR, Salimi G, 2006. Effects of arbuscular mycorrhizal fungi and Bradyrhizobium japonicum on drought stress of soybean ***Biologia*** 61, 324-328

Al-Karaki G, Al-Omoush M, 2002. Wheat response to phosphogypsum and mycorrhizal fungi in alkaline soil ***Journal of Plant Nutrition***, 25, 873–883

Al-Karaki G, McMichael B and Zak J, 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress ***Mycorrhiza*** 14,263-269

Allen MF, 1996. The ecology of arbuscular mycorrhizas: A look back into the 20th century and a peek into the 21st ***Mycological Research*** 100, 769-782

Allen SC, Jose S, Nair PKR, Brecke BJ, Ramsey CL, 2004. Competition for ^{15}N-labeled fertilizer in a pecan (*Carya illinoensis* K Koch)-cotton (*Gossypium hirsutum* L) alley cropping system in the southern United States ***Plant and Soil*** 263, 151−164

Allsopp N, 1998. Effect of defoliation on arbuscular mycorrhizas of three perennial pasture and rangeland species ***Plant and Soil*** 202, 117–124

Allsopp N, Stock WD, 1992. Density dependent interactions between VA mycorrhizal fungi and even-aged seedlings of two perennial *Fabaceae* species ***Oecologia*** 91, 281-287

Almeida JP, Hartwig UA, Frehner M, Nösberger J, Lüscher A, 2000. Evidence that P deficiency induces N feedback regulation of symbiotic N_2 fixation in white clover (*Trifolium repens* L) ***Journal of Experimental Botany*** 51, 1289-97

Altieri MA, 1994. Sustainable Agriculture ***Encyclopaedia of Agriculture Science*** 4, 239-247

Ames RN, Bethlenfalvay GJ, 1987. Mycorrhizal fungi and the integration of plant and soil nutrient dynamics ***Journal of Plant Nutrition*** 10, 1313-1321

Ames RN, CPP Reid, LK Porter, C Cambardella, 1983. Hyphal uptake and transport of nitrogen from two15 N-labelled sources by *Glomus mosseae*, a vesicular arbuscular mycorrhizal fungus ***New Phytologist*** 95, 381–396

Amora-Lazcano E, Vazquez MM, Azcón R, 1998. Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi ***Biology and Fertility of Soils*** 27, 65-70

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ, 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi ***Plant and Soil*** 192, 71–79

Andrade G, Milhara KL, Linderman RG, Bethlenfalvay GJ, 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere ***Plant and Soil*** 202, 89-96

Andrews JH, Harris RF, 1986. r- and K-selection in microbial ecology ***Adv Microb Ecol*** 9, 99–147

Antunes PM, D Deaville, MJ Goss, 2006a. Effect of two AMF life strategies on the tripartite symbiosis with Bradyrhizobium japonicum and soybean ***Mycorrhiza*** 16, 167–173

Antunes PM, de Varennes A, Rajcan I, Goss MJ, 2006b. Accumulation of specific flavonoids in soybean (*Glicine max* L Merr.) as a function of the early tripartite symbiosis with arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* (Kirchner) Jordan ***Soil biology & biochemistry*** 38, 1234-1242

Artursson V, Finlay RD, Jansson JK, 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth ***Environmental Microbiology*** 8, 1–10

Artursson, V, Finlay, RD, Jansson, JK, 2005. Combined bromodeoxyuridine immunocapture and terminal restriction fragment length polymorphism analysis highlights differences in the active soil bacterial

metagenome due to *Glomus mosseae* inoculation or plant species ***Environmental Microbiology*** 7, 1952–1966

Artursson, V, Jansson, JK, 2003. Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae ***Applied and Environmental Microbiology*** 69, 6208–6215

Asimi, S, Gianinazzi-Pearson, V, Gianinazzi, S, 1980. Influence of increasing soil-phosphorus levels on interactions between vesicular arbuscular mycorrhizae and *Rhizobium* in soybeans ***Canadian Journal of Botany***, 58, 2200-2205

Atkinson, S, Berta, G, Hooker, J E, 1994. Impact of mycorrhizal colonisation on root architecture, root longevity and the formation of growth regulators In S Gianinazzi and H Schüepp (Eds), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems (pp 47-60) Basel, Switzerland: Birkhäuser Verlag

Augé RM, 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis ***Mycorrhiza*** 11:3–42

Augé RM, Duan X, Ebel RC, Stodola AJ, 1994. Nonhydraulic signaling of soil drying in mycorrhizal maize ***Planta*** 193, 74–82

Augé RM, Sylvia DM, Park S, Buttery BR, Saxton AM, Moore JM, Cho K, 2004. Partitioning mycorrhizal influence on water relations of *Phaseolus vulgaris* into soil and plant components ***Canadian Journal of Botany*** 82, 503-514

Avice JC, G Lemaire, A Ourry, J Boucaud, 1997. Effects of the previous shoot removal frequency on subsequent shoot regrowth in two *Medicago sativa* L. cultivars ***Plant and Soil*** 188, 189−198

Azcón R, 1987. Germination and hyphal growth of *Glomus mosseae* in vitro: effects of rhizosphere bacteria and cellfree culture media ***Soil Biology and Biochemistry*** 19, 417–419

Azcón R, El-Atrash F, Barea JM, 1988. Influence of mycorrhiza versus soluble phosphate on growth, nodulation, and N_2 fixation (^{15}N) in alfalfa under different levels of water potential ***Biology and Fertility of Soils*** 7, 28-31

Azcón R, El-Atrash, 1997. Influence of arbuscular mycorrhizae and phosphorus fertilization on growth, nodulation and N_2 fixation (^{15}N) in *Medicago sativa* at four salinity levels ***Biology and Fertility of Soils,*** 24:81-86

Azcón R, Ocampo JA, 1981. Factors Affecting The Vesicular arbuscular Infection And Mycorrhizal Dependency Of Thirteen Wheat Cultivars ***New Phytologist*** 87, 677-685

Azcón R, Rodríguez R, Amora-Lazcano E, Ambrosano E, 2008. Uptake and metabolism of nitrate in mycorrhizal plants as affected by water availability and N concentration in soil ***European Journal of Soil Science*** 59, 131 - 138

Azcón R, Rubio, R, Barea, J M, 1991. Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, N_2 fixation (^{15}N) and nutrition of Medicago sativa L ***New Phytologist***, 117, 399-404

Azcón R, Ruiz-Lozano JM, Rodríguez R, 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil ***Canadian Journal of Botany*** 79, 1175–1180

Azcón-Aguilar C, Barea JM, 1992. Interactions between mycorrhizal fungi and other rhizosphere microorganisms In M J Allen (Ed), Mycorrhizal Functioning An Integrative Plant-Fungal Process Routledge (pp 163-198) New York, NY: Chapman and Hall Inc

Bååth E, Hayman DS, 1984. Effect of soil volume and plant density on mycorrhizal infection and growth response ***Plant and Soil*** 77, 373-376

Bago B, 2000. Putative sites for nutrient uptake in arbuscular mycorrhizal fungi ***Plant and Soil*** 226:263-274

Bago B, Azcón-Aguilar C, 1997. Changes in the rhizosphere pH induced by arbuscular mycorrhiza formation in onion (*Allium cepa* L) ***Zt Pflanzenern Bodenkde*** 160, 333-339

Bago B, Pfeffer PE, Shachar-Hill Y, 2000. Carbon Metabolism and Transport in Arbuscular Mycorrhizas ***Plant Physiology***, 124, 949–957

Baon JB, Smith SE, Alston AM, Wheeler RD, 1992. Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection ***Australian Journal of Agricultural Research*** 43, 479–491.

Barea JM, Azcón R, 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants ***Advances in Agronomy*** 36, 1-54

Barea JM, Azcón R, Azcón-Aguilar C, 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality ***Anton Leeuw Int J G*** 81:343 – 351

Barea JM, Azcón-Aguilar C, Azcón R 1987. Vesicular–arbuscular mycorrhiza improve both symbiotic N_2-fixation and N uptake from soil as assessed with a ^{15}N technique under field conditions ***New Phytologist*** 106, 717–721

Barea JM, Werner D, Azcón-Aguilar C, Azcón R 2005. Interactions of arbuscular mycorrhiza and nitrogen fixing simbiosis in sustainable agriculture In: Werner D, Newton WE (eds) Agriculture, forestry, ecology and the environment The Netherlands: Kluwer Academic Publishers

Bartels D, 2001. Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance ***Trends in Plant Science*** 6:284–286

Bayne HG, MS Brown, GJ Bethlenfalvay, 1984. Defoliation effects on mycorrhizal colonization, nitrogen fixation and photosynthesis in the *Glicine-Glomus-Rhizobium* symbiosis ***Physiol Plantarum*** 62, 576-580

Behl RK, H Sharma, V Kumar, N Narula, 2003. Interactions amongst Mycorrhiza, *Azotobacter chroococcum* and Root Characteristics of Wheat Varieties ***Journal of Agronomy & Crop Science*** 189, 151—155

Benhamou N, JA Fortin, C Hamel, M St.Arnaud, A Shatilla, 1994. Resistance response of mycorrhizal Ri T-DNA transformed carrot roots to infection by *Fusarium oxysporum* f.sp. *chrysanthemi* ***Phytopathology*** 84,958-968

Berta G, Fusconi A, Hooker J E, 2002. Arbuscular mycorrhizal modifications to plant root systems: scale, mechanisms and consequences In S Gianinazzi, H Schüepp, J M Barea and K Haselwandter (Eds), Mycorrhiza Technology in Agriculture, from Genes to Bioproducts (pp 71-85) Basel, Switzerland, Birkhäuser Verlag

Berta G, Fusconi A, Trotta A, Scannerini S, 1990a. Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L ***New Phytologist*** 114, 207-215

Berta G, Sgorbati S, Soler V, Fusconi A, Trotta A, Citterio A, Bottone MG, Sparvoli E, Scannerini S, 1990b. Variations in chromatin structure in host nuclei of a vesicular-arbuscular mycorrhiza ***New Phytologist*** 14, 199-205

Bethlenfalvay GJ, Linderman RG, 1992. Mycorrhizae in Sustainable Agriculture (Special Publication No 54) Agronomy Society of America, Madison, Wisconsin

Bever JD, Morton J, 1999. Heritable variation and mechanisms of inheritance of spore shape within a population of *Scutellospora pellucida*, an arbuscular mycorrhizal fungus ***American Journal of Botany*** 86, 1209–1216

Bever JD, Morton JB, Antonovics J & Schultz PA, 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland ***Journal of Ecology*** 84, 71-82

Bever, JD, 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth ***Plant and Soil*** 244:281–290

Bianciotto V, Bonfante P, 1993. Evidence of DNA replication in an arbuscular mycorrhizal fungus in the absence of a host plant ***Protoplasma***, 176, 100-105

Bianciotto V, Bonfante P, 2002. Arbuscular mycorrhizal fungi: a specialised niche for rhizospheric and endocellular bacteria ***Antonie van Leeuwenhoek*** 81, 365–371

Bianciotto V, Lumini E, Bonfante P, Vandamme P, 2003. *'Candidatus Glomeribacter gigasporarum'* gen nov, sp nov, an endosymbiont of arbuscular mycorrhizal fungi ***International Journal of Systematic and Evolutionary Microbiology*** 53, 121–124

Bianciotto V, Minerdi D, Perotto S, Bonfante P, 1996a Cellular interactions between arbuscular mycorrhizal fungi and rhizosphere bacteria ***Protoplasma*** 193, 123–131

Bianciotto, V, Andreotti, S, Balestrini, R, Bonfante, P, Perotto, S, 2001. Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscular mycorrhizal structures ***European Journal of Histochemistry***, 45, 39-49

Bianciotto, V, Bandi, C, Minerdi, D, Sironi, M, Tichy, HV, and Bonfante, P, 1996b An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria ***Applied and Environmental Microbiology*** 62, 3005–3010

Bianciotto, V, Genre, A, Jargeat, P, Lumini, E, Becard, G, Bonfante, P, 2004. Vertical transmission of endobacteria in the arbuscular mycorrhizal fungus *Gigaspora margarita* through generation of vegetative spores ***Applied and Environmental Microbiology*** 70, 3600–3608

Black RLB, Tinker PB, 1977. Interaction between effects of vesicular-arbuscular mycorrhizas and fertiliser phosphorus on the yields of potatoes in the field ***Nature***, 267, 510-511

Blee K, Anderson A, 1998. Regulation of arbuscule formation by carbon in the plant ***The Plant Journal*** 16, 523–530

Bolan NS, 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants ***Plant and Soil***, 134, 189-207

Bonanomi A, Wiemken A, Boller T, Salzer P, 2001. Local induction of a mycorrhiza-specific class III chitinase gene in cortical root cells of *Medicago truncatula* containing developing or mature arbuscules ***Plant Biology*** 3, 194–199

Bonfante P, Genre A, Faccio A, Martini I, Schauser L, Stougaard J, Webb J, Parniske M, 2000. The *Lotus japonicus* LjSym4 gene is required for the successful symbiotic infection of root epidermal cells ***Molecular Plant-Microbe Interactions*** 13:1109-1120

Bonfante P, Perotto S 1992. Plants and endomycorrhizal fungi: the cellular and molecular basis of their interaction In: Verma DPS, (ed) Molecular Signals in Plant-Microbe Communications Boca Raton: CRC Press, 445470

Bonfante P, Perotto S 1995. Strategies of Arbuscular Mycorrhizal Fungi when Infecting Host Plants ***New Phytologist***, 130, 1, 3-21

Bonmatí M, B Ceccanti, P Nannipieri, J Valero, 2009. Characterization of humus–protease complexes extracted from soil ***Soil Biology and Biochemistry*** 41, 1199–1209

Bowen GD, Rovira AD, 1999. The rhizosphere and its management to improve plant growth ***Advances in Agronomy***, 66 1-102

Breuninger M, Trujillo CG, Serrano E, Fischer R, Requena, N, 2004. Different nitrogen sources modulate activity but not expression of glutamine synthetase in arbuscular mycorrhizal fungi ***Fungal Genetics and Biology*** 41, 542-552

Brundrett MC, 2002. Coevolution of roots and mycorrhizas of land plants ***New Phytologist*** 54, 275-304

Brundrett MC, Abbott LK, Jaspe, DA, 1999. Glomalean mycorrhizal fungi from tropical Australia I: Comparison of the effectiveness and specificity of different isolation procedures ***Mycorrhiza*** 8:305–314

Bucking H, Shachar-Hill Y, 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability ***New Phytologist*** 165:899 – 912

Burleigh SH, Harrison MJ, 1999. The Down-Regulation of Mt4-Like Genes by Phosphate Fertilization Occurs Systemically and Involves Phosphate Translocation to the Shoots ***Plant Physiology*** 119, 241-248

Butlin R, 2002. The costs and benefits of sex: new insights from old asexual lineages ***Nature Reviews Genetics*** 3, 311-317

Cappellazzo G, L Lanfranco, M Fitz, D Wipf, P Bonfante, 2008. Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae* ***Plant Physiology*** 147, 429–437

Cappellazzo G, L Lanfranco, P Bonfante, 2007. A limiting source of organic nitrogen induces specific transcriptional responses in the extraradical structures of the endomycorrhizal fungus *Glomus intraradices* ***Current Genetics*** 51, 59–70

Carvalho SM, Caçador I, Martins-Loução A, 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal) ***Mycorrhiza*** 11:303-309

Cavagnaro TR , FA Smith, SM Ayling, SE Smith, 2003. Growth and Phosphorus Nutrition of a Paris-Type Arbuscular Mycorrhizal Symbiosis ***New Phytologist***, 157, 127-134

Chabot S, Bel-Rhlid R, Chênevert R, Piché Y, 1992. Hyphal growth promotion in vitro of the VA mycorrhizal fungus, Gigaspora margarita Becker & Hall, by the activity of structurally specific flavonoid compounds under CO_2-enriched conditions ***New Phytologist*** 122:461-467

Chalk PM, Souza R de F, Urquiaga S, Alves BJR, Boddey RM, 2006. The role of arbuscular mycorrhiza in legume symbiotic performance ***Soil Biology and Biochemistry*** 38, 2944–2951

Chalot M, Blaudez D, Brun A, 2006. Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface ***Trends in Plant Science*** 11, 263–266

Chen B, P Roos, OK Borggaard, YG Zhu, I Jakobsen, 2005. Mycorrhiza and Root Hairs in Barley Enhance Acquisition of Phosphorus and Uranium from Phosphate Rock but Mycorrhiza Decreases Root to Shoot Uranium Transfer ***New Phytologist*** 165, 591-598

Christensen H, Jakobsen I, 1993. Reduction of bacterial growth by a vesicular-arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L) ***Biology and Fertility of Soils*** 15, 253–258

Chrost RJ (ed), 1991. Microbial enzymes in aquatic environments Springer-Verlag, New York

Clark RB, Zeto SK, 2000. Mineral acquisition by arbuscular mycorrhizal plants ***Journal of Plant Nutrition*** 23, 867–902

Cliquet JB, PJ Murray, J Boucaud, 1997. Effect of the Arbuscular Mycorrhizal Fungus *Glomus fasciculatum* on the Uptake of Amino Nitrogen by *Lolium perenne* ***New Phytologist***, 137, 345-349

Corkidi L, Rincón E, 1997. Arbuscular mycorrhizae in a tropical sand dune ecosystem on the Gulf of Mexico I: Mycorrhizal status and inoculum potential along a successional gradient ***Mycorrhiza*** 7:9–15

Crush JR, 1995. Effect of VA mycorrhizas on phosphorus uptake and growth of white clover (*Trifolium repens* L) growing in association with ryegrass (*Lolium perenne* L) ***New Zealand Journal of Agricultural Research***, Vol 38, 303-307

Cui M, MM Caldwell, 1996. Facilitation of plant phosphate acquisition by arbuscular mycorrhizas from enriched soil patches 2 Hyphae exploiting rootfree soil ***New Phytologist*** 133:461-467

Cuttle S, Shepherd M, Goodlass G, 2003. A review of leguminous fertility-building crops, with particular reference to nitrogen fixation and utilization ***Defra Project*** OF0316

Dalpé Y, Diop TA, Plenchette C, Gueye M, 2000. Glomales species associated with surface and deep rhizosphere of Faidherbia albida in Senegal ***Mycorrhiza*** 10:125–129

De Nobili M, Contin M, Mondini C, Brookes PC, 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate ***Soil Biology & Biochemistry*** 33, 1163–1170

Declerck S, JM Risede, G Rufyikiri, B Delvaux, 2002. Effects of arbuscular mycorrhizal fungi on severity of root rot of bananas caused by *Cylindrocladium spathiphylli* ***Plant Pathology*** 51:109-115

Demchenko K, Winzer T, Stougaard J, Parniske M, Pawlowska K, 2004. Distinct roles of *Lotus japonicus* SYMRK and SYM15 in root colonization and arbuscule formation ***New Phytologist*** 163: 381–392

Dilly O, Bloem J, Vos A, Munch JC, 2004. Bacterial diversity in agricultural soils during litter decomposition ***Applied Environmental Microbiology*** 70, 468–474.

Dixon RK, Garrett HE, Cox GS, 1989. Boron fertilization, vesicular-arbuscular mycorrhizal colonization and growth of *Citrus jambhiri Lush* ***Journal of Plant Nutrition****it* 12, 687-700

Djordjevic MA, Mathesius U, Arioli T, Weinman JJ, Gaertner E, 1997. Chalcone synthase gene expression in transgenic subterranean clover correlates with localised accumulation of flavonoids ***Australian Journal of Plant Physiology*** 24:119–132

Dodd JC, CL Boddington, A Rodriguez, C Gonzalez-Chavez, I Mansur, 2000. Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection ***Plant and Soil*** 226:131-151

Dodd JC, Jeffries P, 1989. Effect of fungicides on three vesicular–arbuscular mycorrhizal fungi associated with winter wheat (*Triticum aestivum* L) ***Biology and Fertility of Soils***; 7:120-128

Drevon JJ, Hartwig UA, 1997. Phosphorus deficiency increases the argon-induced decline of nodule nitrogenase activity in soybean and alfalfa ***Planta*** 201:463 – 469

Elias KS, Safir GR, 1987. Hyphal elongation of *Glomus fasciculatus* in response to root exudates ***Applied and Environmental Microbiology*** 53:1928–1933

Emberger L, 1955. Une classification biogéographique des climats. Recueil des Travaux du Laboratoire de Botanique, Géologie et Zoologie de la Faculté des Sciences de l'Université de Montpellier, Série Botanique 7, 3–43

Eom AH, GWT Wilson, DC Hartnett, 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie ***Mycologia*** 93, 233–242

Fester T, Strack D, Hause B, 2001. Reorganization of tobacco root plastids during arbuscule development ***Planta*** 213:864–868

Fitter AH, 1977. Influence of Mycorrhizal Infection on Competition for Phosphorus and Potassium by Two Grasses ***New Phytologist***,79, 119-125

Fitter AH, 1985. Functioning of vesicular–arbuscular mycorrhizas under field conditions ***New Phytologist*** 99, 257–265

Fitter AH, 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas?A null hypothesis for symbiotic function ***New Phytologist*** 172, 3–6

Fitter AH, Garbaye J, 1994. Interactions between mycorrhizal fungi and other soil organisms ***Plant and Soil*** 159:123-132

Fitter AH, Moyersoen B, 1996. Evolutionary trends in root-microbe symbioses ***Philos Trans R Soc B*** 351:1367–1375

Fontaine S, Mariotti A, Abbadie L, 2003. The priming effect of organic matter: a question of microbial competition? ***Soil Biology & Biochemistry*** 35, 837–843

Frank B, 1885. Über die auf Wurselsymbiosen berunhende ErnShrung gewisser BSume durch unterirdisce *Pilze Ber Dtsch Bot Ges*

Frey B, Schüepp H, 1992. Transfer of symbiotically fixed nitrogen from berseem (*Trifolium alexandrium* L) to maize via vesicular arbuscular mycorrhizal hyphae ***New Phytologist*** 122, 447–454

Frey B, Schuepp H, 1993. A role of vesicular-arbuscular (VA) mycorrhizal fungi in facilitating interplant nitrogen transfer ***Soil Biology and Biochemistry***, 25, 651-658

Gandolfi A, Sanders I, Rossi V, Menozzi P, 2003. Evidence of recombination in putative ancient asexuals, ***Mol Biol Evol*** 20, 754–761

Ganry F, 1982. Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans ***Plant and Soil*** 68, 321-329

Ganry F, HG Diem, J Wey, YR Dommergues, 1985. Inoculation with *Glomus mosseae* improves N_2 fixation by field-grown soybeans ***Biology and Fertility of Soils*** 1,15-23

García C, T Hernandez, F Costa, 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils, ***Communications in Soil Science and Plant Analysis*** 28, 123–134

García-Garrido JM, Cabello MN, García-Romera I, Ocampo JA, 1992b. Endoglucanase activity in lettuce plants colonized with the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* ***Soil Biology and Biochemistry*** 24:955 – 959

García-Garrido JM, García-Romera I, Ocampo JA, 1992a. Cellulase production by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol & Gerd) Gerd and Trappe ***New Phytologist*** 121:221 – 226

García-Garrido JM, García-Romera I, Parra-García MD, Ocampo JA, 1996. Purification of an arbuscular mycorrhizal endoglucanase from onion roots colonized by *Glomus mosseae* ***Soil Biology and Biochemistry*** 28:1443 – 1449

García-Garrido JM, Ocampo JA, 2002. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis ***Journal of Experimental Botany***, 373, 1377-1386

García-Romera I, García-Garrido JM, Martinez-Molina E, Ocampo JA, 1991. Production of pectolytic enzymes in lettuce root colonized by *Glomus mosseae* ***Soil Biology and Biochemistry*** 23:597 – 601

Garg N, Geetanjali, Kaur A, 2006. Arbuscular mycorrhiza: Nutritional aspects ***Archives of Agronomy and Soil Science***; 52, 593 – 606

Gaur A, Adholeya, A, 2002. Arbuscular–mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter ***Biology and Fertility of Soils*** 35:214-218

Gazey C, Abbott LK, Robson AD, 2004. Indigenous and introduced arbuscular mycorrhizal fungi contribute to plant growth in two agricultural soils from south-western Australia ***Mycorrhiza*** 14, 355-362

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG, 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection ***Plant Cell*** 17, 3489–3499

George E, 2000. Nutrient uptake p 307-343 In Y Kapulnik and D D Douds Jr (eds) Arbuscular Mycorrhizas: Physiology and Function Kluwer Academic, Dordrecht

Giambalvo D, Stringi L, Scarpello C, Attardo C, Di Miceli G, Amato G, 2005. Effect of defoliation management and plant arrangement on yield and N2 fixation of berseem-annual ryegrass mixture Proceedings of ***EGF 2005 Integrating Efficient Grassland Farming and Biodiversity*** 29-31 August: Tartu (Estonia) pp. 541-545.

Gianinazzi S, Schüepp H, 1994. Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems Basel, Switzerland: ALS Birkhäuser Verlag

Gianinazzi S, Schüepp H, Barea JM, Haselwandter K, 2002. Mycorrhizal Technology in Agriculture, from Genes to Bioproducts Basel, Switzerland: Birkhäuser Verlag

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S, 1989. In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids ***Symbiosis*** 7:243–256

Gianinazzi-Pearson V, Gollotte A, Tisserant B, Franken P, Dumas-Gaudot E, Lemoine MC, Van Tuinen D, Gianinazzi S, Lherminier J, 1995. Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations ***Canadian Journal of Botany*** 73, 526–532

Gill PP, Modi VV, 1981. Induction of extracellular proteases by egg-white in *Aspergillus nidulans* ***Folia Microbiologica*** 2, 78-82

Giovannetti M, Fortuna P, Citernesi AS, Morini S, Nuti MP, 2001. The Occurrence of Anastomosis Formation and Nuclear Exchange in Intact Arbuscular Mycorrhizal Networks ***New Phytologist***, 151,3717-724

Giovannetti M, Mosse B, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection roots ***New Phytologist*** 84, 489-500

Giovannetti M, Sbrana C, Avio L, Citernesi AS, Logi C, 1993. Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages ***New Phytologist*** 125:587-593

Glassop D, Smith SE, Smith FW, 2005. Cereal phosphate transporters associated with the mycorrhizal pathway of phosphate uptake into roots ***Planta*** 222, 688–698

Goicoechea N, Antolín MC, Sánchez-Díaz M, 1997. Influence of arbuscular mycorrhizae and *Rhizobium* on nutrient content and water relations in drought-stressed alfalfa ***Plant and Soil*** 192, 261–268.

Goicoechea N, Szalai G, Antolín MC, Sánchez-Díaz M, Paldi E, 1998. Influence of arbuscular mycorrhizae and *Rhizobium* on free polyamines and proline levels in water-stressed alfalfa ***Journal of Plant Physiology*** 153, 706–711

Gollotte A, Brechenmacher L, Weidmann S, Franken P, Gianinazzi-Pearson V, 2002. Plant genes involved in arbuscular mycorrhiza formation and functioning In S Gianinazzi, H Schüepp, J M Barea and K Haselwandter (Eds), Mycorrhiza Technology in Agriculture, from Genes to Bioproducts (pp 87-102) Basel, Switzerland: Birkhäuser Verlag

Goss MJ, de Varennes A, 2002. Soil disturbance reduces the efficacy of mycorrhizal associations for early soybean growth and N_2 fixation ***Soil Biology and Biochemistry*** 34, 1167-1173

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y, 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis ***Nature*** 435, 819–823

Graham JH, Abbott LK 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi ***Plant and Soil*** 220, 207–218.

Graham JH, Leonard RT, Menge JA, 1981. Membrane-mediated decrease of root-exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation ***Plant Physiology*** 68, 548-552

Guadarrama P, Álvarez-Sánchez FJ, 1999. Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico ***Mycorrhiza*** 8:267-270

Hamel C, U Barrantes-Cartín, V Furlan, DL Smith, 1991. Endomycorrhizal fungi in nitrogen transfer from soybean to maize ***Plant and Soil*** 138, 33-40

Hammer Ø, Harper DAT, Ryan PD, 2001. PAST: paleontological statistics software package for education and data analysis Palaeontologia Electronica 4(1) (9pp) <http://palaeo-electronicaorg/2001_1/past/issue1_01htm>

Hammond JP, Broadley MR, White PJ 2004. Genetic responses to phosphorus deficiency ***Annals of Botany*** 94:323 – 332

Harley JL, Smith SE, 1983. Mycorrhizal Symbiosis Academic Press Inc London and New York

Harris D, Pacovsky RS, Paul EA, 1985. Carbon economy of soybean-*Rhizobium*-*Glomus* associations ***New Phytologist***, 101, 427-440

Harrison MJ 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis ***Annual Review in Plant Physiology and Molecular Biology*** 50:361 – 389

Harrison MJ, Dewbre GR, Liu J, 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi ***Plant Cell*** 14, 2413–2429

Harrison MJ, Dixon RA, 1993. Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula* ***Molecular Plant-Microbe Interactactions*** 6:643–654

Harrison MJ, Dixon RA, 1994. Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme* ***The Plant Journal*** 6:9–20

Harrison MJ, van Buuren ML, 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme* ***Nature*** 378, 626-629

Hause B, Fester T, 2005. Molecular and cell biology of arbuscular mycorrhizal symbiosis ***Planta*** (2005) 221, 184–196

Hawkins HJ, George E, 1999. Effect of plant nitrogen status on the contribution of arbuscular mycorrhizal hyphae to plant nitrogen uptake ***Physiologia Plantarum*** 105, 694–700

Hawkins HJ, Johansen A, George E, 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi ***Plant and Soil*** 226, 275–285

Hayman DS, 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi ***Phytopathology*** 72, 1119-1125

Hayman DS, Mosse B, 1979 Improved growth of white clover in hill grasslands by mycorrhizal inoculation ***Annals of Applied Biology***, 93, 141-148

He X, Critchley C, Bledsoe C, 2003. Nitrogen Transfer Within and Between Plants Through Common Mycorrhizal Networks (CMNs) ***Critical Reviews in Plant Sciences***, 22, 531–567

Hoagland DR, Arnon DI, 1950 Water-culture method for growing plants without soil ***Calif. Agric. Exp. Stn***. Circ. 347.

Hodge A, 2001. Arbuscular mycorrhizal fungi influence decomposition of, but not plant nutrient capture from, glycine patches in soil ***New Phytologist*** 151, 725–734

Hodge A, 2003. Plant Nitrogen Capture from Organic Matter as Affected by Spatial Dispersion, Interspecific Competition and Mycorrhizal Colonization ***New Phytologist*** 157, 303-314

Hodge A, Campbell CD, Fitter AH, 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material ***Nature*** 413, 297-299

Hodge A, Robinson D, Fitter AH, 2000a. Are microorganisms more effective than plants at competing for nitrogen? ***Trends in Plant Science*** 5, 304–308

Hodge A, Robinson D, Fitter AH, 2000b. An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil ***New Phytologist*** 145, 575–584

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH, 1998. Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil ***New Phytologist*** 139, 479-494

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH, 2000c. Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity ***Journal of Ecology*** 88, 150-164

Hoeksema JD, VB Chaudhary, CA Gehring, N Collins Johnson, J Karst, RT Koide, A Pringle, C Zabinski, JD Bever, JC Moore, GW T. Wilson, JN Klironomos, J Umbanhowar, 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi ***Ecology Letters*** 13, 394 – 407

Høgh-Jensen H, 2006. The nitrogen transfer between plants: an important but difficult flux to quantify ***Plant and Soil*** 282, 1-5

Høgh-Jensen H, Schjørring JK, 1994. Measurement of biological dinitrogen fixation in grassland: comparison of the enriched ^{15}N dilution and the natural ^{15}N abundance methods at different nitrogen application rates and defoliation frequencies ***Plant and Soil*** 166, 153–163

Hokka V, Mikola J, Vestberg M, Setala H, 2004. Interactive effects of defoliation and an AM fungus on plants and soil organisms in experimental legume–grass communities ***Oikos*** 106, 73-84

Holford ICR, 1997. Soil phosphorus: its measurement, and its uptake by plants ***Austr J Soil Res*** 35:227–239

Hosny M, Gianinazzi-Pearson V, Dulieu H, 1998. Nuclear DNA content of 11 fungal species in Glomales ***Genome***, 41, 422-428

Hunt S, Layzell DB, 1993. Gas exchange of legume nodules and the regulation of nitrogenase activity in soybean ***Ann Rev Plant Physiology Mol Biol*** 44:483 – 511

Ibekwe AM, CM Grieve, 2004. Changes in developing plant microbial community structure as affected by contaminated water, ***FEMS Microbiology Ecology 48***, 239–248

Ivashuta S, Liu J, Lohar DP, Haridas S, Bucciarelli B, VandenBosch KA, Vance CP, Harrison MJ, Gantt JS, 2005. RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development ***Plant Cell*** 17, 2911–2921

Jackson LE, Schimel JP, Firestone MK, 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland ***Soil Biology & Biochemistry*** 21, 409-415

Jacquelinet-Jeanmougin J, Gianinazzi-Pearson V, Gianinazzi S, 1987. Endomycorrhizas in the Gentianaceae II Ultrastructural aspects of symbiont relationships in *Gentiana lutea* L ***Symbiosis*** 3:269–286

Jakobsen I, Abbott LK, Robson AD, 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L 1. Spread of hyphae and phosphorus inflow into roots ***New Phytologist*** 120, 371-380

Jakobsen I, Chen BD, Munkvold L, Lundsgaard T, Zhu Y-G, 2005. Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant ***Plant, Cell and Env*** 28, 928–938

Jargeat P, Cosseau C, Ola'h B, Jauneau A, Bonfante P, Batut J, Becard G, 2004. Isolation, free-living capacities, and genome structure of '*Candidatus Glomeribacter gigasporarum*', the endocellular bacterium of the mycorrhizal fungus *Gigaspora margarita* ***Journal of Bacteriology*** 186, 6876–6884

Javot H, Pumplin N, Harrison MJ, 2007. Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles ***Plant Cell And Environment*** 30, 310-322

Jayachandran K, Schwab AP, Hetrick BAD, 1992. Mineralization of organic phosphorus by vesicular-arbuscular mycorrhizal fungi ***Soil Biology and Biochemistry*** 24, 897-903

Jeffries P, Craven-Griffiths A, Barea JM, Levy Y, Dodd JC, 2002. Application of arbuscular mycorrhizal fungi in the revegetation of desertified Mediterranean ecosystem In S Gianinazzi, H Schüepp, J M

Barea and K Haselwandter (Eds), Mycorrhiza Technology in Agriculture, from Genes to Bioproducts (pp 151-174) Heidelberg, Germany: ALS, Birkhäuser Verlag

Jin H, PE Pfeffer, DD Douds, E Piotrowski, PJ Lammers, Y Shachar-Hill, 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis ***New Phytologist*** 168, 687–696

Johansen A, Jakobsen I, Jensen ES, 1993. Hyphal transport by a vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate ***Biology and Fertility of Soils*** 16, 66–70

Johansson JF, Paul LR, Finlay RD, 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture ***FEMS Microbiology Ecology*** 48, 1–13

Johnson D, Leake JR, Ostle N, Ineson P, Read DJ, 2002. In situ $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil ***New Phytologist*** 153, 327-334

Judson OP, Normark BB, 1996. Ancient asexual scandals ***Trends in Ecology & Evolution*** 11, 41-46

Jungk A, Claassen N, 1989. Availability in soil and acquisition by plants as the basis for phosphorus and potassium supply to plants ***Zeitschrift für Pflanzenernährung und Bodenkunde*** 152, 151–157

Kabir Z, 2005. Tillage or no-tillage: Impact on mycorrhizae ***Can J Plant Sci*** 85, 23–29

Kandeler E, Gerber H, 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium ***Biology and Fertility of Soils*** 6, 68-72

Kapulnik Y, Douds DD Jr, 2000. Arbuscular Mycorrhizas: Physiology and Function Dordrecht, The Netherlands, Kluwer Academic Publishers

Karagiannidis N, Hadjisavva-Zinoviadi S, 1998. The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils Nutrient ***Cycling in Agroecosystems*** 52, 1–7

Kaschuk G, PA Leffelaar, KE Giller, O Alberton, M Hungria, TW Kuyper, 2010. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses ***Soil Biology and Biochemistry*** 42, 125-127

Kaye JP, Hart SC, 1997. Competition for nitrogen between plants and soil microorganisms ***Trends in Ecology and Evolution*** 12, 139–143

Kennedy AC, 1998. The rhizosphere and spermosphere. In D M Sylvia, J J Fuhrmann, P G Hartel and D A Zuberer (Eds), Principles and Applications of Soil Microbiology (pp 389-407) Upper Saddle River, NJ, Prentice Hall

Khan AG, 1993. Occurrence and importance of mycorrhizae in aquatic trees of New SouthWales, Australia ***Mycorrhiza*** 3:31–38

Kiers ET, Denison RF, 2008. Sanctions, cooperation, and the stability of plant–rhizosphere mutualisms, ***Annual Review of Ecology, Evolution, and Systematics*** 39, 215–236

Killham K, 1994. Soil Ecology, Cambridge university press

Kluge M, D Mollenhauer, E Wolf; A Schüßler, 2002. The Nostoc-Geosiphon Endocytobiosis In AN Rai, B Bergman, U Rasmussen (eds) Cyanobacteria in Symbiosis Kluwer Academic Publishers, pp 19-30

Köhler RH, Hanson MR, 2000. Plastid tubules of higher plants are tissue-specific and developmentally regulated ***J Cell Sci*** 113:81–89

Koide RT 1991. Density-dependent response to mycorrhizal infection in *Abutilon theophrasti* Medic. ***Oecologia*** 85, 389–395

Koide RT, 1985. The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection ***New Phytologist***, 99, 449-462

Koide RT, Elliot G, 1989. Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal symbiosis, ***Functional Ecology*** 3, 252–255

Koide RT, Kabir Z, 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate ***New Phytologist*** 148, 511–517

Koide RT, Li M, 1989. Appropriate controls for vesicular arbuscular mycorrhizal research ***New Phytologist*** 111, 35-44

Kothari SK, Marschner H, Römheld V, 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil ***Plant and Soil*** 131, 177–185

Kristek S, A Kristek, H Pavlović, 2005. The influence of mycorrhizal fungi (*Glomus sp*.) on field pea plant survival and growth in drought caused stress conditions ***Plant and Soil Environ***, 51,, 385–389

Kubikova E, Moore JL, Ownlew BH, Mullen MD, Augé RM, 2001. Mycorrhizal impact on osmotic adjustment in *Ocimum basilicum* during a lethal drying episode ***Journal of Plant Physiology*** 158, 1227–1230

Kucey RMN, Paul EA, 1982. Carbon flow photosynthesis, and N_2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L) ***Soil Biology and Biochemistry***, 14, 407-412

Kuhn G, Hijri M, Sanders IR, 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi ***Nature*** 414, 745-748

Kwapata MB, Hall AE, 1985. Effects of moisture regime and phosphorus on mycorrhizal infection, nutrient uptake, and growth of cowpeas (*Vigna unguiculata* (L) Walp) ***Field Crops Res*** 12:241–250

Ladd JN, Butler JHA, 1972 Short-term assays of soil proteolytic enzyme activities using proteins and dipeptides derivatives as substrates ***Soil Biology and Biochemistry*** 4:19-30

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, Andbothe H, 2002. The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils ***Mycorrhiza*** 12:199–211

LaRue TA, Weeden NF, 1994. The symbiosis genes of the host In: Kiss GB, Endre G, editors Proceedings of the 1st European Nitrogen Fixation Conference Officina Press: Szeged, p 147-151

Lekberg Y, Koide RT, 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003 ***New Phytologist*** 168, 189–204

Li H, Smith AF, Dickson S, Holloway RE, Smith SE, 2008. Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? ***New Phytologist***

Li H, Smith SE, Holloway RE, Zhu Y, Smith FA, 2006. Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses ***New Phytologist*** 172, 536–543

Li HY, Zhu Y, Marschner P, Smith FA, Smith SE, 2005. Wheat Responses to Arbuscular Mycorrhizal Fungi in a Highly Calcareous Soil Differ from those of Clover, and Change with Plant Development and P supply ***Plant and Soil*** 277, 221-232.

Li XL, Marschner H, George E, 1991. Acquisition of phosphorus and copper by VA–mycorrhizal hyphae and root-to-shoot transport in white clover ***Plant and Soil*** 136, 49–57

Linderman RG, 1997. Vesicular-arbuscular mycorrhizal (VAM) fungi In The Mycota Caroll, GC, and Tudzynski, P (eds) Berlin, Germany: Springer-Verlag, pp 117–128

Liu H, Trieu AT, Blaylock LA, Harrison MJ 1998. Cloning and characterization of two phosphate transporters from *Medicago truncatula* roots: Regulation in response to phosphate and to colonization by arbuscular mycorrhizal (AM) fungi ***Molecular Plant-Microbe Interactactions*** 11:14 – 22

Lum MR, Hirsch AM, 2003. Roots and Their Symbiotic Microbes: Strategies to Obtain Nitrogen and Phosphorus in a Nutrient-Limiting Environment ***Journal of Plant Growth Regulation*** 21, 368–382

Mäder P, Vierheilig H, Streitwolf-Engel R, Boller T, Frey B, Christie P, Wiemken A, 2000. Transport of ^{15}N from a Soil Compartment Separated by a Polytetrafluoroethylene Membrane to Plant Roots via the Hyphae of Arbuscular Mycorrhizal Fungi ***New Phytologist***, 146, 155-161

Maeda D, Ashida K, Iguchi K, Chechetka SA, Hijikata A, Okusako Y, Deguchi Y, Izui K, Hata S, 2006. Knockdown of an arbuscular mycorrhiza-inducible phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis ***Plant and Cell Physiology*** 47, 807–817

Mansfeld-Giese K, Larsen J, Bødker L, 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* ***FEMS Microbiology Ecology*** 41, 133-140

Manske GGB, AB Luttger, RK Behl, PLG Vlek, 1995. Nutrient efficiency based on VA mycorrhizae (AMF) and total root length of wheat cultivars grown in India ***Angew Bot*** 69, 108—110

Mantel N, 1967. The detection of disease clustering and a generalized regression approach ***Cancer Research,*** 27, 209-220

Marschner H, Dell B, 1994. Nutrient uptake in mycorrhizal symbiosis ***Plant and Soil*** 159, 89-102

Marschner P, Crowley DE 1996. Root colonization of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum*) by Pseudomonas fluorescens 2-79RL ***New Phytologist*** 134, 115-122

Marschner P, Crowley DE, Higashi RM, 1997. Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L) ***Plant and Soil*** 189, 11-20

Marschner P, Crowley DE, Lieberei R, 2001. Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize ***Mycorrhiza*** 11, 297-302

Marschner P, Timonen S, 2005. Bacterial community composition and activity in rhizospheres of Roots colonised by arbuscular mycorrhizal fungi In: Microbial Activity in the Rhizosphere Eds Mukerji, KG, Manoharachary, C & Singh J Springer Verlag,

Marulanda A, Azcón R, Ruiz-Lozano JM, 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress ***Physiologia Plantarum*** 119, 526-533

McGonigle TP, AH Fitter, 1988. Growth and Phosphorus Inflows of Trifolium repens L with a Range of Indigenous Vesicular-Arbuscular Mycorrhizal Infection Levels Under Field Conditions ***New Phytologist***, 108, 59-65

Meddich A, Oihabi A, Abbas Y, Bizid E 2000. Rôle des champignons mycorrhiziens à arbuscules de zones arides dans la résistance du trèfle (*Trifolium alexandrinum* L.) au déficit hydrique.***Agronomie*** 20, 283–295.

Meuriot F, JC Avice, JC Simon, P Laine, ML Decau, A Ourry, 2004. Influence of initial organic N reserves and residuals leaf area on growth, N uptake, N partitioning and N storage in alfalfa (*Medicago sativa*) during post-cutting regrowth. ***Annals of Botany.*** 94, 311−321.

Miller AJ, Cramer MD, 2004. Root nitrogen acquisition and assimilation ***Plant and Soil*** 274, 1–36

Miller MH, McGonigle TP, Addy HD, 1995. Functional Ecology of Vesicular Arbuscular Mycorrhizas as Influenced by Phosphate Fertilization and Tillage in an Agricultural Ecosystem ***Critical Reviews in Biotechnology*** 15, 241—255

Miller RM, Jastrow JD, 2000. Mycorrhizal fungi influence soil structure In Y Kapulnik and D D Douds, Jr (Eds), Arbuscular mycorrhizas: Physiology and Functions (pp 3-18) Dordrecht, The Netherlands: Kluwer Academic Publishers

Milligan GW, Cooper MC, 1985. An examination of procedures for determining the number of clusters in a data set ***Psychometrika*** 50, 159–179

Minerdi D, Bianciotto V, Bonfante, P, 2002. Endosymbiotic bacteria in mycorrhizal fungi: from their morphology to genomic sequences ***Plant and Soil*** 244, 211–219

Minerdi D, Fani R, Gallo R, Boarino A, Bonfante P, 2001. Nitrogen fixation genes in an endosymbiotic Burkholderia strain ***Applied and Environmental Microbiology*** 67, 725–732

Miyasaka SC, Habte M, 2001. Plant mechanisms and mycorrhizal symbioses to increase phosphorus uptake efficiency ***Communications in Soil Science and Plant Analysis*****t Anal**, 32, 1101–1147

Mosse B, 1986. Mycorrhiza in a sustainable agriculture ***Biol Agric***, 3, 191-209

Mosse B, Stribley DP, Le Tacon F, 1981. Ecology of mycorrhizae and mycorrhizal fungi In Alexander, M (ed) Advances in microbial ecology New York: Plenum Press, vol 5, New York 137-209

Nadelhoffer KJ, JD Aber, JM Melillo, 1985. Fine Roots, Net Primary Production, and Soil Nitrogen Availability: A New Hypothesis ***Ecology*** 66, 1377-1390.

Nannipieri P, B Ceccanti, S Cervelli, E Matarese, 1980. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil, ***Soil Science Society of America Journal*** 44, pp 1011–1016

Nei M, Li W, 1979 Mathematical model for studying genetic variation in terms of restriction endonucleases, ***Proc Natl Acad Sci USA 76***, 5269–5273

Ning J, Cumming JR, 2001. Arbuscular mycorrhizal fungi alter phosphorus relations of broomsedge (*Andropogon virginicus* L) plants ***Journal of Experimental Botany***, 52, 1883-1891

Nishimura R, Hayashi M, Wu GJ, Kouchi H, Imaizumi-Anraku H, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M, Harada K, Kawaguchi M, 2002. HAR1 mediates systemic regulation of symbiotic organ development ***Nature*** 2002, 420:426-429

O'Hara G W, 2001. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review ***Australian Journal of Experimental Agriculture***, 41, 417–433

Oehl F, Sieverding E, 2004. *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes ***Journal of Applied Botany and Food Quality*** 78, 72-82

O'Keefe, DM, DM Sylvia, 1992. Chronology and mechanisms of P uptake by mycorrhizal sweet potato plants ***New Phytologist*** 122:651-659

Olesniewicz KS, Thomas RB, 1999. Effects of mycorrhizal colonization on biomass production and nitrogen fixation of black locust (*Robinia pseudoacacia*) seedlings grown under elevated atmospheric carbon dioxide ***New Phytologist*** 142, 133–140.

Osonubi O, Mulongoy K, Awotoye OO, Atayese MO, Okali DUU, 1991. Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings ***Plant and Soil*** 136:131–143

Ourry A, TH Kim, J Boucaud, 1994. Nitrogen reserves mobilization during regrowth of *Medicago sativa* L: relationships between their availability and regrowth yield ***Plant Physiology*** 105, 831−837.

Pang PC, Paul EA, 1980. Effects of vesicular arbuscular mycorrhiza on ^{14}C and ^{15}N distribution in nodulated fababeans ***Canadian Journal of Soil Science***, 60, 241-250

Parniske M, 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis Current Opinion in ***Plant Biology*** 7, 414–421

Parniske M, 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses ***Nature Reviews Microbiology*** 6, 763-775

Paszkowski U, 2006. A journey through signaling in arbuscular mycorrhizal symbioses 2006. ***New Phytologist*** 172, 35–46

Paszkowski U, Jakovleva L, Boller T, 2006. Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis ***The Plant Journal*** 47, 165–173

Paszkowski U, Kroken S, Roux C, Briggs SP 2002. Rice phosphate transporters include an evolutionary divergent gene specificity activated in arbuscular-mycorrhizal symbiosis ***Proc Natl Acad Sci USA*** 99:13324 – 13329

Pawlowska TE, Taylor JW, 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi ***Nature*** 427, 733-737

Paynel F, Murray PJ, Cliquet JB, 2001. Root exudates: a pathway for short-term N transfer from clover and ryegrass ***Plant and Soil*** 229, 235–243

Pedersen CT, DM Sylvia, 1997. Limitations to using benomyl in evaluating mycorrhizal functioning ***Biology and Fertility of Soils*** 25,163–168

Perotto S, Vandenbosch KA, Brewin NJ, Faccio A, Knox JP, Bonfante-Fasolo P, 1990. Modifications of the host cell wall during root colonization by Rhizobium and VAM fungi In: Nardon P, Gianinazzi-Pearson V, Grenier AM, Margulis M, Smith DC, editors Endocytobiology IV INRA Press: Paris, 114–117

Phillips JM, Hayman DS, 1970. Improved procedure for clearing roots and staining parasitic and vesicular–arbuscular fungi for rapid assessment of infection Transactions of the ***British Mycological Society*** 55, 158–161

Pietikäinen A, Mikola J, Vestbergc M, Setäläb H, 2009. Defoliation effects on *Plantago lanceolata* resource allocation and soil decomposers in relation to AM symbiosis and fertilization ***Soil Biology and Biochemistry*** 41, 2328-2335

Pirozynski KA, Malloch DW, 1975. The origin of land plants: a matter of mycotrophism *Biosystems* 6, 153-164

Po C, Cumming JR, 1997. Mycorrhizal fungi alter the organic acid exudation profile of red clover rhizospheres In: Flores HE, Lynch JP, Eissenstat D (eds) Radical biology: advances and perspectives of the function of plant roots ***Am Soc Plant Physiology***, pp 517-519

Porcel R, Barea JM, Ruiz-Lozano JM, 2003. Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence ***New Phytologist***, 157, 135-143

Porcel R, Ruiz-Lozano JM, 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress ***Journal of Experimental Botany*** 55, 1743-1750

Posta K, Marschner H, Römheld V, 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize ***Mycorrhiza*** 5, 119-124

Pozo MJ, Azcón-Aguilar C, 2007. Unraveling mycorrhiza-induced resitance ***Current Opinion in Plant Biology,*** 4 , 393-398

Pozo MJ, Slezack-Deschaumes S, Dumas-Gaudot E, Gianinazzi S, Azcón-Aguilar C, 2002. Plant defense responses induced by arbuscular mycorrhizal fungi In S Gianinazzi, H Schüepp, JM Barea and K Haselwandter (Eds), Mycorrhiza Technology in Agriculture, from Genes to Bioproducts (pp 103-111) Basel, Switzerland: Birkhäuser Verlag

Provorov NA, Borisov AY, Tikhonovich IA, 2002. Developmental genetics and evolution of symbiotic structures in nitrogenfixing nodules and arbuscular mycorrhiza ***Journal of Theoretical Biology*** 214, 215–232

Rabie GH, 1998. Induction of fungal disease resistance in *Vicia faba* by dual inoculation with Rhizobium leguminosarum and vesicular-arbuscular mycorrhizal fungi ***Mycopathologia*** 141, 159–166

Radojkovica D, J Kusic 2000. Silver Staining of Denaturing Gradient Gel Electrophoresis Gels ***Clinical Chemistry*** 46, 883-884

Raghothama KG 2005. Phosphorus In: Broadley MR, White PJ, (eds) Plant nutritional genomics Oxford: Blackwell, 112 – 126

Rangeley A, Daft MJ, Newbould P, 1982. The inoculation of white clover with mycorrhizal fungi in unsterile hill soils ***New Phytologist***, 92, 89-102

Rao NSS, Tilak KVBR, Singh CS, 1986. Dual inoculation with *Rhizobium sp* and *Glomus fasciculatum* enhances nodulation, yield and nitrogen fixation in chickpea (*Cicer arietinum* Linn) ***Plant and Soil*** 95, 351–359

Raup D, Crick RE, 1979 Measurement of faunal similarity in paleontology, ***J Paleontol*** 53**,** 1213–1227

Recourt K, van Tunen AJ, Mur LA, van Brussel AAN, Lugtenberg JW, Kijne JW, 1992. Activation of flavonoid biosynthesis in roots of Vicia sativa subsp nigra plants by inoculation with Rhizobium leguminosarum biovar viciae ***Plant Mol Biol*** 19:411-420

Redecker D, Hijri I, Wiemken A, 2003. Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems ***Folia Geobotanica***, 38, 113–124

Redecker D, Kodner R, Graham LE, 2000. Glomalean fungi from the Ordovician Science 289, 1920-1921

Redecker D, Raab P, 2006. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers ***Mycologia***, 98, 885-895

Rejon-Palomares A, García-Garrido JM, Ocampo JA, García-Romera I, 1996. Presence of xyloglucan-hydrolyzing glucanases (xylogucanases) in arbuscular mycorrhizal symbiosis ***Symbiosis*** 21:249 – 261

Ribet J, Drevon JJ, 1995. Increase in permeability to oxygen and in oxygen uptake of soybean nodules under deficient phosphorus nutrition ***Physiol Plantarum*** 94:298 – 304

Rillig MC, Mummey DL, 2006. Mycorrhizas and soil structure ***New Phytologist*** 171, 41–53

Rillig MC, Wright SF, Eviner VT, 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species ***Plant and Soil*** 238, 325-333

Rogers JB, A S Laidlaw, P Christie, 2001. The role of arbuscular mycorrhizal fungi in the transfer of nutrients between white clover and perennial ryegrass ***Chemosphere*** 42, 153-159

Rohlf FJ, 1998. NTSYS-PC 2.0 Numerical Taxonomy and Multivariate Analysis System. Exeter Software, NY, USA

Römer W, Schilling G, 1986. Phosphorus requirements of the wheat plant in various stages of its life cycle ***Plant and Soil*** 91, 221-229

Rowan AK, JR Snape, D Fearnside, MR Barer, TP Curtis and IM Head, 2003. Composition and diversity of ammonia-oxidising bacterial communities in wastewater treatment reactors of different design treating identical wastewater, ***FEMS Microbiology Ecology 43***, 195–206

Ruiz-Lozano J, Azcón R, Palma JM 1996. Superoxide dismutase activity in arbuscular-mycorrhizal *Lactuca sativa* L. plants subjected to drought stress. ***New Phytologist*** 134, 327–333

Ruiz-Lozano JM, Azcón R, Gomez M, 1995. Effects of Arbuscular-Mycorrhizal *Glomus* Species on Drought Tolerance: Physiological and Nutritional Plant Responses ***Applied And Environmental Microbiology*** 61, 456–460

Ruiz-Lozano JM, Bonfante, P, 2000. A *Burkholderia* strain living inside the arbuscular mycorrhizal fungus *Gigaspora margarita* possesses the vacB gene, which is involved in host cell colonization by bacteria ***Microbial Ecology*** 39, 137–144

Ruiz-Lozano JM, Collados C, Barea JM, Azcón R 2001b. Cloning of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorhizal symbiosis and by drought stress ***Journal of Experimental Botany*** 52, 2241–2242

Ruiz-Lozano JM, Collados C, Barea JM, Azcón R, 2001a .Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants ***New Phytologist***, 151, 493-502

Ryan MH, Angus JF, 2003. Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield ***Plant and Soil*** 250, 225–239

Ryan MH, Graham JH, 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? ***Plant and Soil*** 244:263–271

Salzer P, Corbiere H, Boller T; 1999. Hydrogen peroxide accumulation in *Medicago truncatula* root colonized by the arbuscular mycorrhiza-forming fungus ***Planta*** 208, 319–325

Sanchez L, S Weidmann, L Brechenmacher, M Batoux, D van Tuinen, P Lemanceau, S Gianinazzi, V Gianinazzi-Pearson, 2004. Common gene expression in *Medicago truncatula* roots in response to *Pseudomonas fluorescens* colonization, mycorrhiza development and nodulation ***New Phytologist*** 161, 855–863

Sanders FE, Tinker BP, Black RLB, Palmerly SM, 1977. The development of endomycorrhizal root systems I Speed of infection and growth-promoting effects with four species of vesicular–arbuscular endophyte ***New Phytologist*** 78:257–268

Sanders IR, 2002a. Ecology and Evolution of Multigenomic Arbuscular Mycorrhizal Fungi ***American naturalist*** 160, 128-141

Sanders IR, 2002b. Specificity in the arbuscular mycorrhizal symbiosis In MGA van der Heijden, IR Sanders, eds, Mycorrhizal Ecology Springer-Verlag, Berlin 415-437

Sanders IR, Alt M, Groppe K, Boller T, Wiemken A, 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities ***New Phytologist*** 130, 419-427

SAS/STAT, 2002. User'S Guide : Version 91 SAS Institute Inc, Cary, NC, USA

Schachtman DP, Reid RJ, Ayling SM, 1998. Phosphorus uptake by plants: from soil to cell ***Plant Physiology*** 116, 447–453

Schellenbaum L, Sprenger N, Schüepp H, Wiemken A, Boller T, 1999. Effects of drought, transgenic expression of a fructan synthesizing enzyme and of mycorrhizal symbiosis on growth and soluble carbohydrate pools in tobacco plants ***New Phytologist*** 142:67–77

Scheublin TR, van der Heijden MGA, 2006. Arbuscular mycorrhizal fungi colonize nonfixing root nodules of several legume species ***New Phytologist*** 172, 732–738

Schoeneberger MM, Volk RJ, Davey CB, 1989. Factors influencing early performance of leguminous plants in forest soils ***Soil Science Society of America Journal*** 53, 1429–1434

Schroeder MS, Janos DP, 2005. Plant growth, phosphorus nutrition, and root morphological responses to arbuscular mycorrhizas, phosphorus fertilization, and intraspecific density ***Mycorrhiza*** 15, 203–216

Schüßler A, Kluge M, 2001. *Geosiphon pyriforme*, and endocytosymbiosis between fungus and cyanobacteria, and its meaning as a model system for arbuscular mycorrhizal research In The Mycota IX Hock, B (ed) Berlin, Germany: Springer Verlag, pp 151–161

Schüβler A, Schwarzott D, Walker C, 2001. A new fungal phylum, the Glomeromycota, phylogeny and evolution ***Mycological Research***, 105, 1413-1421

Schweiger P, Robson A, Barrow N, Abbott L, 2007. Arbuscular mycorrhizal fungi from three genera induce two-phase plant growth responses on a high P-fixing soil ***Plant and Soil*** 292, 181-192

Schweiger PF, Jakobsen I, 1998. Dose-response relationships between four pesticides and phosphorus uptake by hyphae of arbuscular mycorrhizas ***Soil Biology and Biochemistry*** 30, 1415-1422

Schweiger PF, Spliid NH, Jakobsen I, 2001. Fungicide application and phosphorus uptake by hyphae of arbuscular mycorrhizal fungi into field-grown peas ***Soil Biology and Biochemistry*** 33, 1231-1237

Scullion J, Eason WR, Scott EP, 1998. The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass-arable rotations ***Plant and Soil*** 204, 243–254

Secilia J, Bagyaraj DJ, 1987. Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas ***Canadian Journal Microbiology*** 33, 1069-1073

Seligman NG, Feigenbaum S, Feinerman D, Benjamin RW, 1986. Uptake of nitrogen from high C-to-N ratio, ^{15}N-labelled organic residues by spring wheat grown under semi-arid conditions ***Soil Biology and Biochemistry*** 18, 303-307

Sengupta A, Chaudhuri S, 2002. Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India ***Mycorrhiza*** 12:169–174

Shannon CE, Weaver W, 1963. The Mathematical Theory of Communication, The University of Illinois Press, Urbana, Illinois

Shivaram S, Rai PV, Hegde SV, 1988. Nitrogen fixation and nitrogen balance studies in Rhizobium–VA mycorrhiza inoculated legume–grass association by ^{15}N isotope dilution technique under a field condition ***Plant and Soil*** 111, 11–16

Simard SW, Durall D, Jones M 2002. Carbon and nutrient fluxes within and between mycorrhizal plants In: M. van der Heijden and I. Sanders. Mycorrhizal Ecology. Springer-Verlag, Heidelberg. Ecological Studies, Vol. 157, 33-61., Springer

Simon L, Bousquet J, Lévesque RC, Lalonde M, 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants ***Nature*** 363, 67-69

Simpson D, Daft MJ, 1990. Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum ***Plant and Soil*** 121, 179-186

Simpson EH, 1949. Measurement of diversity ***Nature*** 163, 688

Siqueira JO, Saggin-Junior OJ, 2001. Dependency on arbuscular mycorrhizal fungi and responsiveness of some Brazilian native woody species ***Mycorrhiza*** 11:245-55

Smith SE, Gianinazzi-Pearson V, 1990. Phosphate uptake and vesicular-arbuscular activity in mycorrhizal *Allium cepa* L: effect of photon irradiance and phosphate nutrition ***Australian Journal of Plant Physiology***, 17, 177-188

Smith SE, Nicholas DJD, Smith FA, 1979. Effect of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum* L ***Australian Journal of Plant Physiology***, 6, 305-316

Smith SE, Read DJ, 1997. Mycorrhizal symbiosis San Diego, CA, USA: Academic Press

Smith SE, Smith FA, Jakobsen I, 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses ***Plant Physiology*** 133, 16–20

Smith SE, Smith FA, Jakobsen I, 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake ***New Phytologist*** 162, 511-524

Solaiman MZ, Senoo K, Kawaguchi M, Imaizumi-Anraku H, Akao S, Tanaka A, Obata H, 2000. Characterization of mycorrhizas formed by *Glomus sp* on root of hypernodulating mutants of *Lotus japonicus* ***Journal of Plant Research*** 113:443-448

St. John TV, Coleman DC, Reid CPP, 1983. Association of Vesicular-Arbuscular Mycorrhizal Hyphae with Soil Organic Particles ***Ecology*** 64, 957-959

Staehelin C, Charon C, Boller T, Crespi M, Kondorosi A, 2001. *Medicago truncatula* plants overexpressing the early nodulin gene enod40 exhibit accelerated mycorrhizal colonization and enhanced formation of arbuscules ***Proc Natl Acad Sci USA*** 98:15366-15371

Strack D, Fester D, Hause B, Schliemann W, Walter MH, 2003. Arbuscular mycorrhiza: biological, chemical, and molecular aspects ***Journal of Chemical Ecology***, 29, 1955-1979

Sukarno N, Smith SE, Scott ES 1993. The effect of fungicides on vesicular-arbuscular mycorrhizal symbiosis I The effects on vesicular-arbuscular mycorrhizal fungi and plant growth ***New Phytologist*** 25, 139–147

Ta TC, FDH Macdowall, MA Faris, 1990. Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N_2-fixation and shoot regrowth ***Plant and Soil*** 127, 231−236.

Tabatabai MA, 1994. Soil enzymes In: Weaver RW, Angel JS, Bottomley PS (eds) Methods of soil analysis Part 2 Microbial and biochemical properties Soil Science Society of America, Madison, pp 775–833

Tamasloukht MB, Sejalon-Delmas N, Kluever A, Jauneau A, Roux C, Becard G, Franken P, 2003. Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea* ***Plant Physiology*** 131:1468–1478

Tang C, Hinsinger P, Drevon JJ, Jaillard B, 2001. Phosphorus deficiency impairs early nodule functioning and enhances proton release in roots of *Medicago truncatula* L ***Annals of Botany*** 88:131 – 138

Tarafdar JC, Marschner H, 1994. Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus ***Soil Biology and Biochemistry*** 26, 381-395

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, Gollote, A, 1993. In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular endomycorrhizal infections ***Mycological Research***, 97, 245-250

Titus JH, Titus PJ, Nowak RS, Smith SD, 2002. Arbuscular mycorrhizae of Mojave Desert plants ***Western North American Naturalist*** 62:327–334

Tobar R, R Azcón, JM Barea, 1994. Improved Nitrogen Uptake and Transport from ^{15}N-Labelled Nitrate by External Hyphae of Arbuscular Mycorrhiza Under Water-Stressed Conditions ***New Phytologist***, 126, 119-122

Toljander JF, Artursson V, Paul LR, Jansson JK, Finlay RD, 2006. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species ***FEMS Letters***, 34–40

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD, 2007. Infuence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure ***FEMS Microbiology Ecology*** 61, 295–304

Toro M, Azcón R, Barea JM, 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling ***Applied and Environmental Microbiology*** 63, 4408–4412

Trappe JM, 1987. Phylogenetic and ecologic aspects of mycotrophy in angiosperms from an evolutionary standpoint. In Safir, GR Boca Raton (Eds) Ecophysiology of VA mycorrhizal plants: Florida : CRC Press, cop, Florida 5-25

Trenbath BR, 1976 Plant interaction in mixed crop communities In Multiple Cropping Papendick RI, PA Sanchez e GB Triplett Eds 129-169 ***Amer Soc Agron Madison***, Wisconsin (Spec Publ N 27)

Udaiyan K, Manian S, Muthukumar T, Greep S, 1995. Biostatic effect of fumigation and pesticide drenches on an endomycorrhizal-*Rhizobium*-legume tripartite association under field conditions ***Biology and Fertility of Soils*** 1995, 20, 275-283

Vadez Rodier F, Payee H, Drevon JJ, 1996. Nodule permeability to O_2 and Nitrogenase-linked respiration in bean genotypes varying in the tolerance of N_2 fixation to P deficiency ***Plant Physiology Biochem*** 34:871 – 878

van der Heijden MAG, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A, Sanders IR, 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland ***New Phytologist*** 172, 739–752

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity ***Nature*** 396, 69-72

van der Heijden MGA, Sanders IR, 2002. Mycorrhizal Ecology Berlin and Heidelberg, Germany: Springer-Verlag

Vance CP, 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources ***Plant Physiology***, 127, 390-397

Vance CP, Graham PH, Allan DL, 2000. Biological nitrogen fixation: phosphorus Ba critical future need? In FO Pederosa, M Hungria, MG Yates, WE Newton, eds, Nitrogen Fixation from Molecules to Crop Productivity Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 509–518

Vance CP, Heichel GH, Barnes DK, Bryan JW, Johnson LE, 1979. Nitrogen Fixation, Nodule Development, and Vegetative Regrowth of Alfalfa (*Medicago sativa* L) following Harvest ***Plant Physiology*** 64, 1-8

Vance CP, Uhde-Stone C, Allan DL, 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a non-renewable resource ***New Phytologist*** 157:423 – 447

Vande Broek A, Vanderleyden J, 1995. The role of bacterial motility, chemotaxis and attachment in bacteria-plant interactions ***Molecular Plant-Microbe Interactions*** 8, 800–810

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW, 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem ***Molecular Ecology***, 11, 1555-1564

Viera G, Glenn M, 1990. DNA content of vesicular arbuscular mycorrhizal fungal spores ***Mycologia***, 82, 263-267

Vierheilig H, Ocampo JA, 1991. Receptivity of various wheat cultivars to infection by VA-mycorrhizal fungi as influenced by inoculum potential and the relation of VAM-effectiveness to succinic dehydrogenase activity of the mycelium in the roots ***Plant and Soil*** 133, 291-296.

Vierheilig H, Schweiger P, Brundrett M, 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots ***Physiologia Plantarum*** 125, 393–404

Volenec JJ, A Ourry, BC Joern, 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. ***Physiol. Plant.*** 97, 185−193

Walker C, 2008. *Ambispora* and *Ambisporaceae* resurrected ***Mycological Research*** 112, 297-298

Walker C, Blaszkowski J, Schwarzott D, Schüβler A, 2004. *Gerdemannia* gen nov, a genus separated from *Glomus*, and Gerdemanniaceae fam nov, a new family in the Glomeromycota ***Mycological Research*** 108:707–708

Wallace LL, Mc Naughton SJ, Coughenour MB, 1982. The Effects of Clipping and Fertilization on Nitrogen Nutrition and Allocation by Mycorrhizal and Nonmycorrhizal *Panicum coloratum* L., a C4 Grass ***Oecologia*** 54, 68-71

Walter MH, Fester T, Strack D, 2000. Arbuscular mycorrhizal fungi induce the non-mevalonate methylerythritol phosphate pathway of isoprenoid biosynthesis correlated with accumulation of the 'yellow pigment' and other apocarotenoids ***The Plant Journal*** 21, 571–578

Wang B, Qiu YL, 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants ***Mycorrhiza*** 16:299–363

Werner D, 1998. Organic signals between plants and microorganisms. In R Pinton, Z Varanini and P Nannipieri (Eds), The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interfaces New York, NY: Marcel Dekker

Werner D, Barea JM, Brewing NJ, Cooper JE, Katinakis P, Lindström K, O'Gara F, Spaink HP, Truchet G, Müller P, 2002. Symbiosis and defence in the interaction of plants with microorganisms ***Symbiosis*** 32, 83-104

Whiteside MD, Treseder KK, Atsatt PR, 2009. The brighter side of soils: quantum dots track organic nitrogen through fungi and plants ***Ecology*** 90, 100–108

Wirsel SGR, 2004. Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi ***FEMS Microbiology Ecology*** 48, 129-138

Wright DP, Read DJ, Scholes JD, 1998a. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L ***Plant, Cell and Environment*** 21, 881-891

Wright DP, Scholes JD, Read DJ, 1998b. Effects of VA mycorrhizal colonization on photosynthesis and biomass production of Trifolium repens L ***Plant, Cell and Environment*** 21, 209–216

Wright SF, A Upadhyaya, 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi ***Plant and Soil***:97-107

Wulfsohn D, Nyengaard JR, 1999. Simple stereological procedure to estimate the number and dimensions of root hairs ***Plant and Soil*** 209:129-136

Xavier LJC, Germida JJ, 2002. Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy ***Soil Biology and Biochemistry*** 34, 181–188

Xie ZP, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ, Vogeli-Lange R, Boller T, 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans ***Plant Physiology*** 108:1519-1525

Yin C, Pang X, Chenc K, 2009. The effects of water, nutrient availability and their interaction on the growth, morphology and physiology of two poplar species ***Environmental and Experimental Botany*** 67, 196-203

Zahran HH, 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in a arid climate ***Microbiology and molecular biology reviews*** 63, 968-989

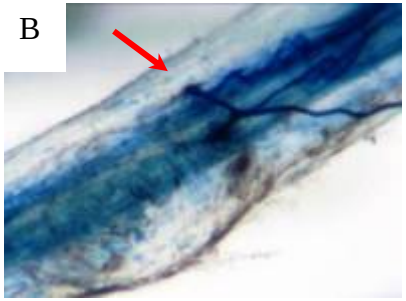
Zhao ZW, Xia YM, Qin XZ, Li XW, Cheng LZ, Sha T, Wang GH, 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China ***Mycorrhiza*** 11:159-62

Zhu Y-G, Smith SE, 2001. Seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi ***Plant and Soil*** 231, 105–112

Zhu Y-G, Smith SE, Barritt AR, Smith FA, 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars ***Plant and Soil*** 237, 249–255

A

B

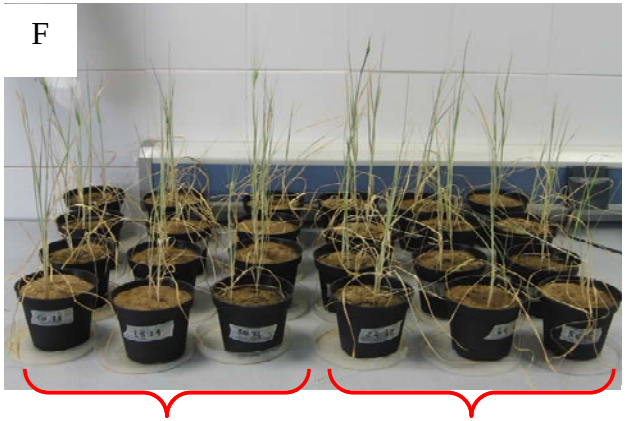


C

D

E

F



Some pictures of the experiments:

A: experimental field.
B: wheat root after staining with trypan blue (red arrow indicates a mycorrhizal infection point).
C: berseem plot after cut.
D: berseem sample area after removal of roots.
E: sample areas in a berseem plot.
F: not infected and mycorrhizal wheat plants (the common “little plant/big plant” effect was probably impaired by the nitrogen lack).