Effect of soil tillage on arbuscular mycorrhizal fungi and on their role in nutrient uptake by crops

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Effect of soil tillage on arbuscular mycorrhizal fungi and on their role in nutrient uptake by crops

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SWISS FEDERAL INSTITUTE OF TECHNOLOGY (ETH) ZÜRICH

For the degree of
DOCTOR OF NATURAL SCIENCES

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Zürich, 2002
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<tr>
<td>AMF</td>
<td>arbuscular mycorrhizal fungus</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BEG</td>
<td>La Banque Européenne des Glomales, <a href="http://www.ukc.ac.uk/bio/beg">www.ukc.ac.uk/bio/beg</a></td>
</tr>
<tr>
<td>CH</td>
<td>chisel tillage</td>
</tr>
<tr>
<td>CP</td>
<td>concentration of water extractable orthophosphate (mg P/L)</td>
</tr>
<tr>
<td>CT</td>
<td>conventional tillage</td>
</tr>
<tr>
<td>CZn</td>
<td>concentration of water extractable free Zn (mg P/L)</td>
</tr>
<tr>
<td>DW</td>
<td>dry weight</td>
</tr>
<tr>
<td>E_{1min-P}</td>
<td>amount of orthophosphate isotopically exchangeable within 1min (mg P/kg soil)</td>
</tr>
<tr>
<td>E_{1min-Zn}</td>
<td>amount of free Zn isotopically exchangeable within 1min (mg Zn/kg soil)</td>
</tr>
<tr>
<td>INVAM</td>
<td>International collection of arbuscular and vesicular-arbuscular mycorrhizal fungi, Morgantown, FL, USA, <a href="http://invam.caf.wvu.edu">http://invam.caf.wvu.edu</a></td>
</tr>
<tr>
<td>LSD</td>
<td>least significant difference</td>
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<tr>
<td>MANOVA</td>
<td>Multivariate analysis of variance</td>
</tr>
<tr>
<td>NAE</td>
<td>nutrient acquisition efficiency, mg acquired nutrient per plant</td>
</tr>
<tr>
<td>NT</td>
<td>no-tillage</td>
</tr>
<tr>
<td>NUE</td>
<td>nutrient uptake efficiency, mg acquired nutrient per unit AMF hyphae length</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>(r_1/R)_P</td>
<td>proportion of $^{33}$P remaining in soil solution after 1 minute of isotopic exchange</td>
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<tr>
<td>(r_1/R)_Zn</td>
<td>proportion of $^{65}$Zn remaining in soil solution after 1 minute of isotopic exchange</td>
</tr>
<tr>
<td>SA</td>
<td>specific activity of labelled nutrient</td>
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<td>SSCP</td>
<td>single-strand conformation polymorphism</td>
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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) were studied in a field experiment evaluating the effects of conventional tillage (CT), chiselling (CH), and no-till (NT) on crop performance and soil properties in a Swiss agrosystem. It was hypothesised that a reduction in tillage intensity would increase the amount, the diversity and/or the symbiotic efficiency of the AMF in the field, and would in consequence result in a higher nutrient acquisition by the mycorrhizal crops.

NT as compared with CT increased mycorrhizal colonisation of the wheat roots and slightly increased AMF infection potential. The effect of soil tillage on plant growth and nutrient uptake remained limited. By using specific molecular markers, increased abundance of *Scutellospora* sp. was observed within maize roots sampled in the NT treatment, while *Glomus* spp. dominated in roots sampled in the CT and CH treatments and *Gigaspora* sp. was present in the roots sampled in all treatments. The reduction of abundance of *Scutellospora* in CT and CH treatments was probably due to the effects of soil disturbance on the *Scutellospora* hyphae.

The effects of AMF on plant dry matter production, P and Zn uptake and on the exploitation efficiency of the soil by AMF were studied in different experimental set-ups (single pots, cuvette, star-pots) for different *Glomus* isolates originating from the differently tilled soils. These experiments showed three strategies for the soil P exploitation by *Glomus* spp, differing in the mycelium spreading capacity and efficiency of nutrient uptake from the soil. No systematic effect of the selection pressure due to the exposure of the AMF to the different tillage practices on their functionality was observed.

It is concluded that although the AMF communities changed under long-term application of the different tillage treatments, these changes did not unequivocally translate into different nutrient acquisition efficiency by the plants. This lack of effect can be explained i) by the high level of available P in this soil; ii) by the fact that AMF having the three different strategies to exploit soil P were present in NT, CH and CT soils.
ZUSAMMENFASSUNG

Arbuskuläre Mykorrhizapilze (AMF) wurden in Böden untersucht, die langfristig drei verschiedenen Bodenbearbeitungssystemen ausgesetzt waren: Pflügen (CT), Schichtengrubber (CH) und Direktsaat (NT). Es besteht die Hypothese dass sich eine Verringerung der Pflügintensität positiv auf die Menge, Diversität und symbiotische Effizienz der AMF im Feld auswirken und die Nährstoffaufnahme durch mykorrhizierte Kulturpflanzen erhöhen kann.


Der Einfluss der AMF auf die Produktion von pflanzlicher Trockenmasse, auf die P- und Zn-Aufnahme und auf die Nährstoffausnutzung des Bodens wurde in verschiedenen experimentellen Ansätzen unter Verwendung von Glomus-Isolaten aus Böden mit den unterschiedlichen Pflügverfahren geprüft. Die Ergebnisse zeigten drei Strategien der P ausnutzung durch Glomus spp., die sich in der Ausbreitung des Myzels im Bodenraume und in der Effizienz der Nährstoffaufnahme unterschieden. Die verschiedenen Pflügverfahren äusserten sich nicht als Selektionsdruck auf die Eigenschaften der AMF.

Aufgrund der Ergebnisse lässt sich schliessen, dass sich trotz Langzeitwirkung des verschiedenen Pflügverfahren auf die Zusammensetzung der AMF keine Wirkung auf die Höhe des Nährstoffaufnahme durch die Pflanzen ergab. Dies erklärt sich (1) aus den hohen Gehalt des Bodens am verfügbarem P und (2) aus den drei Strategien der Ausnutzung des P im Boden durch die AMF, die im NT, CH wie auch im CT Verfahren vorhanden waren.
RÉSUMÉ

Le présent travail porte sur l’étude du développement de champignons mycorhiziens à arbuscules (AMF) dans une expérience au champ évaluant les effets du labour conventionnel (CT), du chisel (CH) et du semis-direct (NT) sur la croissance des cultures et les propriétés du sol dans un agrosystème suisse. L’hypothèse de travail est la suivante : une réduction de l’intensité du travail du sol se traduit par une augmentation de la quantité, de la diversité et/ou de l’efficacité symbiotique des AMF, augmentant ainsi l’acquisition d’éléments nutritifs par les cultures mycorrhizées.

Comparé à CT, NT induit une augmentation de l’infection des racines par les AMF et une légère augmentation du potentiel d’infection. L’effet du travail du sol sur la croissance végétale et le prélèvement d’éléments nutritifs est limité. A l’aide de marqueurs moléculaires, il fut possible de montrer une augmentation de l’abondance de *Scutellospora* sp. dans les racines de maïs cultivé dans le traitement NT, alors que *Glomus* spp. étaient prédominants dans CT et CH. *Gigaspora* était présente dans les racines prélevées dans tous les traitements. La réduction de l’abondance de *Scutellospora* dans les traitements CT et CH est probablement due aux effets de la perturbation du sol sur les hyphes.


Nous concluons que, malgré un changement observé dans la communauté des AMF occasionné par différents types de travail du sol, ces modifications ne se traduisent pas de façon évidente en une capacité différente des plantes à prélever des éléments nutritifs. Ceci peut s’expliquer par 1) un niveau élevé de P disponible dans ces sols, et 2) par la présence de AMF possédant les trois stratégies d’exploitation du sol aussi bien dans NT, CH que dans CT.
1. GENERAL INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ancient plant symbionts, which establish a symbiotic relationship with the majority of the contemporary land plant species (Smith and Read 1997, Smith et al. 2001). They evolved with plants since the Devonian (Remy et al. 1994). AMF colonise the roots of plants and because of an extensive development of their extraradical mycelium, they increase the uptake by the plant of nutrients such as P, Zn and Cu, which are strongly adsorbed to soil particles (Sander and Tinker 1971, Li et al. 1991a, Marschner and Dell 1994, Smith and Read 1997, Harrison 1997, Dodd 2000). In exchange, reduced carbon compounds from the plant photosynthesis are transferred to the AMF (Ho and Trappe 1973, Jakobsen and Rosendahl 1990). Besides their effect on plant nutrition, AMF have been suggested to play other important roles in terrestrial ecosystems. They might affect plant species coexistence, diversity of plant communities, plant reproduction, plant health, and soil aggregation (Newsham et al. 1995, Zobel and Moora 1995, Subramanian and Charest 1997, Streitwolf-Engel et al. 1997, van der Heijden 1998a, van der Heijden et al. 1998b, Wright and Upadhyaya 1998, Marschner 1998, Dodd 2000).

Agricultural practices such as tillage, fertilisation, and crop rotation affect the size, composition and/or functioning of the AMF communities (Thompson et al. 1992, Gavito and Varela 1993, Johnson 1993, Douds et al. 1993, Miller et al. 1995, Miller and Jackson 1998). It has been suggested that heavy mineral fertilisation, intensive tillage, or heavy use of pesticides would be detrimental to AMF, while low-input agricultural practices would enhance the size and activity of the AMF communities (Gavito and Varela 1993, Johnson 1993, Munyanzya et al. 1997). Whereas AMF are known to be indispensable for a sustainable production of agricultural crops having a limited root system such as carrots or leek, the importance of these fungi for crops with a well developed root system (such as cereals) is, however, still unclear (Plenchette et al. 1983).
We can hypothesise that a link exists between different AMF activities in the soil under field conditions and the nutrient acquisition efficiency of the crops growing in that soil. If an agricultural practice would result in a decrease of the AMF activity, this should be reflected in a reduced nutrient acquisition by the plants. And in contrast, an increase in AMF activity would be reflected in an increased nutrient acquisition. This is, however, very difficult to demonstrate under field conditions because plant roots are normally colonised by the AMF (Smith and Read 1997) and appropriate non-mycorrhizal controls, where only AMF would be removed from the system without affecting other biota, are hard (if not impossible) to produce (Fitter and Nichols 1988, Kahiluoto et al. 2000).

The present work aimed at verifying the above-mentioned link between AMF activities and nutrient acquisition in a field subjected to different tillage practices. It was specifically hypothesised that a reduction in tillage intensity would increase the amount, the diversity and/or the symbiotic efficiency of the AMF in the field, and would in consequence result in a higher nutrient acquisition by the mycorrhizal crops.

After a literature review on AMF and on soil tillage effects, the specific objectives, hypotheses, and the scientific approach of the work are presented. Then a chapter dealing with the effect of soil tillage on plant growth, nutrient uptake and root infection by AMF is given. The identification of different AMF species in the roots of maize grown in the different tillage treatments is presented in the next chapter. The following chapter deals with the effect of AMF inoculation on plant dry matter production and P and Zn uptake measured under controlled conditions. The strategies of P uptake by different *Glomus* spp. isolates are studied in the next chapter followed by a chapter assessing the uptake of P and Zn by a single *Glomus* isolate. Finally the general discussion and the conclusions are presented.
2. LITERATURE REVIEW

2.1. ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

2.1.1. AMF biology

The arbuscular mycorrhizal fungi (AMF) are placed in the taxonomic order Glomales. It is a small group of coenocytic fungi, which currently comprises 6-9 genera (Smith and Read 1997, Morton and Redecker 2001, Schwarzott et al. 2001). AMF establish a symbiotic relationship with a majority of terrestrial plant species (estimated to reach up to 80%, Smith et al. 2001). AMF are found in almost every dryland ecosystem, from virgin climax ecosystem to intensively managed agrosystem, saline or heavy metal polluted soils (Read 1991, Giovannetti and Gianinazzi-Pearson 1994, Smith and Read 1997, Bothe et al. 2001). AMF fungi had evolved already in the Devonian period, some 450 M years ago (Simon et al. 1993, Remy et al. 1994), and since then they remain morphologically conserved probably as a consequence of their symbiotic lifestyle (Giovannetti and Gianinazzi-Pearson 1994). AMF are obligate symbionts of plants, and they can not yet be cultivated in absence of a host plant (Smith and Read 1997). In the absence of a living host root, the growth and development of AMF is very limited (Logi et al. 1998).

Colonisation of roots by AMF can arise from three sources of inoculum: spores, infected root fragments, and hyphae – collectively termed propagules (Smith and Read 1997). Spores are the best defined source of inoculum and are the only propagules, by which AMF species can be morphologically identified with any degree of certainty (Walker 1992, Morton and Bentivenga 1994). The spores of AMF are relatively large as compared with other fungi, their diameter ranges from 20 to 500 µm (Schenck and Perez
The spores contain many nuclei and large amount of storage lipids and polysaccharides (Viera and Glenn 1990, Becard and Pfeffer 1993, Shachar-Hill et al. 1995, Giovannetti et al. 1999). The spores can germinate under suitable conditions and form the so-called primary mycelium, whose growth is stimulated by the presence of host roots (Giovannetti et al. 1993). The hyphae of the primary mycelium are capable of forming an appressorium on the surface of host plant roots and proliferate further to the root cortex layer (Nagahashi and Douds 1997, Giovannetti and Sbrana 1998), where they establish intraradical infectious structures. Hyphae, hyphal coils, arbuscules, and vesicles might be formed inside the roots, depending on both the fungal and the host plant species (Brundrett et al. 1996, Smith and Smith 1996, Smith and Smith 1997). Hyphae and arbuscules are considered to be the active state of the AMF, responsible for the nutrient exchange between the plant and the fungus (Smith and Smith 1997). The vesicles are, on the other hand, considered to be the dormant stadium of the AMF analogous to the extraradical spores, responsible for survival under adverse environmental conditions (frost, dryness etc.) (Smith and Read 1997, Staddon and Fitter 2001).

Once the roots have been penetrated by the AMF hyphae, extraradical mycelium is formed, which colonises the soil up to several cm from the host roots (Jakobsen et al. 1992a, Smith and Read 1997, Smith et al. 2000, Smith et al. 2001). The extraradical hyphae of the AMF usually undergo extensive branching (Jakobsen 1995, Bago et al. 1998, Bago 2000), and by their direct contact with the soil particles are very efficient in taking up mineral nutrients similarly as the root hairs (Smith et al. 2001). In this sense, the AMF are fully integrated to the plant body, enlarging its absorption surface for ions (Sander and Tinker 1971, Smith and Read 1997, Marschner 1998, Dodd et al. 2000). Extraradical AMF structures carry asexual spores (Bentivenga et al. 1997, Sanders 1999) which are formed for surviving under adverse conditions (Staddon and Fitter 2001). A sexual stage of AMF has not been identified yet (Sanders 1999). However, parasexual processes, which enable exchange of protoplasm and nuclei among hyphae through anastomoses within some AMF species have recently been shown (Giovannetti et al. 1999).
2.1.2. AMF diversity

About 130-160 species of AMF have been described based on the morphology of their spores so far (Walker and Trappe 1993, Giovannetti and Gianinazzi-Pearson 1994, Morton and Bentivenga 1994). The other AMF structures (mycelium, arbuscules, and vesicles) are morphologically very conserved and provide very limited number of characters useful for taxonomy (Morton and Bentivenga 1994). Some AMF species might preferentially establish symbiotic relationships with certain plant species (such as *Acaulospora* sp. preferentially establishing symbiosis with *Allium* sp. or *Glomus* sp. with *Plantago* sp.) (Bever et al. 1996). No clear evidence for absolute (species to species) specificity has been yet recognised (Vanderplank 1978, Smith and Read 1997). However, the processes controlling the recognition between the plant and the AMF partner in the arbuscular mycorrhizal symbiosis and the factors involved in this interaction are still poorly known (Giovannetti et al. 1994, Giovannetti et al. 1996, Douds et al. 1996, Roussel et al. 2001).

Although no pronounced diversity can be observed at the morphological level by the AMF, a high level of diversity can be found at the molecular level, because of the specific genetical (multinucleated) cellular structure of these fungi (Morton 1990, Sanders et al. 1995, Pringle et al. 2000, Kuhn et al. 2001). Large genetic diversity within AMF spores has been recognised (Sanders et al. 1995, Lloyd-Macgilp et al. 1996, Redecker et al. 1997, Antoniolli et al. 2000). However, the range of the molecular diversity in AMF has been studied in depth for only a few species (Antoniolli et al. 2000, Pringle et al. 2000, Clapp et al. 2001).

Recently, it was suggested, based on sequencing of amplified and cloned rDNA from the spores, that certain AMF isolates might share genomes previously ascribed to different AMF species or genera (Hosny et al. 1999, Clapp et al. 2001, Rodriguez et al. 2001). However, among the sequences obtained from the AMF spores there appeared to be also sequences, which were associated with entries from saprophytic zygo- and
ascomycetous fungi in the sequence database (Redecker et al. 1999, Schüssler et al. 2001, Rodriguez et al. 2001). This points to the necessity of confirming the findings by using completely sterile AMF spores obtained from in-vitro cultures (Becard and Piche 1992, Declerck et al. 1998, Karandashov et al. 1999, Fortin et al. 2002).

Many studies report interspecific differences in the physiological properties of the AMF such as symbiotic effectiveness, environmental tolerance etc. These reports indicate that different AMF might have both negative and positive influence on plant growth and nutrient uptake and/or respond differently to environmental conditions (e.g. Giovannetti and Gianinazzi-Pearson 1994, Johnson et al. 1997, Smith and Read 1997, Streitwolf-Engel et al. 1997, Dickson et al. 1999, Graham and Abbott 2000, Klironomos 2000, Smith et al. 2000, Taylor and Harrier 2000, Klironomos et al. 2001). On the other hand, the diversity of functional properties of AMF on the intraspecific level (i.e. among isolates belonging to the same AMF species) is not well known and begins only to be researched (Knudsen et al. 2001, Larsen et al. 2001, McGonigle, pers. comm.).

The study of van der Heijden et al. (1998a) has shown that there is a link between the diversity of the AMF community in the soil and the diversity of the plant cover aboveground. That study has also shown that the higher diversity of AMF, the higher the total P uptake by the plant community. This supports the expectation that different functional groups of AMF might co-exist within an ecosystem and their composition might affect plant community structure and /or performance through multiple interactions (Newsham et al. 1995, Streitwolf-Engel et al. 1997, van der Heijden et al. 1998b, Hartnett and Wilson 1999, Klironomos et al. 2000, Klironomos et al. 2001). Depending on plant/AMF genotype combination, different plant species may benefit in different ways (nutritional and non-nutritional) from the symbiosis with AMF (Kuncl and Elhottova 1995, Newsham et al. 1995, Smith and Goodman 1999). Therefore Bever (1999) proposed that the mechanism underlying the mutual influence of plant and AMF communities on the ecosystem diversity could be based on the diversity of interactions among different AMF and plant species.
2.1.3. Plant nutrition and AMF

AMF improve the uptake by the host plants of nutrients such as P, Zn, and Cu that are strongly sorbed on soil particles (Bowen et al. 1974, Cooper and Tinker 1978, Schüpp et al. 1987a, Gnekow and Marschner 1989, Marschner and Dell 1994, Smith and Read 1997, Marschner 1998). This improved nutrition has been explained by the extension of the AMF hyphae in zones remote from the root system, allowing the exploration of nutrients spatially inaccessible for the uptake by roots (Li et al. 1991a, Sander and Tinker 1971, Jakobsen et al. 2001, Smith et al. 2001). Mycorrhizal hyphae of *Glomus* sp. and *Acaulospora* sp. can transport P from distances of up to several cm from host plant roots (Li et al. 1991a, Jakobsen et al. 1992a, 1992b). That is much further than the range of several millimetres, which root hairs can exploit (Gahoonia and Nielsen 1998). It was hypothesised that the most pronounced effects on plant nutrient uptake could be observed in plants with coarse root system or little developed root hairs (Baylis 1972, StJohn 1980, Schweiger et al. 1995).

Until now, a lot of research has been focused on the effect of AMF on P, Zn, Cu, Pb, and Cd uptake and transport to the plants and/or immobilisation in the rhizosphere (Smith and Read 1997, Schweiger and Jakobsen 2000, Joner and Leyval 2001) while the effects of AMF on the uptake of other mineral nutrients (Fe, Co, Mn, N, K) or contaminants (e.g. Cs) from the soil have been less studied (Clark and Zeto 2000, Mäder et al. 2000, Berreck and Haselwandter 2001, Suzuki et al. 2001).

AMF deliver mineral nutrients to the host plant and obtain in return reduced carbon compounds derived from plant photosynthesis. Ho and Trappe (1973) performed one of the earliest experiments directly showing the transfer of labelled carbon from the plant to the AMF. Since then, a vast body of literature on this topic has accumulated (see Smith and Read 1997 for review). Jakobsen and Rosendahl (1990) calculated that *Glomus fasciculatum* used up to 20% of the total $^{14}$CO$_2$ fixed in photosynthesis by cucumber. Reduction of the growth rate of plants infected by AMF during the early stages of plant
growth or under low light intensity or low temperature has been frequently observed (Son and Smith 1988, Smith and Smith 1996, Dickson et al. 1999) and ascribed to the strong sink strength of the AMF for organic carbon (Dickson et al. 1999). A whole continuum of interactions thus exists among different AMF and their host plants. The nature of those interactions depending on both AMF and plant genotype and/or on the growth conditions ranges from mutualistic (beneficial for both partners) to parasitic (beneficial for one and detrimental for the other partner) (Johnson 1993, Smith and Smith 1996, Johnson et al. 1997).

Because most of the functional experiments have been performed with only a limited number of AMF isolates, it is now important to assess the level of diversity in the functional properties within species/genus/family levels and within the communities of AMF co-occurring in the ecosystem.

2.1.4. Mineral nutrient uptake and transport by AMF

As plants take up P, Cu, Zn, the soil solution surrounding the roots becomes depleted in these nutrients (Nye and Tinker 1977, Bolan 1991, Rengel 1993, Raghothama 2000), which have then to move by diffusion from the solid phase of the soil to the soil solution (Marschner 1995). However, the diffusion processes become rapidly kinetic-limited. The movement of ions by diffusion can become very slow if the ions are strongly sorbed onto soil surface and for crops that have a limited root system, the rate of nutrient delivery might become insufficient, triggering the apparition of deficiency (Geelhoed et al. 1997). The presence of extraradical AMF hyphae might improve plant nutrition by two processes: i) exploring soil located beyond the depletion zone or ii) accessing more efficiently the nutrients remaining in the depletion zone (Zhu et al. 2001, Smith et al. 2001).

2.1.4.1. AMF mycelium growth

The length and distribution of external hyphae vary for different AMF genera (Jakobsen et al. 1992a). The length density of hyphae of Scutellospora calospora...
declined approximately exponentially with increasing distance from the roots, whereas *Acaulospora laevis* maintained a plateau of constant hyphal density up to 7 and 11 cm from the roots. *Glomus* sp. had an intermediate pattern of spread with a plateau closest to the roots followed by an exponential decline (Jakobsen et al. 1992a). Whether such a variation also exists within the genera/species of the AMF is not known.

The density of AMF hyphae in the soil is important for the mycorrhiza-mediated nutrient uptake by the plants (Dodd et al. 2000, Schweiger et al. 2001). Although hyphae of *Scutellospora calospora* did not grow as far as *G. caledonium*, they formed denser extraradical mycelium in the vicinity of the roots, resulting in higher P acquisition efficiency for the *Scutellospora*- than for *Glomus*-inoculated *Medicago truncatula* (Smith et al. 2000). It is still unclear whether the density of the mycelium alone is responsible for the rate of P uptake by the AMF from the soil or whether some other factors such as the density of nutrient transporters or the affinity of nutrient transporter molecules on the surface of the AMF hyphae play an important role too (Smith et al. 2001).

The AMF extraradical mycelium in the soil can directly affect soil aggregate stability (Jastrow et al. 1998) by physically binding the soil particles (Miller and Jastrow 1990). Glomalin, an exudation product of AMF hyphae (Wright and Upadhyaya 1998) could also bind the soil particles together (Wright et al. 1999). The survey of soil properties performed in the central Great Plains showed large variation of glomalin concentrations in the soils subjected to different management intensities. The highest and lowest aggregate stability and glomalin values were seen in perennial grassland and in soils cropped with triticale, respectively (Wright and Anderson 2000).

### 2.1.4.2. Mechanism of nutrient uptake by the AMF from the soil

The phosphate is taken up by the AMF hyphae from the soil in a form of orthophosphate (Marschner and Dell 1994, Smith et al. 2001) by specialised P transporter molecules located in the plasma membrane of the hyphae. The uptake mechanism is thus principally similar to that by the plants (Smith et al. 2001, Zhu et al. 2001). High-affinity P transporters were recently cloned from several *Glomus* spp. (Harrison and van Buuren
1995, Maldonado-Mendoza et al. 1997), which were also shown to be specifically expressed in the external mycelium of the AMF and to respond to the actual environmental conditions (Maldonado-Mendoza et al. 2001). Similar transporters specialised for uptake of other mineral nutrients such as Zn by the AMF hyphae from the soil have not been found yet.

In contrast to the ectomycorrhizal fungi, AMF apparently take up the nutrients only in forms, which are also available for plants (Marschner and Dell 1994, Joner and Jakobsen 1995, Perez-Moreno and Read 2000). It is still not known whether AMF have any important effect on the solubilisation of nutrients (mainly P) bound in sparingly soluble mineral or organic forms (Joner and Jakobsen 1994, Joner and Jakobsen 1995, Smith and Read 1997). Although phosphatases have been recently found to be exuded by axenically growing mycelium of *Glomus intraradices* (Koide and Kabir 2000), it is not known whether this plays any important role in unsterile soil. The improved uptake of P from sparingly soluble forms might be rather due to the interactions of AMF with other soil microbiota (e.g. P-solubilising bacteria) (Kim et al. 1998, Joner and Johansen 2000).

2.1.4.3. P transport through AMF hyphae and P transfer to the plant

Phosphate is transported through the AMF extraradical mycelium in P-rich pleiomorphic vacuoles by cytoplasmic streaming (Smith et al. 2001). The actual form of transported P is not clear. It was previously suggested that P might be transported in a form of polyphosphate (polyP) (Cox and Tinker 1976, Capaccio and Callow 1982, Solaiman et al. 1999, Smith et al. 2001). This however remains uncertain because the proportion of polyP to the total P in the AMF is rather small, reaching only up to 17% in 9 weeks old mycelium of *Gigaspora margarita* (Solaiman et al. 1999). Moreover, Boddington and Dodd (1999) failed to detect any polyP in the extraradical mycelium of *Glomus manihotis* by histo-chemical staining.

The transfer of P from AMF to the plants takes place at the interface between plant cells and the intraradical structures of the AMF such as arbuscules, hyphal coils, and intraradical hyphae (Smith et al. 2001). Whether there are active or passive processes
involved on the fungal side is not known yet. The uptake of P by the plant cells is an 
active process, which involves P-transporters (Rausch et al. 2001). The transport 
processes responsible for transporting carbon compounds from the plant to the AMF are 
less well understood than the processes controlling mineral nutrient transfer in the 
opposite direction (Bago et al. 2000, see Smith et al. 2001 for review).

Zinc is also taken up by AMF and transported by the mycelium to the host plant 
roots, increasing the plant Zn content in many soils (Clark and Zeto 2000). The efficiency 
of AMF to take up and transport Zn from the soil to the roots, and the effects on 
partitioning of Zn between shoots and roots appears to be dependent on AMF species 
(Mehravaran et al. 2000). The variability of the Zn uptake efficiency among different 
AMF as well as the processes involved in uptake and transport of Zn through the AMF 
hyphae must now be studied to achieve a better understanding.

The processes involved in uptake, hyphal translocation and AMF-plant transfer of 
mineral nutrients other than P and Zn are understood only to a very limited extent. There 
is a need to elucidate the role and mechanisms involved in uptake of other elements such 
as Cu, Pb, Co, Cs etc. by the plants through AMF.

**2.2. Effect of land use on the AMF**

Land is used by mankind for different purposes such as extracting raw materials, 
producing food and fibres etc. Up to a half of the land surface has been already 
systems such as forest, pasture, crop production field, horticultural field and glasshouses 
present a scale from extensively to very intensively used ecosystems (Nair 1991, Shriar 
2000, Sauerbeck 2001). Intensification of land use is characterised by increased 
frequency of cropping, shift towards more value-added production, and increased 
amounts and frequency of inputs (Bender 2000, Dumanski and Pieri 2000).
With increasing intensity of land use the composition of AMF communities in soils changes and AMF diversity usually declines (Dodd et al. 1990a, 1990b, Sieverding 1991, An et al. 1993, Douds and Millner 1999, Boddington and Dodd 2000a, Egerton-Warburton et al. 2001). Nwaga et al. (2000) observed a significant decrease in AMF spore diversity in tropical soils in response to the transition of the ecosystem from the rain forest to agricultural land. AMF diversity in the temperate woodland, assessed by amplification of fungal rDNA sequences from the roots, was also significantly higher than in the nearby arable fields (Helgason et al. 1998, Helgason et al. 1999, Daniell et al. 2001). Furthermore, land use intensity might also have pronounced effects on the composition of AMF communities, as suggested for example by Abbott and Robson (1977), Blaszkowski (1993), Talukdar and Germida (1993) etc. These authors observed an increased abundance of *Glomus mosseae*, *G. clarum* and *G. deserticola* in cropped soils, whereas AMF communities containing also *Gigaspora* spp., *Scutellospora* spp. and *Acaulospora* spp. were observed in less intensively used soils such as pastures or woodlands.

The loss of diversity and change in community composition of AMF due to agricultural use of the land could be attributed to the effects of fertilisation, cropping history, ploughing or fungicide application on the AMF in the soil (Sattelmacher et al. 1991, Kurle and Pfleger 1996, Helgason et al. 1998).

### 2.2.1. Fertilisation

Johnson (1993) showed that the application of mineral fertilisers resulted in a loss of non-*Glomus* fungi and increase in population of *Glomus intraradices* in the AMF community in the fields. Egerton-Warburton and Allen (2000) and Egerton-Warburton et al. (2001) also reported significant changes in diversity, species richness and evenness of the AMF communities in the soil in response to anthropogenic nitrogen deposition derived from vehicular emissions as well as from nitrogen fertilisers. Low AMF infection
potential in the soil together with lower incidence of some AMF in high-input agricultural soils was also reported by Douds et al. (1993). Johnson (1993) demonstrated that fertilisation selected for “inferior mutualists”, which exerted higher carbon costs to the host plants than the AMF from unfertilised fields. This indicates that application of fertilisers might be an important factor affecting both AMF diversity and their symbiotic function.

2.2.2 Crop rotation

The positive effect of pre-cropping with AMF host-plant on the AMF inoculum potential in the soil has been long recognised (Ocampo 1980, Dodd et al. 1990b, Douds et al. 1997, Gavito and Miller 1998, Boswell et al. 1998, DeSousa et al. 1999, Oliveira and Sanders 1999). There might also be significant differences in the efficiency of certain plant species in increasing the inoculum potential of the AMF in the soils (Dodd et al. 1990b). Bagayoko et al. (2000) reported higher root AMF colonisation in cereals grown in rotation with legumes than under continuous cereals. Continuous soybean enhanced the spore population of *Gigaspora* spp. in the soil, while *Glomus* spp. dominated the AMF communities when soybean was included in a rotation with maize, millet or fescue (An et al. 1993, Hendrix et al. 1995). It was also shown that different *Glomus* spp. might be similarly stimulated or inhibited by different cropping histories (Johnson et al. 1991). Positive influence of pre-cropping with certain plant species such as cassava, *Pueraria phaseoloides* (a legume) or sorghum on AMF colonisation of roots, P uptake and yield of cowpea and *Stylosanthes capitata* was observed by Dodd et al. (1990a) in contrast to the savanna control.

2.2.3 Soil tillage

Tillage is a method of soil preparation aiming at preparing optimal seedbed conditions for fast and reliable establishment and growth of crops and for weed control (Rieger 2001). It basically combines soil cutting and mixing of the upper soil layer
Alternative tillage practices such as chisel tillage or no-till have been developed mainly to reduce the erosion of agricultural land. Fifteen million hectares in the USA, and 14 million hectares in Brazil are annually planted with no-tillage management (Six et al. 2001). Besides reducing soil erosion, reduced tillage also affects different physical, chemical, and biological properties of soil. For example, no-tillage increases bulk density of topsoil, and may also lead to decrease of soil macroporosity (Kaspar et al. 1991, Hussain et al. 1998). Improved soil aggregation (Hussain et al. 1999, Wright et al. 1999), higher soil penetration resistance (Unger and Jones 1998), increase in water retaining capacity in the topsoil (0-15 cm) and a decrease in soil temperature in the spring (Lal 1995, Arshad and Azooz 1996, Hussain et al. 1998) were observed in the no-till soils as compared with their tilled counterparts. Streit et al. (2000) observed an increase in the proportion of perennial weeds and O’Donovan and McAndrew (2000) observed a respective decrease in the proportion of annual weeds in the no-till soils as compared with the tilled soils. However, many of the effects are dependent on the duration of application of the no-till soil management, on the soil type, and on the climatic conditions (Blevins and Frye 1993).

No-till can also reduce water pollution by reducing the nutrient leaching and might lower production costs (Lal 1991, Ehlers and Claupein 1994). The yield of some crops under no-till practice might be higher or lower than in the conventionally tilled soil, depending on many factors such as soil type, climatic conditions, crop plant species etc. (Anken et al. 1997, Gavito and Miller 1998, Dos Santos et al. 2000, Ogban and Babalola 2002). Crop growth under no-till can be slower during the early-season (McGonigle et al. 1999), because of the lower soil temperature in the no-till fields (Chassot 2000).

No-tillage results in higher concentration of organic carbon, available P, K, and Ca in the topsoil (0-5cm) as compared with the tilled soils (Hussain et al. 1999, Matowo et al. 1999). Lower concentrations of organic carbon, and of available P, Ca, Mg, and K are found in the deeper layers (under 10 cm) of no-tilled as compared with conventionally tilled soils (Holanda et al. 1998, Hussain et al. 1999, Matowo et al. 1999). Agricultural
management practices have been shown to influence the composition of decomposer community in soils, with no-tillage favouring fungi over bacteria, as compared with the conventional tillage systems (Guggenberger et al. 1999). Increase in the fungal biomass under no-till conditions was mainly due to pronounced development of arbuscular mycorrhizal fungi (AMF), as shown by the analysis of fatty acid profiles in differently tilled soils (Drijber et al. 2000). No effect of no-till was observed on the total amount of bacterial biomass in the soil as compared with conventional tillage (Frey et al. 1999). Carbon mineralization and microbial biomass, as well as earthworm and collembolan communities were higher under no-till conditions than in conventionally tilled fields (Pankhurst et al. 1995, Palma et al. 2000).

Faster AMF development in the roots of maize and wheat, and greater rates of P absorption by plants have been observed in the no-tilled soils as compared with the tilled soils (Miller et al. 1995, Mozafar et al. 2000). In the soils that have not been tilled during three years, 1.5 times more glomalin have been detected than in the counterpart tilled soils. And in the nearby grassland non-disturbed for 15 years, about three times more glomalin was found than in the tilled soils (Wright et al. 1999). This could be explained by a higher activity of the AMF in the non-disturbed soils (Wright and Upadhyaya 1999). An experimental exclusion of fungi from the soil by fungicides has lead to a decrease in aggregate stability of the no-tilled as compared with the tilled soils (Beare et al. 1997) suggesting that AMF (as the major part of fungal assemblage in the soil, Drijber et al. 2000) would play a major role in the build-up of soil structure under no-till management.

Mechanical disturbance of the soil was shown to reduce the density of spores, species richness and the lengths of extraradical mycelium of AMF as compared with the undisturbed soil (Boddington and Dodd 2000a). The disturbance had, however, different effects on different AMF species. Disturbance (cutting) of a pre-established extraradical mycelium reduced root colonisation by *Gigaspora rosea* and increased that by *Glomus manihotis* on *Desmodium ovalifolium* plants (Boddington and Dodd 2000b).
The effects of soil tillage on the reduction of the AMF infection potential in the soils as well as on the slower development of AMF colonisation of roots and the consequent effects of tillage on plant nutrition have been extensively studied (Miller et al. 1995, Galvez et al. 2001). However, the effect of soil tillage on the diversity and structure of AMF communities colonising crop plant roots has not been clarified yet.

Improved P nutrition of mycorrhizal crop plants growing in the no-till soil as compared with the tilled soil has frequently been reported (Fairchild and Miller 1990, Miller 2000, Mozafar et al. 2000). This improved P nutrition was suggested to be partly due to enhanced AMF activity and partly due to the changes in soil physical and chemical properties accompanying the different tillage treatments (Miller and McGonigle 1992, Holanda et al. 1998, Hussain et al. 1999, Matowo et al. 1999, Mozafar et al. 2000). Whether the improved nutrient uptake of the plants growing under the no-till conditions is due to improved AMF activity or to the changes in soil properties/nutrient stratification established in response to the different tillage treatment is, however, still unclear.

2.2.4. Role of AMF in agricultural soils

Whether AMF presence in intensively used agricultural soil is of a vital importance for the growth of cultural plants with well-developed root system (cereals, maize, bean etc.) is not clear. The unequivocal proof that AMF contribute to nutrient uptake and yield of the field grown plants is difficult to obtain, because under the field conditions the roots are normally colonised by the AMF and appropriate non-mycorrhizal control are hard to produce (Fitter and Nichols 1988, Kahiluoto et al. 2000). McGonigle (1988) evaluated 78 field trials with AMF and found that inoculation with AMF resulted in an average yield increase of 37%, which provided an indirect evidence of AMF contribution to the crop yield. The results obtained by the use of fungicides such as Benomyl (Graham and Eissenstat 1998, Thingstrup et al. 2000) must be always interpreted with caution because fungicide application affects many more components of the soil ecosystem than only AMF. Comparison of the performance of mycorrhizal and non-mycorrhizal crop plants
provides a better estimation of mycorrhiza-derived benefits among fields/plots, but this method still provides only an indirect evidence for AMF activity (Evans and Miller 1988, Mozafar et al. 2000). Recently, methods were developed, which enable direct observation of AMF-mediated P uptake and transport to the plants growing under the field conditions (Schweiger and Jakobsen 2000, Jakobsen et al. 2001). These methods might in the future provide more objective assessment of AMF contribution to the mineral nutrition of plants under different field management practices.

AMF might be an important component of the natural or semi-natural ecosystems (Smith and Read 1997, Dodd et al. 2000, Klironomos et al. 2000). It is probable that AMF might also play a range of important roles in intensively managed agrosystems, taking part in improving mineral nutrition of cultural plants by various elements, increasing drought tolerance, protecting plants from uptake of heavy metals, increasing soil aggregate stability etc. (Bethlenfalvay and Schüepp 1994, Bagyaraj and Varma 1995, Schreiner and Bethlenfalvay 1995, Smith and Read 1997, Dodd 2000). However, the quantitative estimation of those beneficial effects remains in most cases still to be done.
3. OBJECTIVE, HYPOTHESES AND APPROACHES

3.1. OBJECTIVE

The objective of this work was to elucidate the effect of a specific agricultural practice, soil tillage, on the composition of AMF communities in plant roots and the consequences of the tillage-induced changes in AMF communities for the nutrient acquisition by crop plants.

3.2. HYPOTHESES

3.2.1. Hypothesis 1

Soil tillage affects (1.1.) the size and activity of the AMF communities in the soil, (1.2.) the species composition of AMF in the roots of fields grown plants, and (1.3.) also affects the physiological properties of the AMF species.

3.2.2 Hypothesis 2

Through the change in AMF communities, the nutrient acquisition efficiency (NAE) by the plants growing in differently tilled soils is changed.
3.3. EXPERIMENTAL APPROACH

A proper experimental approach was needed to test the above-given hypotheses. Since all different possible situations could not be studied it was decided to focus on exemplary model systems from which results could then be generalised.

The first step was to identify a well-established field experiment comparing the effects of different tillage practices on crop performance and on soil properties. Having access to such an experiment would have the following advantages: i) to obtain results reflecting correctly the complex relationships occurring in a single agroecosystem, ii) to study the changes in AMF community/AMF activity/plant nutrient acquisition as affected by a single, well characterised, modification of the agrosystem: soil tillage. Finally, to assess the influence of isolated AMF on plant nutrition, maize was chosen as a model plant and P and Zn as model nutrients and it was decided to study these interactions under controlled glasshouse conditions.

3.3.1. Description of the field experiment

The growth and nutrient uptake of the plants, AMF development and community structure in the roots as well as nutrient availability in differently tilled soils was studied in the long-term field tillage experiment in Tänikon-Hausweid. This experiment is located in north-east Switzerland (N 47°29' 10.0", E 8°55' 10.1", altitude 540m), The experiment was established in 1987 on a well-drained stony Orthic Luvisol (topsoil 0-25 cm: 51% sand, 31% silt, 16% clay, pH\textsubscript{H2O} \textsuperscript{*} 5.8) and was thoroughly described by Anken et al. (1997). Selected soil chemical properties at different soil depths are given in Table 3-1.

Several different tillage practices are included in the field experiment, in combination with two plant-residue managements. The plots (72 m\textsuperscript{2} each, 6m × 12m) are organised in a completely randomised block design with four blocks. The 4-year crop rotation for all soil treatments is the same and consists of winter wheat (*Triticum*).

\* pH measured in a suspension of 1g of dry soil to 2.5 mL deionized H\textsubscript{2}O
*aestivum* L.) - maize (*Zea mays* L.) – winter wheat – oil seed rape (*Brassica napus* L.).

The fertilisers (mineral fertiliser only were used) and herbicides were applied in the same rate for all the soil tillage treatments with the exception of glyphosate, which was applied specifically on the no-tilled soil surface once per two years (Anken et al. 1997). There were no insecticide or fungicide inputs to the field since 1987. Mineral fertilisers were applied according to the recommendations of FAL and RAC (2001), the average rates of fertilisers applied between 1987 and 1999 was 112 kg N/ha/a, 75 kg P₂O₅/ha/a, and 122 kg K₂O/ha/a.

**TABLE 3-1**

The effects of soil tillage treatment (conventional tillage, chisel, no-till) and soil depth (0-40 cm) on soil chemical properties (data from 1999 courtesy of Thomas Anken).

### a) Mean values

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Depth (cm)</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
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<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong>&lt;sub&gt;total&lt;/sub&gt; (mg/kg)</td>
<td>Till</td>
<td>964.4</td>
<td>980.2</td>
<td>659.1</td>
<td>616.4</td>
<td>1093</td>
<td>992.3</td>
<td>659.3</td>
<td>620.0</td>
<td>1076</td>
<td>950.4</td>
<td>689.5</td>
<td>617.4</td>
</tr>
<tr>
<td></td>
<td>Chisel</td>
<td>1093</td>
<td>992.3</td>
<td>659.3</td>
<td>620.0</td>
<td>1076</td>
<td>950.4</td>
<td>689.5</td>
<td>617.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No-till</td>
<td>1076</td>
<td>950.4</td>
<td>689.5</td>
<td>617.4</td>
<td>1076</td>
<td>950.4</td>
<td>689.5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zn</strong>&lt;sub&gt;total&lt;/sub&gt; (mg/kg)</td>
<td>Till</td>
<td>37.0</td>
<td>39.4</td>
<td>33.5</td>
<td>38.2</td>
<td>37.1</td>
<td>41.2</td>
<td>33.6</td>
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<td></td>
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<td>41.2</td>
<td>33.6</td>
<td>38.1</td>
<td>36.5</td>
<td>39.4</td>
<td>33.6</td>
<td>36.0</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No-till</td>
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<td>39.4</td>
<td>33.6</td>
<td>36.0</td>
<td>36.5</td>
<td>39.4</td>
<td>33.6</td>
<td>36.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P</strong>&lt;sub&gt;available&lt;/sub&gt; (mg/kg)</td>
<td>Till</td>
<td>1.70</td>
<td>1.75</td>
<td>1.20</td>
<td>0.41</td>
<td>2.32</td>
<td>1.44</td>
<td>0.56</td>
<td>0.25</td>
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<td></td>
<td>Chisel</td>
<td>1.75</td>
<td>1.44</td>
<td>0.56</td>
<td>0.25</td>
<td>4.01</td>
<td>1.47</td>
<td>0.62</td>
<td>0.19</td>
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<tr>
<td></td>
<td>No-till</td>
<td>1.75</td>
<td>1.47</td>
<td>0.62</td>
<td>0.19</td>
<td>1.75</td>
<td>1.47</td>
<td>0.62</td>
<td>0.19</td>
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<td></td>
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</tr>
<tr>
<td><strong>K</strong>&lt;sub&gt;available&lt;/sub&gt; (mg/kg)</td>
<td>Till</td>
<td>24.3</td>
<td>18.5</td>
<td>20.8</td>
<td>16.6</td>
<td>47.9</td>
<td>25.5</td>
<td>12.2</td>
<td>8.3</td>
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<td>23.5</td>
<td>12.7</td>
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<tr>
<td></td>
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<td>24.3</td>
<td>18.5</td>
<td>20.8</td>
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<td>25.5</td>
<td>12.2</td>
<td>8.3</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>No-till</td>
<td>24.3</td>
<td>18.5</td>
<td>20.8</td>
<td>16.6</td>
<td>47.9</td>
<td>25.5</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Organic C %</strong></td>
<td>Till</td>
<td>1.54</td>
<td>1.52</td>
<td>1.24</td>
<td>0.82</td>
<td>1.91</td>
<td>1.49</td>
<td>0.95</td>
<td>0.69</td>
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<td>1.57</td>
<td>0.99</td>
<td>0.68</td>
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<td>1.24</td>
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<td>1.91</td>
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<td>0.69</td>
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<tr>
<td></td>
<td>No-till</td>
<td>1.54</td>
<td>1.52</td>
<td>1.24</td>
<td>0.82</td>
<td>1.91</td>
<td>1.49</td>
<td>0.95</td>
<td>0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### b) Two-way ANOVA results and comparison of slopes of the regression curves

Comparison of correlation coefficients, which are describing nutrient and organic matter contents of differently tilled soils with increasing depths.

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>F – value</th>
<th>Comparison of corre. coeff.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong>&lt;sub&gt;total&lt;/sub&gt; (mg/kg)</td>
<td>d&lt;sub&gt;T&lt;/sub&gt; 2</td>
<td>d&lt;sub&gt;D&lt;/sub&gt; 3</td>
</tr>
<tr>
<td>Tillage (T)</td>
<td>Depth (D)</td>
<td></td>
</tr>
<tr>
<td>0.50 n.s.</td>
<td>47.44 ***</td>
<td>0.48 n.s.</td>
</tr>
<tr>
<td>0.47 n.s.</td>
<td>7.47 ***</td>
<td>0.18 n.s.</td>
</tr>
<tr>
<td>0.65 n.s.</td>
<td>10.83 ***</td>
<td>1.54 n.s.</td>
</tr>
<tr>
<td>0.67 n.s.</td>
<td>25.31 ***</td>
<td>3.94 **</td>
</tr>
<tr>
<td>0.13 n.s.</td>
<td>64.03 ***</td>
<td>2.62 *</td>
</tr>
</tbody>
</table>

Measure of significance: n.s. not significant, (*) P<0.1; * P<0.05, ** P<0.01, *** P<0.001.

1- estimated in 2000 by the method modified from Saunders and Williams (1955). The soil was extracted by 5.6M HCl after incineration at 660°C for 6h.

2- estimated in 1999 by the method of Dirks and Scheffer (1930) according to FAL, RAC, FAW (1996).

3- estimated in 1999 by the bichromate-oxidation method according to FAL, RAC, FAW (1996).

4- comparison of slopes of the linear regression lines for soil chemical properties of differently tilled soils with increasing depths.
Three different soil tillage treatments were included in this study: (1) conventional tillage (CT), (2) chisel treatment (CH) and (3) no-tillage (NT). The study was carried out during the three seasons, 1999 (wheat), 2000 (maize), and 2001 (wheat). We only studied soils from the plots where organic residues had been left in the fields.

In the CT treatment, the soil is ploughed annually to the depth of 25 cm with a mouldboard plough. This soil in the upper horizon is cut and mixed upside-down by the tillage in the CT treatment. In the CH treatment, the soil is loosened annually with a wing share chisel, which cuts the soil to the depth of 25 cm, but does not turn it upside-down. Soil in the NT treatment was neither cut nor mixed at all. The level of mechanical disturbance imposed on the soil was highest in the CT, intermediate in the CH, and virtually zero in the NT treatment (Blevins and Frey 1993, Lal 1995, Anken et al. 1997). The three tillage treatments studied together might also be used to dissect the effects of soil cutting and soil mixing on the soil physical, chemical and/or biological properties. CT combines soil cutting with soil mixing, CH represents soil cutting in the absence of soil mixing, and NT represents absence of both soil cutting and mixing.

This latter hypothesis is backed up by the results of soil analyses made in 1999 and 2000 in the field trial (Table 3-1). These results show that the concentrations of available P, K, and organic C in the different depths of the NT and CH soils were almost always identical, whereas those observed in the CT treatment were different. The available K and organic C contents of the CH and NT soils were highest in the 0-10 cm layer compared with those measured in the CT treatment whereas the contrary was observed in the horizons deeper than 10cm. Although the available P content was lower in the 0-10 cm horizon of the CH than in the NT treatment, it remained higher than in the CT treatment and followed a similar trend with depth as the content of available K and organic C. The highest concentration of available P, K, and organic C observed in the NT and CH in the 0-10 cm layer and the steep concentration gradient observed from the 0-10 cm to the deeper horizons support the hypothesis that the soil remained unmixed in both of those
treatments. In contrast, the similar organic C, available P and K concentrations observed in the first 0-20 cm layer of the CT treatment support the hypothesis that the soil is regularly mixed.

3.3.2. Model plants

The field experiment was studied during maize (cultivar “LG 22.65”) and wheat (cultivars “Terza” and “Levis” for the seasons 1999 and 2001, respectively) cropping seasons. Furthermore maize is a well-known mycorrhizal host plant species (Kaeppler et al. 2000, Liu et al. 2000). Therefore, in further glasshouse experiments studying P and Zn uptake, maize (cv. “Corso”, which is a traditional Swiss cultivar) was kept as the model plant.

3.3.3. Model nutrients

This study concentrated on the uptake of P and Zn by the plants since AMF are supposed to play an important role in the uptake of both of these elements (Mosse 1957, Marschner and Dell 1994).

P and Zn were chosen as model nutrients for three other reasons: i) they have a low mobility in the soil, both of them can be strongly adsorbed onto the surface of soil particles, ii) their availability in the soil can be precisely characterised by isotopic techniques (Frossard and Sinaj 1997), and iii) in the field experiment, P is annually added as fertiliser and accumulates in the first 5 cm of the NT soil whereas no Zn is added with fertilisers and the concentration of available Zn is similar in the first 30 cm of all the different tillage treatments.

3.3.4 Greenhouse studies

It was decided to conduct experiments in a glasshouse for obvious reasons: this allowed to work with controlled climatic conditions, with single AMF strains, in systems where AMF hyphae could grow far away from the root system and where radioactive P and Zn could be used.
Different AMF strains were isolated from the different tillage treatments by combining trap culturing and monosporic isolate culture establishment (Brundrett et al. 1999). The monosporic AMF cultures were then used for development of specific molecular markers to identify the AMF in the roots and for functional tests.

3.4. SPECIFIC OBJECTIVES STUDIED IN EACH EXPERIMENTAL CHAPTER

3.4.1. Effect of soil tillage on plant growth, nutrient acquisition and root colonisation by AMF (chapter 4)

This part aimed at monitoring crop plant (wheat and maize) growth, nutrient uptake, and AMF development in differently tilled soils during three consecutive seasons (1999, 2000, and 2001). This was done concomitantly with the analysis of soil nutrient availability and the assessment of AMF infection potential in the soils. The specific objective of this work was to determine whether different tillage regimes had affected the size/infectivity of the AMF communities in the fields and if this had translated in different P and Zn uptake by plants.

3.4.2. Effect of soil tillage on the community of AMF within maize roots (chapter 5)

Using the spores of different AMF genera obtained from trap cultures, and the spores from the pure monosporic isolates of genus *Glomus*, specific molecular markers were developed to identify the distinct species or species-groups of *Glomales* based on their 28S-rDNA sequences. These markers were used for the identification of AMF in the field-grown maize roots. The specific objective of this study was to compare the impact of different tillage regimes on the functionally relevant (=root-colonising) AMF community composition in the fields.
3.4.3. Effects of AMF from differently tilled soils on plant growth and nutrient acquisition in pots with a single compartment (chapter 6)

Glasshouse experiments were conducted in single pots to study the effect of AMF on maize growth and P and Zn uptake. Both natural AMF communities and single spore AMF isolates from differently tilled soil were used as inoculum. The specific objective of this chapter was to test whether different tillage practices had influenced some functional properties of AMF.

3.4.4. Strategies of soil exploration by AMF from genus *Glomus* (chapter 7)

The objective of this study was to determine the differences in P uptake strategies among eight different *Glomus* strains isolated from either CT or NT treatment and to determine if those differences were related to the AMF species or to the tillage treatment of the origin of the AMF isolate. This study was performed in a glasshouse using compartmented cultivation containers, in which root-free compartments had been labelled with radioactive P. These experiments allowed to characterise the distance, from which AMF hyphae were capable of taking up and transporting P from the soil to the plants.

3.4.5. Uptake and transport of P and Zn by *Glomus intraradices* Schenck & Smith growing in symbiosis with maize (chapter 8)

As tillage practices had a stronger influence on Zn uptake than on P uptake by crops in the field, the specific objective of this chapter was to test whether this could be explained by a differential element uptake by AMF. This study was done in a glasshouse using compartmented cultivation containers and soil labelled with P and Zn radioisotopes. One single-spore isolate of *Glomus intraradices* was used for inoculation of maize grown in the compartmented containers.
4. EFFECT OF SOIL TILLAGE ON PLANT GROWTH, NUTRIENT ACQUISITION AND ROOT COLONISATION BY AMF

ABSTRACT

Plant growth, nutrient uptake and arbuscular mycorrhizal fungal (AMF) development in the roots of wheat and maize were studied in a field experiment. This study was performed in 1999, 2000 and 2001 in soils subjected to three different soil tillage treatments (conventional mouldboard ploughing, wing-share chiselling, and no-till), representing a gradient in tillage intensity. Total and available P and Zn contents in the topsoil (0-10 cm) were measured in 1999 and 2000. Mycorrhizal infection potential of the soils was estimated in 1999 and 2001, following the rapeseed and the maize seasons, respectively.

Higher concentrations of Mg and Ca were observed in plants from conventionally tilled plots early in the season, while later in the season, increased concentrations of Mn, Zn, Cu, K, and P were sporadically found in plants growing in no-tilled soils. Total and available P contents were higher in the 0-10 cm layer of the no-tilled and the chiselled soils as compared with the tilled soils. Zinc content of the soils was not influenced by tillage practice. No differences were observed in mycorrhizal infectivity of the soils among the different soil tillage treatments, although the infectivity tended to be higher in the no-tilled soil. The development of mycorrhizal colonisation (hyphae and arbuscules) in the roots of field grown plants was usually faster and more pronounced under no-tilled conditions.

KEYWORDS
tillage, chisel, no-till, nutrient uptake, field, maize, wheat, fertilisation, phosphorus, zinc, arbuscular mycorrhiza
4.1. INTRODUCTION

Soil tillage affects AMF by disrupting of their extraradical mycelium (Kabir et al. 1998), leading to a loss of mycorrhizal infectivity (Galvez et al. 2001), lower roots colonisation rates (McGonigle and Miller 1993a) and decreased spore densities in the soils (Galvez et al. 2001). Soil disturbance reduces mycorrhiza-mediated P acquisition by plants (McGonigle et al. 1990a, McGonigle and Miller 1999). Thus it was suggested that the decrease in P uptake by crops observed in annually ploughed soils as compared with no-tilled soils might be related to the lower AMF colonisation of roots in the ploughed soils or to slower development of AMF colonisation (Miller et al. 1995, Gavito and Miller 1998). This lower mycorrhiza-mediated nutrient acquisition might also lead to yield reductions, especially under nutrient limiting conditions (McGonigle and Miller 1996a, Miller 2000, Grant et al. 2001).

The objective of this work was to monitor soil nutrient (P and Zn) content and availability, mycorrhizal infectivity of the field soil, and the growth, nutrient uptake and mycorrhiza development in the roots of crop plants growing in soils which had been subjected to different tillage regimes for more than 12 years.

The following hypotheses given at the beginning of the dissertation were tested in this chapter:

1) **Soil tillage affects the size and activity of the AMF communities in the soil (hypothesis 1.1.).**
This hypothesis was approached by answering the following specific questions: Does the soil tillage affect AMF infection potential in the soil? Does reduced tillage promote faster colonisation of the roots by the AMF?

2) **Through the change in AMF communities the nutrient acquisition efficiency by the plants growing in differently tilled soils is changed.**
This hypothesis was approached by assessing plant dry matter production and nutrient uptake as affected by the different tillage regimes.

The results in this chapter are presented in two parts:
1. Plant growth, nutrient acquisition and mycorrhiza development in their roots throughout the three seasons. Topsoil nutrient concentrations throughout the season.
2. AMF infectivity of the topsoil as affected by the previous crop and by the tillage treatment of the soil.

4.2. MATERIAL AND METHODS

4.2.1 Plant growth and nutrient uptake, AMF colonisation, total and available P and Zn in soils

4.2.1.1. Plant growth and nutrient uptake

Samples of field grown plants from the long-term tillage experiment described in chapter 3.3.1. were collected several times a year in three consecutive seasons: wheat in 1999 (cultivar Terza, planting density 410 seeds m\(^{-2}\), following rapeseed), silage maize in 2000 (cultivar LG22.65, 11 plants m\(^{-2}\)), and wheat in 2001 (cultivar Levis, 420 seeds m\(^{-2}\)). Wheat is sown under Swiss conditions at the first half of October, whereas maize is sown at the end of April. Wheat is usually harvested during end of July - beginning of August, whereas maize is usually harvested in the middle of September.

Wheat samples were taken at tillering (end of March – mid of April), heading (end of April – beginning of May), and flowering (middle – end of June) stages. Maize was sampled at the 2-leaf (end of May), 9-leaf (end of June) and the flowering (end of July) stages. Additional sampling was performed just before the harvest in the seasons 1999 and 2000.

Aerial parts and roots (sampling depth 0 - 20 cm) of several plants (4 or more), randomly chosen in the fields, were collected from each plot, washed in deionized water, and pooled. The biomass of the aerial parts per plant was recorded after drying at 105°C
for 48h, and a subsample of the top biomass was ground in a centrifuge mill (Retsch ZM100, Wuppertal, Germany) for chemical analysis. Half-a-gram ground samples were ashed for 6h at 550°C and extracted with 2ml of 5.6M HCl, filtered through a Whatman-40 ashless cellulose filters and diluted to 50 mL with deionized water. Element concentration in the extracts was analyzed by ICP emission-spectrometry (Varian Liberty 220 equipped with an Ultrasonic Nebulizer CETAC U-5000 AT+, Varian Inc., Palo Alto, CA, USA).

4.2.1.2. AMF colonisation of roots

Mycorrhizal colonisation structures in the roots were stained after clearing the roots in 1.8M KOH (90°C, 1h) in a mixture of Trypan- and Methylene Blue (each 0.05% in lactic acid: glycerol: water, 1:1:1 v: v: v), following a procedure modified from Phillips and Hayman (1970). Percentage of root length colonisation with AMF and nonmycorrhizal fungi was estimated by the grid-line intersect method (Giovannetti and Mosse 1980), observing 50 intersects at 200x magnification under a compound microscope Olympus Provis AX70 (Olympus Optical Co., Tokyo, Japan) as recommended by McGonigle et al. (1990b). We recorded AMF hyphal colonisation, and the percentage of root length occupied by the arbuscules, which provided a rough estimate of the active AMF colonisation (Sanders and Fitter 1992, Smith and Smith 1997).

4.2.1.3. Total and available P and Zn content of the soil

Samples of topsoil from the field were collected throughout the seasons 1999 and 2000 at the same sampling dates as the plant samples were taken. Thirty individual soil cores (diameter 3cm, depth 0-10 cm) were taken randomly from each experimental plot at each sampling date. The cores were pooled and thoroughly mixed to obtain a representative sample.

Total phosphorus and zinc contents in the soil were estimated throughout the seasons 1999 and 2000. Elements were extracted from homogenised and sifted soil samples ( > 2mm) with 5.6M HCl after incineration at 550°C for 6h (Saunders and
Williams 1955). Phosphorus concentration was estimated by the malachite green method (Ohno and Zibilske 1991), while the zinc concentration was measured by atomic absorption spectrometry at 213.9 nm (AAS Perkin-Elmer – 5000, Wellesley, MA, USA).

The amounts of readily available P (measured in samples taken throughout the season 2000) and Zn (measured only in samples taken in September 2000) were assessed by measuring the amount of isotopically exchangeable element within 1 minute ($E_{1\text{ min}}$) using the isotope exchange kinetic technique (Fardeau, 1996, Frossard and Sinaj 1997, Sinaj et al. 1999). In this method, $^{33}\text{PO}_4$ or $^{65}\text{Zn}$ ions are added carrier-free to an aqueous soil suspension (1:10, w:v) at a steady-state equilibrium for orthophosphate and free Zn (pre-incubated on a shaker for 20 and 72 hours in a room temperature). After the addition of radioisotope (carrier-free $^{33}\text{P}$-orthophosphate, Amersham Pharmacia Biotech, Piscataway, NJ, USA; or carrier free $^{65}\text{ZnCl}_2$, NEN, Perkin-Elmer Life Sciences, Boston, MA, USA), the radioactivity in solution decreases with time according to the equation given by Fardeau (1996) which can be simplified as follows:

\[
\frac{(r_t/R)_P}{(r_1/R)_P} = \frac{(r_t/R)_P}{(r_1/R)_P} \times t(\text{n})_P \quad \text{[1]}
\]

\[
\frac{(r_t/R)_Zn}{(r_1/R)_Zn} = \frac{(r_t/R)_Zn}{(r_1/R)_Zn} \times t(\text{n})_Zn \quad \text{[2]}
\]

Where:

- $(r_t/R)_P$ is the proportion of radioactive P remaining in the solution after $t$ minutes of isotopic exchange
- $(r_t/R)_Zn$ is the proportion of radioactive Zn remaining in the solution after $t$ minutes of isotopic exchange
- $(r_1/R)_P$ is the proportion of radioactive P remaining in the solution after 1 minute of isotopic exchange
- $(r_1/R)_Zn$ is the proportion of radioactive Zn remaining in the solution after 1 minute of isotopic exchange
- $t$ is the time of isotopic exchange duration in minutes
- $(\text{n})_P$ is the rate of decrease of the radioactive P concentration in the soil solution after 1 minute of exchange calculated as proposed by Frossard and Sinaj (1997)
(n)Zn is the rate of decrease of the radioactive Zn concentration in the soil solution after 1 minute of exchange calculated as proposed by Frossard and Sinaj (1997).

The radioactivity remaining in the solution after \( t \) min of incubation \( (r_t) \) is measured in the filtrate of the soil suspension through a 0.2 \( \mu \)m filter (Sartorius AG, Göttingen, Germany).

Total orthophosphate \( (C_p) \) and free Zn \( (C_{Zn}) \) concentrations in the soil filtrate were measured by the malachite green method (Ohno and Zibilske 1991) and by ion-chromatography (Dionex DX500 system, DIONEX, Chicago, IL, USA), respectively. Samples for ion-chromatography (100 \( \mu \)L) were injected and separated on guard- and analytical columns Ionpac CG5 and Ionpac CS5, respectively. The eluent was 50 mM oxalic acid/LiOH (pH 4.80), which had a flow rate of 1.0 mL/min. Ions were detected by absorbance at 520 nm after post-column reaction with \( 4 \times 10^{-4}M \) 4-(2-pyridylazo)-resorcinol in 3.0M H\( \text{N}_4\)OH/1.0M CH\(_3\)COOH. The flow rate of the reagent was 0.5 mL/min. Zn peak appeared after approximately 12 min.

Assuming that the radioactive elements (orthophosphate and free Zn) are exchanged in the soil at the same rate as the stable elements, the amount of orthophosphate respectively of free Zn exchangeable within one minute can be deduced according to the following equations:

\[
E_{1\text{min}-P} = C_p \times 10^7 / (r_t/R)_P \tag{3}
\]

\[
E_{1\text{min}-Zn} = C_{Zn} \times 10^7 / (r_t/R)_{Zn} \tag{4}
\]

Where:

\( E_{1\text{min}-P} = \) amount of orthophosphate isotopically exchangeable within 1min (mg P/kg soil)
\( E_{1\text{min}-Zn} = \) amount of free Zn isotopically exchangeable within 1min (mg Zn/kg soil)
\( C_p = \) concentration of water extractable orthophosphate (mg P/L)
\( C_{Zn} = \) concentration of water extractable free Zn (mg Zn/L)
The factor 10 allows to translate the concentration of water soluble orthophosphate or free Zn per L of solution in mg of water soluble orthophosphate or free Zn per kg of soil (the experiment was conducted using aqueous soil suspension 1:10).

According to Frossard and Sinaj (1997), the fraction of orthophosphate or free Zn isotopically exchangeable within 1 minute is instantaneously and totally available to plant roots and represents therefore a relevant indicator for soil P and Zn availability. According to Gallet (2001), the yield of crops grown under Swiss conditions could decrease due to a P deficiency when $E_{1\text{min},P}$ gets lower than 5 mg P kg$^{-1}$ soil.

4.2.2. Mycorrhizal infectivity in the field soils

Most probable number (MPN) tests of soil AMF infectivity were performed in two wheat seasons (1999 and 2001), following the rapeseed and maize seasons, respectively.

In 1999, AMF propagule number was estimated in the tilled and no-tilled soils only. The soil samples were collected from the eight plots of Tänikon-Hausweid field experiment in January 1999 and combined for each of the two soil tillage treatments. Field soil was first mixed with an equal volume of sterile quartz sand. This unsterile soil-sand mixture (1:1) was then stepwise diluted with a field soil: sand mixture (1:1 v: v), sterilised by $\gamma$-irradiation (minimum of 10 kGy, Studer AG Werk Hard, Däniken, Switzerland). Two dilution series were used: (1) Two-fold dilution series, which included seven dilution treatments (soil diluted by 2, 4, 8, 16, 32, 64, and 128 times) and (2) a ten-fold dilution series, including five treatments (soil diluted by 10, 100, 1000, 10000, and 100000 times). Diluted soil was filled into 250 mL pots (four replicates per treatment), planted with sugar-maize (Zea mays L. cv. Tasty Sweet F1) and after 5 weeks roots were collected and stained for AMF colonisation (see above). The presence/absence of AMF colonisation in the roots was recorded for the 10-fold dilution series, and the absolute AMF colonisation rates (according to Giovannetti and Mosse 1980) were recorded for the 2-fold dilution series. Propagule counts in the undiluted soil were estimated from the presence/absence data by comparison with tabulated values for MPN tests (Woomer...
1994), or inferred from the log-linear regression of absolute colonisation values as proposed by Adelman and Morton (1986). This regression provided an estimation of the ultimate dilution, which would yield a zero colonisation, and which would indicate a propagule count of maximum 1 propagule per pot.

The MPN estimation of infective propagules in the soil sampled in January 2001 was performed separately for each of the twelve plots (managed under conventional tillage, chisel tillage or no-till, respectively) of the field tillage experiment. The soil was diluted as described above, but only the ten-fold soil dilution was used, yielding 10, 100, 1000, 10000, and 100000 times dilution of the original soil. The soil was filled into 300 mL containers and planted with maize (Zea mays L. cv. Corso), which was then grown for 5 weeks in the greenhouse. Four replicate pots per each dilution and soil plot were established. The propagule counts were estimated from AMF presence/absence data by comparison with tabulated values for MPN tests (Woomer 1994).

4.2.3. Statistical analysis

One-way ANOVA analysis (Statgraphics® software version 3.1, Manugistics 1997) was used to test the effect of the different tillage treatments on plant and soil data at each sampling date or at each soil depth. If the ANOVA was significant, LSD-based F test was performed on 5% significance level. Two-way ANOVAs were performed for the soil nutrient content data throughout the seasons 1999 and 2000 (with soil tillage treatment and sampling date as factors). AMF propagule densities in the soil were compared among the different soil tillage treatments in the 2001 season by one-way ANOVA.

4.3. RESULTS

4.3.1. Wheat growth and nutrient uptake in 1999

The dry matter production of aerial parts of wheat was once in March lower in the NT treatment than in the other 2 treatments whereas at the last sampling date in August higher dry matter production was observed in NT and CH compared with the CT
treatments (Fig. 4-1). A higher seed biomass was also observed per ear in the NT and CH than in the CT treatment (Fig. 4-1). The similar yields observed this year in all three tillage treatments (5.21, 5.17, 5.32 t/ha in CT, CH, and NT, respectively, P (ANOVA) = 0.96) suggest that the lower seed weight per ear observed in the CT treatment was compensated by a higher number of ears per hectare. Highest root colonisation with AMF was observed under the no-till treatment, followed by the chisel in April and June. Both AMF hyphal and arbuscule colonisation of the roots was lowest in the tilled treatment, what might indicate a lower AMF activity in soils under this treatment. The absolute AMF colonisation rates reached about 5% of the root length in the spring and up to 30% later in the season (Fig. 4-1). The concentrations of Mg, Fe, and Ca in plant tops were highest in the tilled plots in January (Fig. 4-2). On the other hand, higher concentrations of Mn, Mg, Cu, Zn, and K were observed in the aerial parts of wheat sampled in June (in case of Mn and Zn also in August) in the no-till plots. No differences in P concentration in plant tissue among the tillage treatments were observed throughout the season. Element concentration in the grains of wheat was not affected by the tillage treatments (Fig. 4-2).

4.3.2. Maize growth and nutrient uptake in 2000

The three studied treatments did not affect the production of dry matter by maize, the production of seeds per plant (Fig. 4-3), nor the final grain yield (17.2, 16.4, 16.7 t/ha in CT, CH, and NT, respectively, P (ANOVA) = 0.17). The percentage of roots colonised by AMF hyphae was only once in June lower in the CH treatment than in the other two treatments, and no differences were observed throughout the season for arbuscule colonisation (Fig. 4-3). The concentration of Ca was highest in the plants sampled in May in CT compared with CH and NT treatments. The concentration of P and Cu was highest in plant aerial parts sampled in September in the NT treatment. Similarly, Zn, P, and K, and Mn concentrations were highest in the seeds from the NT treatments as compared with the CH and CT treatments (Fig. 4-4).
Growth and mycorrhizal colonisation of wheat grown in differently tilled soils in 1999. Means and standard errors of four field replicates are given. F-value from the one-way ANOVA comparing the tillage treatments and/or measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P <0.05, ** P< 0.01, *** P<0.001. CT – conventional tillage, CH – chisel, NT – no-till. Error bars indicate ± 1 SE.

The differences among the treatment means are labeled in the figures as follows: Symbols > or < denote significantly different treatments (larger or smaller on 5% probability level). Symbols >= or <= denote no significant differences among two treatments, while a difference to the third treatments was significant. Symbol = was used to label no significant difference between treatments.
FIGURE 4-2
Element concentrations in the wheat top biomass and in grains sampled in 1999 from differently tilled soils. Means and standard errors of four field replicates are given. The measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P <0.05, ** P< 0.01, *** P<0.001. Error bars indicate ± 1 SE.
FIGURE 4-3

Growth and mycorrhizal colonisation of maize, grown in differently tilled soils in 2000. Means and standard errors of four field replicates are given. F-value from the one-way ANOVA comparing the tillage treatments and/or measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P <0.05, ** P< 0.01, *** P<0.001. Error bars indicate ± 1 SE.
Figure 4-4
Element concentrations in maize top biomass and in grains sampled in 2000 from differently tilled soils. Means and standard errors of four field replicates are given. The measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P<0.05, ** P<0.01, *** P<0.001. Error bars indicate ± 1 SE.
4.3.3. Wheat growth and nutrient uptake in 2001

No effect of tillage intensity was observed on the dry matter production of the aerial parts of wheat (Fig. 4-5) nor on the final grain yield (5.52, 5.05, 5.28 t/ha in CT, CH, and NT, respectively, \( P \) (ANOVA) = 0.65). AMF hyphal colonisation of roots was slightly higher in the NT treatment compared with both other tillage treatments in April, while root colonisation by arbuscules was slightly higher in the NT treatment compared with both other tillage treatments in June (Fig. 4-5). Aerial parts of wheat sampled in April in the CT treatment had a higher Ca and Mg concentration than plants sampled in the CH treatment, while plants sampled in June in the NT treatment had slightly higher Zn concentration than the plants sampled in the CT treatment (Fig. 4-6).

**FIGURE 4-5**
Growth and mycorrhizal colonisation of wheat, grown in differently tilled soils in 2001. Mean values and standard errors of four field replicates are given. The measure of the significance of the difference among the tillage treatments is given: (*) \( P < 0.1 \), *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \). Error bars indicate ± 1 SE.
FIGURE 4-6
Element concentrations in wheat top biomass sampled in the 2001 from differently tilled soils. Mean values and standard errors of four field replicates are given. The measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P <0.05, ** P< 0.01, *** P<0.001. Error bars indicate ± 1 SE.
4.3.4. P and Zn content in topsoil

Total P content in the topsoil (0-10 cm) was significantly higher in the NT and CH soils compared with the CT soils, while it was not influenced by the sampling date (Table 4-1, Fig. 4-7). The content of immediately available P in the topsoil (E_{min-P}) was significantly higher in the NT and the CH treatments than in the CT treatment, and the difference became more pronounced later in the season (Fig. 4-7). This was probably related to the P uptake by maize plants, which was more efficient later in the season. No differences were found in the total and available Zn contents in the topsoil (0-10 cm) among the different soil tillage treatments (Table 4-1, Fig. 4-7). Results of total P and Zn contents in the soil, obtained in 2000 were similar to those observed in 1999 (data not shown).

4.3.5. Mycorrhizal infection potential in the field soil

In January 1999, the infection potential of AMF was estimated in soils from the CT and NT treatments under wheat coming after rapeseed. The estimated numbers of infective propagules per gram of soil were 1.33 and 1.85 for the tilled and no-tilled soil, respectively, as assessed using the 10-fold soil dilution series. The numbers of infective propagules estimated by using the 2-fold dilution series were 1.43 and 1.86 propagules per gram of tilled and no-tilled soil, respectively.

In 2001, when the soil was sampled under wheat after maize, the number of infective propagules in soils was about one order of magnitude higher than in the previous check. MPN test yielded approximately 5 and 15 propagules per gram in the tilled and no-tilled soil, respectively (Fig. 4-8). However, no significant differences among the soil tillage treatments were detected.
**TABLE 4-1**  
Effect of tillage and sampling date on total P and Zn content, as well as on immediately available P (as assessed by isotope exchange kinetic method) in the topsoil (0-10 cm) under maize in 2000.

<table>
<thead>
<tr>
<th>Variable</th>
<th>df&lt;sub&gt;t&lt;/sub&gt;</th>
<th>df&lt;sub&gt;d&lt;/sub&gt;</th>
<th>df&lt;sub&gt;x d&lt;/sub&gt;</th>
<th>df&lt;sub&gt;residual&lt;/sub&gt;</th>
<th>F value for factor</th>
<th>Tillage</th>
<th>Sampling Date</th>
<th>TxD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P total</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>45</td>
<td>12.18</td>
<td>n.s.</td>
<td>0.38</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(*** P&lt;0.001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E&lt;sub&gt;1min&lt;/sub&gt;-P</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>45</td>
<td>17.85</td>
<td>n.s.</td>
<td>1.51</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(*** P&lt;0.001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn total</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>45</td>
<td>0.53</td>
<td>n.s.</td>
<td>2.14</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(*) P&lt;0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F values and respective df are shown, with measure of significance: ns not significant, (*)P<0.1, *P<0.05, **P<0.01, ***P<0.001

**FIGURE 4-7**  
Total and available phosphorus and zinc contents in the topsoil (0-10 cm) of differently tilled soils, in 2000. Total P and Zn data as well as the data on P availability were collected for several dates of the year, while Zn availability was estimated only in the samples taken on September 18, 2000. F-value from the one-way ANOVA comparing the tillage treatments and/or measure of the significance of the difference among the tillage treatments is given: (*) P<0.1, *P <0.05, ** P< 0.01, *** P<0.001. The results of mean comparison (LSD-based F test, P<0.05) are also given. Error bars indicate ± 1 SE.
4.4. DISCUSSION

4.4.1. Plant growth and nutrient acquisition

In the three seasons, no pronounced effects of the tillage management on the growth of maize and wheat plants were observed. Anken et al. (1997) have shown that no consistent yield difference was observed among the different tillage treatments in the experimental field at Hausweid since 1987. This is in contrast to other tillage experiments in Switzerland, where no-till management caused significant yield depressions, especially by maize (Rieger 2001). Growth depression was probably related to the reduced soil temperature under no-till in the spring, affecting either the meristem physiology of the plants (Chassot et al. 2001) and/or soil N mineralisation rate (Chapman et al. 2001).
The higher Ca concentration observed early in the season in both maize and wheat in the tilled soil is in agreement with the observation of Mozafar et al. (2000). In wheat, Mg concentration in biomass also increased early in the season in the tilled as compared with the no-tilled soils. This might be linked to the increased availability of Ca and Mg in the uppermost 15cm soil layer in tilled soils observed by Hussain et al. (1999) and to a faster colonisation of this horizon by roots of young plants in the tilled soil (Swift 1997). Later in the season, higher Zn concentrations were observed in plants growing in the no-tilled soil. Higher Mn, P, K, Mg, and Cu concentrations in plant tops were sometimes observed for the crops growing under NT conditions as compared with the CT, but the effects were usually rather limited. Our results are in agreement with previous observations by Mozafar et al. (2000), who observed higher Mn, Zn, K, and P concentrations in the biomass of mycorrhizal crops growing in the NT soils as compared with the CT ones, while no differences in nutrient uptake were observed among different tillage systems for nonmycorrhizal plant such as rapeseed. However, the effects we observed were more variable.

**4.4.2. Nutrient concentration in the soil (0-10 cm)**

The concentration of immediately available P in the soil was well above the critical level of 5mg P/kg soil, under which P becomes limiting for crops (Gallet 2001). Higher available P and K contents as well as accumulation of soil organic matter, which we observed in the upper soil layers of NT and CH soils were due to the lack of incorporation of fertilisers and crop residues to the deeper soil layers in both of those treatments (see also Table 3-1). These observations are in agreement with those of Robbins and Voss (1991), Holanda et al. (1998), Hussain et al. (1999) and Matowo et al. (1999), who observed an accumulation of P and K in the topsoil in no-tilled and chiselled fields, as compared with the ploughed soils. On the other hand, no effect of tillage on the concentration and availability of Zn were observed in our study site. This is probably because no Zn fertiliser has been applied to the fields since 1987, and all the Zn in the soil originated from the previously applied fertilisers (manure), or from the soil matrix.
4.4.3. AMF development in crop roots

The development of AMF in wheat roots was usually faster under the no-till conditions than under chisel- or conventional tillage. This was observed in wheat roots sampled from the topsoil (0-10 cm) throughout the season and also in the roots collected from different soil depths (data not shown). This points to possible adverse effects of mechanical disturbance imposed by tillage on AMF development (McGonigle and Miller 1993a, 1993b, McGonigle and Miller 1996b, Boddington and Dodd 2000b, Kabir et al. 1999). However, no negative effects of soil tillage on AMF colonisation of maize roots could be found in this study in contrast to Mozafar et al. (2000). In this study, the development of AMF was not measured during the autumn periods for winter wheat because of time limitations. Faster development of AMF in the NT soil as a consequence of rapid connection to an undisturbed extraradical mycelium of AMF (Boddington and Dodd 2000a) might have resulted in significantly higher colonisation rates in the wheat roots during early spring period. This should be most pronounced if the activity of AMF in the soil is high, such as following maize season. This hypothesis is well backed-up by our data (see AMF development in the seasons 1999 and 2001). Checking of AMF development during the early growth stage of winter crops (Dodd and Jeffries 1986) appears therefore to be important in order to elucidate which role do the AMF play in affecting growth and nutrition by the crop plants in the agrosystems.

Opposite to root colonisation by AMF, we observed a negative effect of reduced tillage on wheat root colonisation by nonmycorrhizal fungi (data not shown). It is known that soil tillage may have detrimental effects on many filamentous fungi (Stahl et al. 1999, Gill et al. 2001). It is possible that in our case, pronounced growth of AMF in no-tilled soils resulted in a competitive exclusion of nonmycorrhizal fungi from the roots (Azcon-Aquilar and Barea 1996, Sturz et al. 1997).

A reduction of both AMF and nonmycorrhizal fungal colonisation rates of wheat roots was observed with increasing soil depth at wheat flowering in June 1999 (data not
shown). Since AMF are strictly aerobic (Kabir et al. 1998), the decrease in AMF root colonisation with increasing soil depth might be related to the lower partial pressure of oxygen in the deeper soil horizon of the soil (Brady and Weil 1996). Another possibility is that both AMF and nonmycorrhizal fungi do not find enough host roots in the deeper layers to establish enough infective communities, merely because most of the roots are concentrated in the upper 30 cm (data not shown). Finally no significant differences were observed in wheat root distribution with depth between the different tillage treatments at wheat flowering in June 1999 (data not shown).

4.4.4. AMF infectivity in the soil

We observed a trend towards increased AMF infectivity in the no-tilled soil, which was however not significant. The reason why we could not detect any significant differences in infectivity among the soil tillage treatments is probably due to the low sensitivity of the MPN method which was used (Maldonado et al. 2000). However, there is no alternative method available for determining AMF infectivity in the soils yet.

The higher root colonisation rates by AMF of the wheat in 2001 were attributable to the improved AMF infectivity in soils following maize season (up to 15 propagules g⁻¹). On the other hand, relatively low colonisation rate in 1999 might be attributable to the deleterious effect of the previous rapeseed crop on soil AMF infectivity (1.5 propagule g⁻¹ soil).

4.5. CONCLUSIONS

1. AMF colonisation of roots of wheat plants in NT soils was faster as compared with the CH and CT treatments. Similar effect was, however, not observed for AMF colonisation of maize roots in this study, but was reported in the previous AMF
monitoring at the same field site (Mozafar et al. 2000). The infection potential of the field soil varied in the range of one order of magnitude, depending on the previous crop plant species grown in that soil, but was only slightly affected by the tillage treatment of the soil.

We therefore conclude that the activity of AMF communities in the soil was affected by soil tillage.

2. Soil tillage intensity sporadically affected uptake of mineral nutrients by the crops in the fields, depending on the element, season, and the crop plant species. The only systematic changes in crop uptake in response to different tillage regimes was higher Ca concentration early in the season in plants growing in the tilled soil, and higher Zn concentration in plants from the no-till soils later in the season. In contrast to Miller et al. (1995), we could not show a systematic effect of soil tillage on plant uptake of P. Because differences in soil chemical properties at different soil depths were shown among different tillage regimes in the studied field, different root development might be responsible for some of the nutritional effects observed on the plants under different tillage treatments. Therefore root development as affected by various tillage treatments should be further studied.

We conclude that differences in AMF activity induced by different tillage history of the soils did not unequivocally translate to differences in nutrient acquisition by the crop plants grown in the fields. However, the role of AMF on crop nutrition could not be realistically assessed because of the many confounding factors occurring in a field experiment.
5. EFFECT OF SOIL TILLAGE ON THE COMMUNITY OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) WITHIN MAIZE ROOTS*

ABSTRACT

We tested whether communities of AMF colonising maize roots were affected by soil tillage practices (conventional tillage, chisel, and no-till) in a long-term field experiment.

AMF were identified in the roots by specific PCR markers, which were developed using AMF that had previously been isolated from the study site. A nested PCR procedure, employing primers of increased specificity (eucaryotic > fungal > AMF species-group specific) was used. DNA sequencing confirmed that the DNA amplified from roots was of AMF origin. Presence of particular AMFs were scored as a presence of a DNA product after PCR with specific primers. Single-strand conformation polymorphism analysis (SSCP) of amplified DNA samples was used to check whether the amplification of AMF DNA from maize roots matched to the expected profiles for a particular AMF isolate with a given specific primer pair.

Presence of the genus *Scutellospora* in maize roots was strongly reduced in tilled and chiselled soils. Fungi from the suborder *Glominae* were most prevalent colonisers of maize roots growing in conventionally tilled soils, but were also present in the roots from other tillage treatments. This is the first report on community composition of AMF in the roots of a field-grown crop plant (maize) as affected by soil tillage. Our results provide direct experimental evidence that soil tillage affects community structure of the AMF associated with plant roots.

KEYWORDS

arbuscular mycorrhiza, root colonisation, maize, community structure, molecular identification, specific primers, soil tillage, field experiment

* submitted to Ecological Applications
5.1. INTRODUCTION

Up to now, studies on the community composition of AMF under different tillage regimes have all been based on the spore counts of the different AMF species in the soil (Douds et al. 1995, Jansa et al. 2002, see Appendix p.166). Shifts in composition of AMF spore communities associated with crops growing in differently tilled soils have been observed. For example, *Glomus etunicatum* was found to be more abundant in tilled soils, while *Glomus occultum* was more frequent in soil subjected to no-tillage for several years (Douds et al. 1995). Spores of various AMF species from the families *Acaulosporaceae* and *Gigasporaceae* were more abundant in soils under no-till for 12 years whereas in the conventionally tilled soils, almost only spores belonging to *Glomus* spp. were found (Jansa et al. 2002). Although the AMF spore composition in the fields was affected by soil tillage, no differences in the diversity of AMF spore communities in differently tilled soils were found (Jansa et al. 2002).

The studies based on the AMF spores isolated from the fields have only a limited relevance to the physiologically active stage of AMF, i.e. the intraradical and extraradical mycelium that colonises roots and grows out into the soil (Miller and Kling 2000). The observation of AMF colonisation structures in the roots (Merryweather and Fitter 1998) does however not allow reliable identification of AMF species (van Tuinen et al. 1998a). This limitation can be overcome by the use of molecular identification tools (van Tuinen et al. 1998a, 1998b). Their development in AMF research has been slow for two reasons. Firstly, AMF are obligate symbionts and can not be cultured without host plant roots (Smith and Read 1997). Thus, the amount and purity of material available for study is severely limited (Horn et al. 1993). Secondly, there exists a large variability of sequences within an individual AMF isolate (Sanders et al. 1995, Lloyd-Macgilp et al. 1996, Clapp et al. 2001), which is due to the specific cellular (multinucleate) organisation and the absence of sexual reproduction in this fungal group (Sanders 1999, Kuhn et al. 2001).
The development of molecular methods has recently allowed the establishment of a reliable protocol for identification of AMF species within plant roots and enabled also ecological studies in the field. Most of recent studies are based on a nested PCR procedure, targeted to both 18S (Clapp et al. 1995, Redecker et al. 2000) or 28S ribosomal DNA (vanTuinen et al. 1998a, 1998b). However, the limited number of available primers has hindered the use of this technique in highly diverse systems. The majority of the studies published so far were carried out under greenhouse conditions (vanTuinen et al. 1998a, Kjøller and Rosendahl 2000) or in rather low-diversity, polluted environments (Jacquot et al. 2000, Turnau et al. 2001, Jacquot-Plumey et al. 2001). Only little work has been done in complex natural communities such as agricultural fields or woodlands (Clapp et al. 1995, Helgason et al. 1998, Helgason et al. 1999, Kjøller and Rosendahl 2001, Daniell et al. 2001). These studies have indicated a higher diversity of mycorrhizal symbionts in undisturbed environments as compared with cropped land.

Because the information about the composition of AMF communities in the roots as affected by soil tillage is still missing, the objectives of this study were:

i) To isolate different AMF species from the field tillage experiment.

ii) Based on the freshly formed AMF spores produced in trap culture pots, to adapt a molecular technique for identification of AMF species in the roots of field grown crop plants. This identification technique will be based on the 28S-rDNA sequence variation among different AMF species.

iii) To use the method for identification of AMF in the roots of field grown maize in order to describe how the different AMF species colonising roots were affected by soil tillage intensity. Three different soil tillage practices representing a gradient in tillage intensity were tested: conventional tillage, chisel, and a no-till treatment.

The following hypothesis given at the beginning of this dissertation was tested in this chapter:
1) Soil tillage affects the species composition of AMF in the fields (hypothesis 1.2.).
This was done by addressing the following specific questions: Which AMF species are colonialising maize roots in the field? Is the abundance of any particular AMF species in maize roots positively or negatively affected by soil tillage?

5.2. MATERIAL AND METHODS

5.2.1. AMF cultures – isolation and propagation

AMF were isolated from the soil from the long-term tillage experiment described in the chapter 3.3.1. Soil samples were collected in the fields from the plots belonging to the three tillage treatments (till, chisel, and no-till). Trap cultures for multiplication of native mycorrhizal populations were established in January 1999, after the rapeseed season. A mixture of original field soil and quartz sand was planted with different host plants. After five months of cultivation in the greenhouse, spores were extracted from the growth substrate by the method of Daniels and Skipper (1982) and single-spore cultures were established on Plantago lanceolata L. Subsequently, maize (Zea mays L. cv. Corso) and wheat (Triticum aestivum L. cv. Albis) growing in 800 mL containers were inoculated with monosporic cultures of AMF and grown in the greenhouse for 2 months. Details about trap and single-spore cultures of AMF, as well as about the identification of the AMF from the studied site were described elsewhere (Jansa et al. 2002).

5.2.2. Development of molecular markers

AMF spores and colonised roots of maize and wheat from twelve single-spore cultures belonging to several species of Glomus spp. (G. mosseae, G. caledonium, G. intraradices, G. claroideum*), and spores isolated from AMF trap cultures (Jansa et al.

* Two different morphotypes of G. claroideum were included in this study, both of them previously isolated from the field site. The morphotype originally recognised as G. claroideum is referred as G. claroideum Type A throughout this dissertation, the morphotype resembling G. clarum or G. luteum is referred as G. claroideum Type B throughout this thesis.
AMF from the suborder Gigasporineae could not be established in pure cultures during the short period of this study, and thus we used fresh single spores recovered from trap cultures.

DNA from single spores was extracted according to Sanders et al. (1995). Samples were heated for 3 min (95°C) during extraction in order to improve the efficiency of DNA extraction. Five µL of the DNA extract were taken as template for PCR amplification of the large ribosomal subunit, 28S. Root pieces of maize and wheat colonised by monosporic isolates of Glomus spp. (5 cm fragments of 2 months old roots) for DNA extraction were carefully cleaned from adjacent spores and mycelium, frozen in a liquid N₂ in a ceramic mortar and powdered immediately. DNA was extracted from the root pieces by DNeasy Plant Mini Kit (Qiagen Sciences, Germantown MD, USA) following manufacturer’s recommendations. Five-µl aliquot was used for PCR amplification of the 28S gene. Root pieces of non-mycorrhizal maize and wheat were used as a negative control.

A nested PCR procedure employing firstly eucaryotic-specific primers ITS3 + NDL22 (White et al. 1990) and secondly fungal specific primers LR1 + FLR2 (vanTuinen et al. 1998b, Turnau et al. 2001) was performed on both spore and root DNA samples. There were 30 cycles with each primer pair. The product of the first PCR was diluted 1000 times, and five µL of the diluted mixture was used for the second reaction. The PCR conditions were the same as those described by vanTuinen et al. (1998b), except that the annealing temperature for both PCR steps was set at 60°C.

Ten µL of reaction mixture was used to check the amplification on 1.2 % agarose (SeaKem® LE, Flowgen, Leicestershire, UK) gel. The remaining product was then purified using QIAquick PCR Purification Kit (Qiagen Sciences), and cloned into a blue script vector (pGEM-T Easy, Promega-Catalys AG, Wallisellen, Switzerland) and transformed into bacterial strain E. coli JM109 by the heat-shock method. The size of the insert in growing bacterial colonies was checked after PCR amplification using M13+
and M13- primers that were targeted to the cloning site of the vector. Plasmid DNA was isolated from transformed bacteria (following standard Miniprep procedure, Sambrook et al. 1989), and used as a template for cycle sequencing using BigDye™ Terminator. Sequencing analysis was performed on ABI-310 Capillary Sequencer (Perkin-Elmer, Wellesley MA, USA). All the sequence data were computer-edited and re-edited manually. Sequences obtained from this screening were deposited in the GenBank under accession numbers AF396782-AF396799.

AMF 28S sequences obtained in this study and from the Genbank (28S sequences for Gigaspora rosea, Scutellospora castanea and several Glomus spp.) were aligned by

**FIGURE 5-1**
Identification of five phylogenetic lineages of AMF (contrasted with gray background), for which specific primers were designed based on sequencing of LR1-FLR2 fragments (700 bp long) of 28S from both spores and plants (maize or wheat) inoculated with single-spore cultures, and from spores recovered from the trap cultures. Numbers refer to isolate code numbers. Sequences of Gigaspora and Scutellospora originate from spores from trap cultures. Sequences from nonmycorrhizal roots belong to asco- and basidiomycetous fungi. The sequence of Sinapis alba obtained from the GenBank was used as an outgroup. Sequences obtained by PCR from the non-mycorrhizal roots showed similarity to basidiomycetous and ascomycetous fungi (analysis not shown).
FIGURE 5-2
Position of primer binding sites for the primers used for detection of AMF within host plant roots for the 3rd PCR of the nested PCR procedure. Binding sites of fungal specific primers LR1 and FLR2 (van Tuinen et al. 1998b) are also shown.

TABLE 5-1
Specificity and length of the expected DNA fragment obtained in PCR with primers constructed for *in-planta* identification of AMF.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
<th>Targeted specificity</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clar1A</td>
<td>CGA TCG AAG TCA GTC GTG CTG GCG</td>
<td><em>Glomus claroideum</em> group</td>
<td>265</td>
</tr>
<tr>
<td>Clar1B</td>
<td>CGA TTG AAG TCA GTC GTG CTG GCG</td>
<td><em>Glomus claroideum</em> group</td>
<td>265</td>
</tr>
<tr>
<td>Clar2A</td>
<td>AAG AGA AGC CAG GTG GAA CAG CCC</td>
<td><em>Glomus intraradices</em></td>
<td>254</td>
</tr>
<tr>
<td>Clar2B</td>
<td>AAG AGA GGC CAG GTG GAA CAG CCC</td>
<td><em>Glomus intraradices</em></td>
<td>254</td>
</tr>
<tr>
<td>Intra1</td>
<td>GGT GCG ATT CTG TGG AGT GTG AGG</td>
<td><em>Glomus intraradices</em></td>
<td>254</td>
</tr>
<tr>
<td>Intra2</td>
<td>CAA GCT TTC ACC AGA GCA ACC</td>
<td><em>Glomus intraradices</em></td>
<td>254</td>
</tr>
</tbody>
</table>
| Cal1        | CCT CTT GAG TTT GGT TCT GTG GG | *Glomus caledonium*
| Cal2        | AGG CAA CGT TTC AGA GAT CAG ACC | *Glomus mosseae* group | 248 |
| Gig1        | GGT ATC ATA GAG GGT GAG AAT CCC | *Gigaspora* sp. (genus) | 308 |
| Gig2        | AAA TCG ACG CTA ACC TGC CAA ACG | *Gigaspora* sp. (genus) | 308 |
| Scut1       | AGT AAT GAC GGA GGG TGA AAG TCC C | *Scutellospora* sp. (genus) | 434 |
| Scut2       | CGT ATT AGA GAC CAG GCG GTT AAC C | *Scutellospora* sp. (genus) | 434 |

ClustalW software (Thompson et al. 1994). Phylogenetic analysis was performed (Phylip software, see statistical analysis section) to identify distinct AMF groups, for which specific primers were then designed (Fig. 5-1).

The specific primers were designed so as to amplify fragments 250 bp to 450 bp long (Fig. 5-2, Table 5-1). DNA extracts from AMF spores were used to crosscheck that
the constructed primers specifically amplified the targeted fungal group, and do not cross-amplify other AMF. The non-specific primers were discarded. Specific primers for detection of *Glomus claroideum* group were partially degenerated in one base each (Table 5-1).

For each AMF group, the specific primers were used separately in a third nested PCR. The second PCR product was diluted 1:1000, and 5 µL of the solution was used as a template for the third reaction. This was run under stringent conditions, denaturation at 95°C for 3', 32 cycles of 93°C for 60", 68°C for 60", and 72°C for 60", final extension at 72°C for 5'. The composition of the PCR mixture was the same as described by vanTuinen et al. (1998b), except for the primers. The amplification product was analyzed on 2% agarose gel. A subsample of these products were cloned and sequenced and compared with the 28S sequences obtained previously. This comparison showed that the number of mutations in the sequences introduced by many PCR cycles did not affect the position of the sequences in the phylogenetic tree (analysis not shown).

### 5.2.3. Field sampling of roots

From each of the 12 field plots (conventional tillage –CT, chisel – CH, no tillage – NT, four replicates) of the field experiment described in the section 3.3.1. of this dissertation, three 90days old maize plants (cultivar LG 22.65) were randomly sampled on 27 July 2000 together with their roots from the depth 0-15 cm. The roots were carefully cleaned with tap water and washed several times with deionized water. Roots were cut into 5-cm pieces, and four fine root pieces (< 1 mm in diameter) were sampled from each plant. Each root piece was separately washed again in molecular-grade sterile water and then the DNA was extracted using the DNeasy Plant Mini Kit as described above. Altogether, 144 root fragments were tested.

### 5.2.4. Molecular identification of the AMF in the roots

DNA samples from root pieces were subjected to the nested PCR protocol as described above. The reaction product of the first PCR reaction was diluted 1000 times
prior to the second PCR. The reaction product of the second PCR was 1000 times diluted for amplification with the specific primer pairs. Five separate PCR reactions were performed with 5 different primer pairs on each DNA sample in the last step. Presence/absence of reaction product of each reaction was scored after electrophoresis of 10 μL aliquot on 2% agarose gel (Fig. 5-3). Root colonisation was calculated as a percentage of presence of a given fungus in root fragments, and the data for each plot were pooled for statistical analysis.

**Fig 5-3a**

(a) PCR reaction product of first (ITS3-NDL22) and second (LR1-FLR2) PCR of the nested procedure on four DNA samples from four root fragments (pieces) of a single field grown maize plant from a no-till plot. (b) Presence and absence of different AMF in those four root pieces from a single plant as determined by visualisation (ethidium bromide staining) of the PCR products after the third nested PCR using five primer pair combinations. Variability of AMF colonising different root pieces from a single root system of a field grown maize plant is shown. The specificity of the primers is indicated above each lane.
A sequencing control was performed on the DNA samples from the field roots after the three PCR steps. Those sequence data were used to check the identity of the samples from roots, and they were later deposited in the GenBank under accession numbers AF396800 – AF396826. Sequence data obtained from the field root samples, the sequences obtained from pure isolates of AMF from the studied field site, and the sequences obtained from the GenBank were used in a phylogenetic analysis so as to confirm the identification of the AMF from the roots.

5.2.5. SSCP analysis of amplified DNA samples

Where positive amplification with specific PCR primers occurred, the amplified DNA samples were further analysed by single-strand-conformation-polymorphism analysis (SSCP) in order to distinguish lower taxonomical units within the species-groups of AMF targeted by those specific primers. The specific primers for each of the five AMF species groups (Table 5-1) were designed so that the size of the DNA fragments obtained after the third PCR would be optimal for SSCP analysis.

DNA was denatured by mixing 4 μL of PCR sample with 8 μL of formamide (containing 10 mM NaOH), heated at 95°C for 5 min, and placed on ice for 5 min. Samples were loaded onto pre-cooled (5°C) SSCP precast gels (GMA™ Wide Mini S-26, Elchrom Scientific, Cham, Switzerland) and resolved in Elchrom SEA-2000 electrophoresis apparatus at 5 V cm⁻¹ for 16 hours. Gels were stained with SYBR Gold II dye (Molecular Probes Inc., Eugene OR, USA) and photographed on a short-wave, 254 nm transluminator (Elchrom).

DNA samples from spores of the monosporic isolates of AMF were also subjected to the same SSCP (Fig. 5-4). The profiles (molecular types) obtained were used as reference to identify the profiles produced from root extracted DNA, and as a further check for successful amplification the targeted AMF. Presence or absence of a specific
SSCP profile in field samples amplified with respective primers was scored and the root colonisation by a given molecular type of AMF was calculated per plot.

An exception was made in case if pure AMF isolates from the studied site were not available (e.g. *Gigaspora* and *Scutellospora*) or if a completely distinct profile was recognised among SSCP of field samples that could not be related to any of the pure isolate samples studied (e.g. *G. caledonium*). In those above given cases, distinct SSCP profiles were described and evaluated independently (see also Fig. 5-7).

### 5.2.6. Statistical analysis

Colonisation of roots was calculated from the AMF presence/absence data in the respective 12 root pieces (4 root pieces per plant, 3 plants per plot) from each of the field plots. Colonisation rates for each AMF in each field plot were used in MANOVA and following one-way ANOVAs with the soil tillage treatment as a factor and four replicate plots for each treatment. Statistical analyses were carried out with SAS software version 6.12 (SAS Institute Inc., Cary, NC, USA) and Statgraphics® software version 3.1 (Manugistics 1997). Acceptable level of significance for this study was set at $P<0.05$. Results with $0.05 \leq P < 0.1$ were considered to be marginally significant, with regard to the fact that the study was carried out under field conditions. LSD Multiple range test ($P < 0.05$) was performed to test significant differences among the means provided ANOVA analysis gave significant results.

Phylogenetic analyses were performed on sequence data. Multiple alignment of sequences was performed using ClustalW software (Thompson et al. 1994). Phylogenetic relationships were inferred using Phylip Program Package (Felsenstein 1993), employing neighbour joining algorithm. The Kimura distance model was used, in which value ratio of transition/transversion was set at 2. The identity of outgroup species used to root the dendrograms is indicated separately for each analysis. Sequences deposited in the GenBank were used for comparison.
FIGURE 5-4
Variability of SSCP profiles of DNA samples amplified with primers specific for two species-groups of AMF. The gels show comparison of samples amplified in three-step PCR reaction from spores of single-spore isolates and samples amplified from field-grown maize roots. Colours on the pictures were inverted to obtain better contrast. (a) Samples amplified with primers Cal1 and Cal2 (specific for *G. caledonium-G. mosseae* group). Lane 1 - spore of *Glomus mosseae* isolate 964, lane 2 - spore of *Glomus caledonium* isolate 658, lanes 3-11 – field root samples. (b) Samples amplified with primers Clar1A+B, Clar2A+B (specific for *G. claroideum* group). Lane1 - spore of *Glomus claroideum* isolate 133, lane 2 - spore of *Glomus claroideum* isolate 360, lane 3 - spore of *Glomus claroideum* isolate 672, lanes 4-13 - field root samples.
5.3. RESULTS

5.3.1. PCR marker study

All PCR markers used in this study were confirmed not to amplify other AMF species from the studied field. This was done by cross-specificity PCR test, in which spore DNA extracts of all available AMF were amplified with all possible PCR primers (analysis not shown).

Nested PCR amplification of root DNA samples from field-grown maize using AMF groups-specific primers showed differences in colonisation profiles even among root pieces from the same individual plant (Fig. 5-3). Some plants contained AMF from all five types that were tested, within their root system (Fig. 5-3). Difference in AMF richness was observed among the soil tillage treatments (ANOVA F = 9.0, P < 0.007), Roots in the conventionally tilled soil were colonised with AMF communities with significantly lower richness than that in the no-tilled soils. These differences were due to higher abundance of *Gigasporaceae* in the no-tilled soils (see also Fig. 5-6).

A subsample of the DNA that had been subjected to the three-step PCR reaction was cloned and sequenced to test the identity of sequences amplified from the field roots. This was necessary because there is a possibility that the primers could amplify DNA from fungi, which had not previously been isolated from the field or for which those particular primers were not designed. Phylogenetic analysis of a selection of those sequences and the respective GenBank accessions is shown in Fig. 5-5. Sequences obtained from roots by primers targeted for three *Glomus* groups fitted into the expected clusters (Fig. 5-5a). Sequences obtained using specific primers targeted to *Scutellospora* and *Gigaspora* did indeed cluster within these AMF genera. However, the diversity of AMF from the genus *Gigaspora* in our experimental field might have been substantially higher than expected from previous analysis of spores formed in trap pots. This conclusion is drawn from the deeply branched tree structure of *Gigaspora* sequences from field-root samples, as compared with the sequences obtained from spores from trap
cultures, which were clustering close to the reference sequence of *Gigaspora rosea* (Fig. 5-5b).

**Fig 5-5a (for legend see next page)**

- **G. fragilistratum** AF145747
- **G. caledonium** 658
- **G. caledonium** AF145745
- **G. constrictum** AF304981
- **G. coronatum** AF145740
- **G. geosporum** AF145744
- **J3-caledonium**
- **J6-caledonium**
- **J5-caledonium**
- **J8-caledonium**
- **J7-caledonium**
- **G. mosseae** 243
- **G. mosseae** AF304994

**G. claroideum - G. mosseae group**

- **G. claroideum** AF236716
- **G. claroideum** 133
- **G. claroideum** AF236743
- **G. claroideum** AF235008
- **G. etunicatum** AF145749
- **G. claroideum** 132
- **J1 claroideum**
- **J2 claroideum**

**G. intraradices group**

- **J12 intraradices**
- **J13 intraradices**
- **G. intraradices** 141
- **G. intraradices** x99640

**Gigaspora rosea** Y12075

**Fig 5-5b**

- **S. castanea** Y12076
- **S. scutellospora**
- **S. scutellospora** Y12076
- **S. scutellospora**
- **S. scutellospora**
- **S. scutellospora**
- **G. rosea** Y12075
- **G. margarita "clone 58"**
- **G. margarita "clone 60"**
- **G. margarita"clone 90"**
- **J19 Gigaspora**
- **J17 Gigaspora**
- **J18 Gigaspora**
- **J14 Gigaspora**
- **J15 Gigaspora**
- **J16 Gigaspora**
- **J11 Gigaspora**
- **J10 Gigaspora**
- **J27 Gigaspora**
- **J26 Gigaspora**
- **J21 Gigaspora**
- **J20 Gigaspora**
- **J24 Gigaspora**
- **J23 Gigaspora**
- **Glomus claroideum** 133
**FIGURE 5-5**

Phylogenetic analysis to check the origin of the sequences obtained from maize roots from the field, using the five group-specific primer pairs. Sequences obtained from the roots are contrasted by grey background and they are labelled with J followed by 2-digit numbers and the name of the fungal group, for which the primers were targeted for (these groups are shown in bold at the right margin). Sequences obtained from GenBank are labelled with their respective entry numbers (AF, X, or Y followed by a number). Sequences labelled with AMF species name and a 3-digit number originate from single-spore cultures isolated from the studied field site. The sequences of *Gigaspora* and *Scutellospora* labelled with the clone number were obtained from spores recovered from trap cultures. All the sequences obtained from AMF spores from the studied field site are additionally labelled with a suffix SP.

(a) Phylogeny of sequences amplified from maize roots by group-specific primer pairs targeted for different *Glomus* sp. groups. *Gigaspora rosea* sequence from GenBank was used as outgroup.

(b) Phylogeny of sequences amplified from maize roots using group specific primer pairs targeted for *Gigaspora* sp. and *Scutellospora* sp. Sequence from a single-spore isolate of *Glomus claroideum* was used as outgroup. Position of branch containing the only 28S sequence of *Gigaspora* currently present in the GenBank (*Gigaspora rosea* Y12075), clustering in the dendrogram close to *Scutellospora* sp. could not be confirmed by parsimony analysis.

MANOVA analysis of the abundance data of AMF detected in the maize roots (Fig. 5-6), showed a significant effect of soil tillage on the community structure of AMF (Hotelling-Lawley Trace statistic $F = 39.5$, $P < 0.0001$; Wilks’ Lambda $F = 20.1$, $P < 0.0001$). The presence of *Scutellospora* sp. in the roots was significantly reduced in both conventionally tilled and chiselled soils as compared with the no-till. Colonisation of roots by *Glomus intraradices* was significantly higher in the tilled and chiselled soils as compared with the no-till. This trend was also true for colonisation by the *Glomus claroideum* group although this was only marginally significant. Root colonisation by the *Glomus mosseae-G. caledonium* group as well as by *Gigaspora* sp. was not affected by tillage practices (Fig. 5-6).
5.3.2. SSCP study

Distinct SSCP profiles within each set of samples obtained from field-grown maize roots were identified and compared with the SSCP profiles obtained from DNA amplified from the spores of AMF previously isolated from the same field site (Fig. 5-7). By doing this, closely related AMF species could be distinguished from each other within samples where they were co-amplified by group-specific primers (as it was the case in the *Glomus mosseae-G. caledonium* and *Glomus claroideum* groups). MANOVA showed significant effects of soil tillage treatments on the abundance of different molecular types of AMF (Hotelling-Lawley Trace statistic F = 10.8, P < 0.088; Wilks’ Lambda F = 7.1, P < 0.009). Root colonisation by both molecular types of *Scutellospora* sp. was significantly reduced in tilled and chiselled soils, as compared with the no-tilled soils. One of the *Scutellospora* types was detected only in the no-tilled soils, and it was fully absent from tilled soils (molecular type A, Fig. 5-7). None of the three different *Gigaspora* types were affected by soil tillage. Tillage intensity also did not affect the abundances of *Glomus mosseae* and *Glomus caledonium* at 95% probability level. However, increase in colonisation by *Glomus caledonium* (type B) with increased tillage intensity was observed at 90% probability level. Increased tillage intensity increased the abundance of *Glomus claroideum* type A, while it did not affect the development of *Glomus claroideum* type B. Colonisation of all three types of *Glomus intraradices* in the roots of field-grown maize increased as a result of increased soil tillage intensity (Fig. 5-7).

5.4. DISCUSSION

This study has shown for the first time that an agricultural management practice, namely soil tillage, affects AMF community structure within the roots of a crop plant (maize) in the fields. This was possible because of the combination of appropriate sampling within an established experimental design, and because reference AMF cultures had previously been isolated from the same field site (Jansa et al. 2002). Several AMF genera were observed to colonise roots of maize growing in intensively agriculturally used soils in contrast to previous studies suggesting very low abundance of non-*Glomus*
FIGURE 5-6
Effect of soil tillage practices on percentage of colonisation of maize roots by five different AMF groups as assessed by AMF-group specific PCR primers. Different letters above bars indicate a significant difference according to LSD test (at the P<0.05 level of significance). F-ratio for the ANOVA showing the tillage treatment effect is given below each graph, with a measure of significance: n.s. not significant, (*) P<0.1; * P<0.05, ** P<0.01, *** P<0.001. Bars represent +1 standard error of mean.
Fig 5-7 (for legend see next page)

SSCP types

1. *Scutellospora* sp.
   - Colonization of roots of maize growing in differently tilled soils by different molecular types of AMF
   - Sampling wells
   - Single stranded DNA bands
   - Double stranded DNA

   Types A, B

   **Scutellospora type A**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 7.38^* \)

   **Scutellospora type B**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 9.10^{**} \)

2. *Gigaspora* sp.
   - Types A, B, C

   **Gigaspora type A**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 0.26 \text{ n.s.} \)

   **Gigaspora type B**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 1.95 \text{ n.s.} \)

   **Gigaspora type C**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 1.50 \text{ n.s.} \)

3. *Glomus mosseae- Glomus caledonium*
   - Types A, B, C

   **Glomus mosseae (type A)**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 0.74 \text{ n.s.} \)

   **Glomus caledonium I (type B)**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 3.10^* \)

   **Glomus caledonium II (type C)**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 2.55 \text{ n.s.} \)

4. *Glomus claroiduem*
   - Types A, B

5. *Glomus intraradices*
   - Types A, B, C

   **Glomus intraradices type A**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 3.13^* \)

   **Glomus intraradices type B**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 6.28^* \)

   **Glomus intraradices type C**
   - Percentage of colonized root fragments
   - \( F_{(2,9)} = 3.20^* \)
Colonisation of roots of maize grown in the field soil subjected to different tillage, by different molecular types of AMF. The types were defined based on SSCP banding pattern of DNA samples of isolated AMF cultures or on the distinct SSCP patterns identified among the field samples, amplified in 3-step PCR procedure. Up to 3 subtypes were distinguished in each AMF species-group. Colours of SSCP images were inverted to obtain better contrast.

SSCP types were identified in the groups of *Scutellospora* and *Gigaspora* based on the DNA samples amplified from the field roots. SSCP types in *Glomus mosseae-Glomus caledonium* group were defined based on profiles both from single-spore isolates (type A and B, corresponding to spores of *Glomus mosseae* and *Glomus caledonium* resp.) and from the field samples (type C). SSCP types in *Glomus claroideum* group were defined based on profiles of single-spore isolates (type A - *Glomus claroideum* type A (isolate 132), type B - *Glomus claroideum* type B (isolate 133)). Within *Glomus intraradices*, only presence/absence of particular bands could be detected, because the band composition was very similar among the samples and the samples differed mostly in intensity of the bands. Three particular bands could be distinguished and separately evaluated. Positions of those bands are shown with arrows in the picture. Different letters denote significant differences between the three tillage treatments according to LSD Multiple Range Test (P<0.05), which was only performed where the ANOVA showed significant differences among tillage treatments. F-ratio for the ANOVA showing the soil treatment effect is given below each graph, with a measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Bars represent ±1 standard error of mean.

AMF genera in the roots of plants from arable land (Helgason et al. 1998, Daniell et al. 2001). However, the statistical analysis in those referred studied was limited due to absence of a suitable sampling design.

The changes, which were observed in AMF community composition in response to different soil tillage practices might be due to i) the direct effects of tillage on AMF hyphae and/or ii) indirect effects of soil tillage such as change in soil physical and chemical properties or change in weed populations in the fields, which might in turn affect the AMF communities in the soil.
Different intensities of mechanical disturbance imposed on the soil by different tillage regimes (due to soil cutting and/or mixing) may directly affect mycorrhizal hyphae by breaking up the extraradical mycelium. Boddington and Dodd (2000a) have shown that soil disturbance reduces the total amount of AMF mycelium in the soil and also decreases AMF species richness. Different AMF might also depend to a different extent on intact hyphal networks for colonising plant roots. Boddington and Dodd (2000b) have shown that colonisation of roots by *Glomus manihotis* was increased by soil disturbance, while the opposite was true for *Gigaspora rosea*. It seems that the undisturbed hyphal network might play an important role in both survival and root colonisation establishment of *Scutellospora* (Jasper et al. 1993) and *Gigaspora* (Boddington and Dodd 2000b). Therefore, the absence and the very low abundance of *Scutellospora* in conventionally tilled and in chiselled fields, respectively, as shown in this study, might be due to the adverse effects of soil tillage (cutting) on integrity of its soil hyphae. *Gigaspora* sp. that was found frequently in roots growing in both tilled and no-tilled soils in this study is probably able to accommodate to the soil disturbance more efficiently than *Scutellospora*. On the other hand, experimentally applied soil disturbance was shown to increase colonisation of roots by *Glomus manihotis* (Boddington and Dodd 2000b), which is in agreement with our finding of higher incidence of *Glomus* spp. in tilled soils. Reduced root colonisation rates of *Glomus* spp. under no-tillage conditions may be the result of competitive exclusion of these fungi by others, such as *Scutellospora*. We are, however, aware that some other AMF (*Acaulospora* and *Entrophospora*) might have been missed in this study, because we could not develop appropriate molecular detection tools for them.

Soil tillage may also have indirect effects on AMF by changing soil properties, nutrient stratification, or composition of microbial communities and aboveground plant communities that in turn affect AMF. Mineral fertilisation has been shown to affect the composition of AMF communities in the soil (Johnson 1993, Douds et al. 1993, Ezawa et al. 2000, Titus and Leps 2000). Particularly, nitrogen fertilisation was shown to have a negative impact on *Gigasporaceae* populations (Egerton-Warburton and Allen 2000,
Egerton-Warburton et al. 2001). In our study the very low abundance of *Scutellospora* spp. observed in the tilled and chiselled soils whereas it was present in the non-tilled soil suggests that this AMF was less sensitive to the difference in nutrient availability than to soil cutting. In the 0-10 cm soil layer nutrient availability in the non-tilled and chiselled treatments were similar and higher than the nutrient availability observed in the tilled treatment whereas the contrary was observed in deeper layer. However nutrient availability was measured in 10 cm layers whereas the presence of AMF in maize roots was assessed in the 0-15 cm layer and therefore results can not be strictly compared. Further work is needed to assess the importance of nutrient stratification on the AMF in roots sampled in differently tilled soils.

Both total microbial activity (Palma et al. 2000) and the structure of microbial communities (Lupwayi et al. 1998, Guggenberger et al. 1999, Lupwayi et al. 2001) can be affected by soil tillage practices. The changes in AMF communities observed in our study in maize roots growing on differently tilled soils might be explained by changes in AMF interactions with other soil micro-organisms. However, only little consistent information is available about interaction of AMF with other micro-organisms, but both positive and negative effects have been reported (Germida and Walley 1996).

Another possible reason explaining our results showing altered AMF community composition in no-tilled fields as compared with the tilled ones might be the changes in composition in weed plant community in response to different tillage practices. Streit et al. (2000) observed an increase in the proportion of perennial weeds and O’Donovan and McAndrew (2000) observed a respective decrease in the proportion of annual weeds in no-tilled as compared with the tilled soils. Therefore, with increased abundance of long-living weeds in the no-tilled fields, *Gigasporaceae* might have established symbiosis with a more suitable plant partner than cereals or maize such as legumes (Hendrix et al. 1995) or grasses (Bever et al. 1996), which would facilitate production of new spores.
The molecular identification methods used in this study have been developed only recently (van Tuinen et al. 1998b, Kjøller and Rosendahl 2000) and that is why they are prone to irregularities. The high reliability of the results was reached by using many controls at every stage of the identification procedure, and by fine tuning of the identification procedure for previously characterised AMF communities, from whose many AMF species had been cultivated in monosporic cultures (Jansa et al. 2002). This was never fully accomplished in the previous studies (Helgason, 1998, Kjøller and Rosendahl 2000, Turnau et al. 2001), in which only a limited AMF diversity could have been studied. However, this approach requires extensive sequencing necessary for development of markers and for the feedback controls. The cross-specificity tests and the sequencing controls performed with the DNA samples amplified from field samples showed that the AMF species-groups, which were targeted by the specific PCR markers, are genetically disjunctive and do not share rDNA sequences among them. This confirmed the use of the PCR technique for the identification of AMF, and allowed to study AMF community within the roots. It has to be, however, mentioned that the markers developed in this study might co-amplify some other AMF from other ecosystems and that much more information is needed for development of universal AMF-specific markers useful in any ecosystem world-wide.

Ribosomal RNA genes within AMF species are highly variable (Sanders et al. 1995, Lloyd-MacGilp et al. 1996, Clapp et al. 2001, Kuhn et al. 2001, Rodriguez et al. 2001). Therefore, the results shown in Fig. 5-3b and Fig. 5-7 must be interpreted as follows: multiple bands on PCR gels (samples amplified with G. claroideum – specific primers) and different SSCP profiles (i.e. different molecular types) are representing different sequences (Sheffield et al. 1993). These different sequences must not necessarily represent different individuals, but might also represent different genotypes within the same individual. For SSCP gels, however, multiple bands might also represent different spatial conformations of the same sequence. This usually does not appear while using ethidium bromide for visualisation of SSCP DNA bands, but is frequently encountered when more sensitive staining methods (such as silver staining) are used.
In this study, a highly sensitive staining method was used and therefore the detection of several conformations of a single sequence type per sample is also possible.

For some AMF species (e.g. *G. mosseae*), identification of species-specific SSCP patterns could have still been done due to extensive calibration with pure cultures. For others such as *Gigaspora* or *Scutellospora*, we prefer to keep the label “molecular type” until more information is available.

### 5.5 Conclusions

1. Five different AMF species-groups could be distinguished by the PCR-based molecular identification within the roots of field grown maize. Further SSCP analysis allowed identification of different molecular types within each of the groups. The relevance of those molecular types to particular AMF species is, however, still unclear, and deserves further study.

2. Soil tillage intensity alters the composition of AMF communities colonising maize roots under field conditions. Colonisation of maize roots by *Scutellospora* sp. was depressed by mouldboard tillage and by chiselling, while the opposite was true for the members of *Glomaceae*. This might be due to the direct effects of soil tillage (mechanical disturbance of the soil) on the performance of different AMF, or due to secondary effects of tillage on the field ecosystem properties such as nutrient stratification, soil physical and chemical properties, microbial and plant community composition etc. The future challenge is to investigate the functional aspect of the tillage-induced AMF community shift on the performance of field grown plants.
6. **Effects of AMF from Differently Tilled Soils on Plant Growth and Nutrient Acquisition in Pots with a Single Compartment**

**ABSTRACT**

Glasshouse experiments were conducted using small pots with a single compartment where both roots and AMF could develop, to study if the AMF from differently tilled soils (tilled, chiselled, no-till) have different effects on root colonisation, maize growth and nutrient uptake. Both natural AMF communities and single spore AMF isolates from differently tilled soils were used as inoculum.

In a first experiment maize was grown for 60 days in the presence of different rates of P fertilisation either in soils sterilised and re-inoculated with soil bacteria (AMF-free substrate) or in non-sterilised soils (AMF-containing substrate). Soil sterilisation, which resulted in the elimination of AMF and of other organisms lead to an increase in maize biomass production and in P uptake, especially under high P supply. This might be related to the removal of soil pathogenic organisms, as well as to the changes in soil properties due to autoclaving. As similar differences were observed in plant P uptake between the different tillage treatments in the presence or in the absence of AMF, it was concluded that the AMF from differently tilled soils did not differ in their effects on plant P uptake, but the effects were due to the differences in soil chemical properties. Root colonisation with either AMF or nonmycorrhizal fungi was less affected by high P supply for the fungi from no-till as compared with the tilled soils. Our results nevertheless suggest that the AMF community from the no-till soil improved Zn acquisition by maize.
In a second experiment maize was grown for 54 days in a low P substrate in the presence of eight monosporic isolates belonging to *Glomus* spp., which were obtained from tilled and no-tilled soils. Maize top biomass production was not influenced when compared with the nonmycorrhizal control plants. However, different species of AMF had significantly different effects on plant growth. The growth of maize roots was influenced by the inoculation with different AMF to a greater extent than the top growth. *Glomus intraradices* and *G. mosseae* strongly reduced maize root growth, while *G. claroideum* did not affect root growth. Total P uptake of the plants was increased only by *G. intraradices*, but not with other AMF. Total P uptake was correlated to the chitin content of the roots and not to the AMF root colonisation estimation obtained by root staining. The chitin content of roots therefore seems to better quantify the amount of functionally relevant AMF biomass in the roots than the semi-quantitative staining methods.

**KEYWORDS**

arbuscular mycorrhizal fungi (AMF), field community, tillage, monosporic isolate, *Glomus*, pot experiment, phosphorus (P) uptake, *Zea mays* (maize), colonisation
6.1. INTRODUCTION

In this chapter we tested the following hypotheses expressed at the beginning of the dissertation:

1) through the change in AMF communities the nutrient acquisition efficiency (NAE) by the plants growing in differently tilled soils is changed (hypothesis 2),

and

2) soil tillage affects functional properties of the AMF species (hypothesis 1.3.);

so as to demonstrate whether AMF from differently tilled soils have different effects on maize growth and nutrient acquisition.

These hypotheses were tested in two experiments assessing the effect of i) the entire AMF communities from the differently tilled soils (ploughed, chiselled, no-till) and ii) of single AMF isolates obtained from the tilled and no-tilled soils on plant growth and nutrient uptake. These experiments were conducted in single pots (i.e. in pots presenting a single compartment where roots and AMF develop). In the first experiment, maize was grown with increasing P fertilisation rates in the presence of non-sterilised soils containing AMF from the different tillage treatments (CT, CH, NT) in comparison to soils from which AMF had been removed by sterilisation (experiment 1). In the second experiment we studied the influence of three Glomus species isolated from NT and CT, which had been observed in the roots of field-grown maize (chapter 5), on root colonisation rate, maize growth, and P uptake in a low P substrate (experiment 2).
6.2. MATERIAL AND METHODS

6.2.1. Growth and nutrient uptake by maize as affected by indigenous AMF from differently tilled soils (experiment 1)

Field soil from the upper horizon (0-15 cm) was collected in October 1999 (after the wheat season) from the long-term field tillage experiment conducted in Tänikon-Hausweid (described in section 3.3.1). The soil was collected from 12 field plots, belonging to 3 different soil tillage treatments: conventional tillage (CT), chisel (CH), and no-till (NT), each replicated four times. Two litres of soil from each of the field plots were autoclaved at 121°C for 1 hour, and then treated with soil washing from the respective field plot and finally incubated at ambient temperature for 2 months. Soil washing was prepared by mixing unsterile field soil with tap water (1:10 w:v), and filtering 3 times through a Whatman No 1 filter. This resulted in the elimination of AMF (but also of some other fungi, nematodes, protozoan etc.). Other soil micro-organisms passing through the paper filter were, however, reintroduced after the sterilisation. Unsterilised soil from each of the field plots was used as a mycorrhizal treatment. The soil samples were mixed with sterile quartz sand (0.7 - 1.2 mm) in a 1:4 (v:v) ratio. This substrate mixture was put into 300 mL pots (410 g dry weight of soil-sand mixture per pot). Twenty pots were prepared for every of the 24 treatments (3 soil tillage treatments, four field replicates, and factorial combinations with mycorrhizal and non-mycorrhizal treatment). Maize (Zea mays L. cv. Corso) was grown in the pots (1 plant per pot) for 60 days (until the apparition of male inflorescence) under greenhouse conditions (16h photoperiod, light intensity min. 400 µmol photon m⁻² s⁻¹, 25/22°C day/night, resp.). The plants were fertilised daily with 2 mL of 7-times concentrated Hoagland nutrient solution (Sylvia and Hubbell 1986). Each treatment was split into four P fertilisation sub-treatments (each containing five replicate pots), receiving 1,10,100, and 1000 µM phosphate in a form of KH₂PO₄ (the total amount of introduced phosphorus in the highest fertilisation level was 3.7 mg of P per pot). In this design, the effects of the tillage treatment from which AMF were obtained, and the effect of removing AMF communities
from the substrate, on maize biomass production and nutrient uptake could be explored under different P-fertilisation levels. P availability in the substrate was low when no P was added (E_{1min-P} = 2.9 mg P/kg substrate, see method in section 4.2.1.3.).

Because the soil from all tillage treatments was exposed to the same mechanical disturbance while sampling and inoculum preparation, only the effect of the tillage history on the functional properties of the AMF under uniform conditions could be tested, not the effect of the actual soil tillage treatment.

Plant top biomass was estimated after drying at 105°C for 48 hours. The dried samples of maize tops were then ground and mixed, and 0.5g samples were incinerated at 550°C for 6 hours. The ashes were solubilised with 2 mL of 5.6M HCl, filtered through Whatman Nr 40 ashless filter papers, and diluted to 50 mL with deionized water. P concentration in the extracts was measured by malachite green method (Ohno and Zibilske 1991), and Zn concentration was measured by atomic absorption spectrometry at 213.9 nm (AAS Perkin-Elmer – 5000, Wellesley, MA, USA).

Mycorrhizal structures in the roots were stained after clearing the roots with 1.8M KOH (90°C, 1h) in a mixture of Trypan- and Methylene Blue (each 0.05%) in a solution containing lactic acid: glycerol: water (1:1:1 v: v: v), following the procedure modified from Phillips and Hayman (1970). Percentage of root length colonisation by AMF was estimated by the modified grid-line intersect method (McGonigle et al. 1990b).

6.2.2. Growth and P uptake by maize inoculated with monosporic isolates of Glomus spp. from tilled and no-tilled soils (experiment 2)

Eight monosporic fungal isolates belonging to three species of Glomus (G. intraradices, G. mosseae, and G. claroideum) were isolated from tilled and no-tilled soils from the field tillage experiment in Tänikon-Hausweid (reference above). These AMF had been observed in the soil and in the roots of crop plants in the studied tillage
experiment (Jansa et al. 2002, chapter 5). AMF isolates were obtained from single spores previously isolated from trap cultures (chapter 5). Single-spore cultures were established on Plantago lanceolata L. and propagated for 3 months in the greenhouse. This germplasm was registered and made available through the INVAM and the BEG culture collections, respective catalogue numbers are given in Table 6-2.

Inoculum for this experiment was produced on wheat for 3 months in a growth chamber. The substrate was soil-quartz sand mixture (1:4 v: v), and final spore density of about 40-50 spores per gram of substrate was reached. The wheat roots were cut into 1 cm pieces, mixed with the spore containing substrate from the original wheat pots and combined with expanded Montmorillonite clay (Oil Dri Chem-Sorb WR24/18, Brenntag, Vitrolles, France) in a ratio of 1:1 (v: v). This inoculum was filled into 50 mL containers, which were planted with pre-germinated maize seeds. For the establishment of non-mycorrhizal control, the growth substrate and roots of nonmycorrhizal wheat plants were mixed with expanded Montmorillonite clay in the same ratio as for the mycorrhizal treatment and used as inoculum.

Maize seeds (Zea mays L. cv. Corso) were surface-sterilised with 5% calcium hypochlorite for 15 minutes and germinated on moist sand at 25°C in dark for 2 days. Germinating seeds were planted into 50ml inoculum containers, as described above (one seed per container), and grown for 2 weeks in the growth chamber (300 µmol photon m⁻² s⁻¹, 16h photoperiod, 25/20°C day/night, 50/80% relative air humidity, resp.). The rooted plantlets were then transferred with the adjacent substrate block to the pots (volume 750 mL), filled with sterilised substrate, and grown for another 6 weeks in the greenhouse under following conditions: 16h photoperiod, 25°C/20°C, resp., atmospheric humidity 40-50%. Illumination (combined of solar and artificial light - 400W DL/BH Lamps, Eye, Japan) was provided at a minimum intensity of 400 µmol photons m⁻² s⁻¹. The substrate mixture consisted of eutrochreptic soil (loamy (22.3 % clay), neutral (pH 7.4), deep Brown-soil from an agricultural field in Eschikon, Switzerland): sand: expanded Montmorillonite clay (1:4:4, v: v: v). All components of the substrate mixture
(expanded Montmorillonite clay, quartz sand 0.7-1.2 mm grain size, and the soil) were first separately autoclaved, and the soil was then treated with an aqueous solution extracted from the original unsterile soil (soil suspension 1:10 w:v) and filtered 3 times through a paper filter Whatman No 1, in order to reintroduce the original soil bacteria. The soil was afterwards incubated for 6 weeks under greenhouse conditions before preparing the substrate mixture. The total P content of the substrate mixture (estimated as described in section 4.2.1.3.) was 196 mg P/kg, and the readily available P pool (E1min-P value, estimated by the isotope exchange method, Fardeau 1996, see section 4.2.1.3.) in the mixture was 2.5 mg P/kg. The experiment was split into two parts and run simultaneously with the radioactive P uptake experiments described in chapter 7. In each experimental part, a separate nonmycorrhizal control treatment was included so that relative values could be compared between the two runs. Six replicate pots were established for each treatment.

Maize plants were harvested at the start of flowering (male inflorescence), at the age of 54 days. Shoot and root biomass was measured after drying the plant material at 105°C for 48 hours. Ground samples of shoot and roots were incinerated for 6 hours at 550°C, and extracted with 2 mL of 5.6M HCl, and the P concentration in the extracts was measured as described above. Chitin content of the roots was assessed as the amount of glucosamine released by acid hydrolysis of milled root material. The root samples were pre-extracted with 0.2M KOH for 5 hours at 100°C and hydrolysed with 1M H2SO4 at 100°C for 4h. After neutralisation with Ba(OH)2, they were derivatised with fluorenylmethyl-chloroformate (FMOC) and analysed with reverse-phase-HPLC equipped with electrochemical detector (DIONEX). Glutamine was used as internal standard. Glucosamine measurements were related to the content of reducing sugars in the roots, measured by anthron method (Deldon et al. 1994).

6.2.3. STATISTICS

Statistical evaluation was performed by Statgraphics® software version 3.1 (Manugistics, 1997), employing following techniques: analysis of variance (ANOVA),
analysis of covariance (ANCOVA), and regression analysis. Significant differences among the treatment means are shown. Different letters denote significant differences at 5% probability level (multiple range LSD-based F test). The differences among treatment means are labeled in the figures as following: to denote no significant differences among two treatments, while a difference to the third treatments was significant, symbols \( \geq \) or \( \leq \) were used, while symbols \( > \) or \( < \) were used to denote significantly different treatments (larger or smaller on 5% probability level).

6.3. RESULTS

6.3.1. Effect of AMF communities from CT, CH, and NT soils on maize growth and nutrient uptake

Plant growth was improved in the substrate that had been sterilised and then inoculated with AMF-free soil filtrate containing soil bacteria, as compared with the untreated (AMF-containing) field soil (Table 6-1, Fig. 6-1). Plant produced significantly more biomass when grown in the NT soils than in the CT soils. The total P content of aerial parts of plants grown in the soils containing the original AMF communities did not differ between the NT, CT, and CH treatments. In contrast, higher P uptake was observed in the top parts of plants in the NT and CH AMF-free soils as compared with the CT AMF-free soil at low and intermediate P levels (Fig. 6-1). At the highest P fertilisation level, the differences among the soil tillage treatments were not significant in the AMF-free substrate (Fig. 6-1). The absolute amount of P taken up by the aerial parts of maize under the highest P fertilisation regime was higher in plants grown in AMF-free substrates than in the mycorrhizal counterparts. The zinc content of the plants tops was not influenced by sterilisation of the substrate (Table 6-1). However, a strong interaction
TABLE 6-1

Biomass production of maize aerial parts, P and Zn uptake, and AMF root colonisation in pots with field soil. Effects of soil tillage history (CT, CH, and NT soils) and soil sterilisation along a P-fertilisation gradient (4 levels).

a) Plant growth and nutrient content. Results of two-way ANCOVA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degrees of freedom</th>
<th>F-value</th>
<th>Covariate (P-level)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d_T, d_S, d_(T,S)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant top dry weight</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P content in the tops</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Zn content in the tops</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

b) AMF colonisation of roots, determined in the mycorrhizal treatment only. Results of one-way ANCOVA are shown.

<table>
<thead>
<tr>
<th>Root length occupied by</th>
<th>Degrees of freedom</th>
<th>F-value</th>
<th>Covariate (P-level)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d_T, d_cov, d_res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF hyphae</td>
<td>2</td>
<td>1</td>
<td>236</td>
</tr>
<tr>
<td>Arbuscules</td>
<td>2</td>
<td>1</td>
<td>236</td>
</tr>
<tr>
<td>Vesicles</td>
<td>2</td>
<td>1</td>
<td>236</td>
</tr>
<tr>
<td>Nonmycorrhizal fungal structures</td>
<td>2</td>
<td>1</td>
<td>236</td>
</tr>
</tbody>
</table>

df: degrees of freedom, n.s.: not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001.

Between the former tillage treatment of the soil and soil sterilisation on the maize top Zn content was observed (Fig. 6-1). The uptake of Zn at all P fertilisation levels was uniform among the soil tillage treatments in the AMF-free substrate, with the only exception of
the lowest P-fertilisation level, where Zn uptake from the CT soils was significantly higher than that from CH and NT soils. On the other hand, the Zn content of the aerial parts of plants grown in the non-sterilised substrate was different among the different soil tillage treatments, and this became more pronounced with increasing P fertilisation level. At the lowest P level, no difference among the soil tillage treatments was observed, while at higher P levels the highest Zn uptake was observed from NT soils, followed by CT soils, and the lowest Zn content was observed in plants growing in CH soils (Fig. 6-1).

Higher AMF colonisation was observed in the NT soils as compared with the CH and CT soils under both the lowest and highest P fertilisation (Table 6-1, Fig. 6-2). Arbuscular colonisation of the roots was higher in the NT soils than in the CH soils under the lowest P- level, while at the highest P-level, incidence of arbuscules in NT soils was higher than in the other two soil tillage treatments (Fig. 6-2). Slight difference in vesicular colonisation of roots has been encountered at medium P fertilisation regime between CT and CH treatments (Fig. 6-2). Colonisation of roots by non-mycorrhizal fungi was different among the soil tillage treatments only under higher P-fertilisation levels. The occurrence of nonmycorrhizal fungi in the roots of plants grown in unsterilised soils was higher in the CH than in the CT soils (Fig. 3-2). The occurrence of nonmycorrhizal fungi in the roots of plants growing in previously NT soil was more variable depending on the P fertilisation level.
FIGURE 6-1
Effects of the different tillage history of the soil and of the presence of indigenous AMF, on maize top biomass production and nutrient (P, Zn) content in maize tops at different P-fertilisation levels. Significant differences, as determined by the LSD test following single-way ANOVAs, are shown, with measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Error bars indicate ± 1 SE.
6.3.2. Maize growth and P uptake as affected by *Glomus* spp. isolates

Root colonisation by different AMF isolates was high enough (>50% root length colonised) to ensure comparability among the treatments, and also the reliability of the experiment (the observed effects could be attributed to the AMF).
Maize top dry matter production was only slightly affected by inoculation with different isolates of AMF. Top biomass production of nonmycorrhizal plants was not different from any of the mycorrhizal treatments (Table 6-2), while significant differences were observed among different AMF species. Inoculation with *Glomus intraradices* and *G. claroideum* type B resulted in better plant growth than inoculation with *G. mosseae* or *G. claroideum* type A (Table 6-3). No effect of the tillage treatment of the soil, from which AMF were isolated, was observed on maize top biomass production (Table 6-3).

Inoculation of plants with AMF affected the growth of maize roots more than the growth of the tops (Table 6-2). In this respect, fungal isolates under study could be separated into two groups. 1) The group of *Glomus intraradices* and *G. mosseae* significantly reduced maize root growth and 2) the group of *G. claroideum* had no significant effect on root growth (Table 6-2, Table 6-3). Root: shoot ratio was affected in a very similar manner as the root growth, because the biomass production of the tops was not much affected by AMF inoculation. No relation was observed between the tillage history of the soils from which the AMF were isolated and their effect on root growth (Table 6-3). Significant interaction between the AMF species and tillage treatment on root biomass production (Table 6-3) was mainly caused by intra-specific differences in root growth response within *G. claroideum* isolates (Table 6-2). Only the inoculation with *Glomus intraradices* significantly increased phosphorus content of the plants (tops + roots) as compared with the nonmycorrhizal controls growing in this low P soil (Table 6-2). Significant effects of AMF species on plant P uptake were observed. Plants inoculated with *Glomus intraradices* acquired more P than the plants inoculated with *G. mosseae* and *G. claroideum* (Table 6-3). Soil tillage treatment where AMF isolates originated had no effect on P uptake by maize (Table 6-3).

AMF isolates were significantly different with respect to the hyphal, arbuscule, and vesicle colonisation of roots. Some of this variability could be due to significant differences among the AMF species (Table 6-3). Significant differences in root AMF
colonisation between the soil tillage treatments, from which the AMF were isolated, as well as significant interactions between the tillage treatment and the species of the AMF were found (Table 6-3). Higher root colonisation by the hyphae and arbuscules of the AMF isolates from the NT soil than from the CT, as well as the opposite trend with respect to the vesicles (Table 6-3), might indicate higher activity of the AMF isolated from the NT soils, which persisted even after the establishment of the AMF isolates.

The chitin content of roots, considered to be a measure of fungal biomass, was significantly higher in roots of mycorrhizal plants as compared with non-mycorrhizal control plants (Table 6-2). AMF species differed significantly in terms of the amount of chitin produced in the roots (Table 6-3). Highest fungal biomass per unit root biomass was produced by *G. intraradices* SW 205, followed by *G. intraradices* SW 206, and by the *G. mosseae* and *G. claroideum* isolates (Table 6-3). Significant difference in chitin content per unit root biomass was observed among the isolates of *Glomus intraradices* (Table 6-2).

The total P content of the plants was significantly correlated with the chitin content of the roots ($R^2 = 0.53$, $P = 0.026$), but not with the colonisation rate as estimated with root staining method ($R^2 = 0.10$, $P = 0.41$) (Fig. 6-3). Neither the AMF colonisation rate (correlation $P = 0.15$) nor the chitin content ($P = 0.10$) of the roots provided, however, significant correlation with the root biomass.
TABLE 6-2
Growth response and phosphate content of maize infected with different isolates of AMF from tilled or no-tilled soils. Results of one-way ANOVA are shown.

<table>
<thead>
<tr>
<th></th>
<th>F value (8, 45)</th>
<th>NMC</th>
<th>G.i.²</th>
<th>G.m.³</th>
<th>G.cl.⁴</th>
<th>G.c.⁵</th>
<th>G.i.</th>
<th>G.m.</th>
<th>G.cl.</th>
<th>G.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SW205⁴</td>
<td>SW211</td>
<td>SW201</td>
<td>SW208</td>
<td>SW206</td>
<td>SW212</td>
<td>SW202</td>
<td>SW209</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BEG157⁷</td>
<td>BEG160</td>
<td>BEG158</td>
<td>BEG161</td>
<td>BEG155</td>
<td>BEG156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Till¹</td>
<td></td>
<td></td>
<td>Till</td>
<td>Till</td>
<td>Till</td>
<td>No-till</td>
<td>No-till</td>
<td>No-till</td>
<td>No-till</td>
<td></td>
</tr>
<tr>
<td>Plant top dry weight (g)</td>
<td>1.95 (*)</td>
<td>1.00 (7.25)</td>
<td>1.00</td>
<td>0.92</td>
<td>0.93</td>
<td>0.99</td>
<td>1.06</td>
<td>0.96</td>
<td>0.94</td>
<td>1.06</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>5.46 ***</td>
<td>1.0 (1.19)</td>
<td>0.74</td>
<td>0.72</td>
<td>1.17</td>
<td>1.09</td>
<td>0.73</td>
<td>0.76</td>
<td>0.83</td>
<td>1.07</td>
</tr>
<tr>
<td>Root: Shoot ratio</td>
<td>5.47 ***</td>
<td>1.0 (0.17)</td>
<td>0.71</td>
<td>0.76</td>
<td>1.24</td>
<td>1.12</td>
<td>0.65</td>
<td>0.76</td>
<td>0.88</td>
<td>1.0</td>
</tr>
<tr>
<td>P content (mg/plant)</td>
<td>5.80 ***</td>
<td>1.0 (9.26)</td>
<td>1.19</td>
<td>1.02</td>
<td>0.97</td>
<td>1.00</td>
<td>1.24</td>
<td>1.13</td>
<td>0.90</td>
<td>1.01</td>
</tr>
<tr>
<td>Chitin content in roots⁸</td>
<td>16.32 ***</td>
<td>1.0 (0.79)</td>
<td>7.20</td>
<td>4.44</td>
<td>2.24</td>
<td>2.89</td>
<td>4.61</td>
<td>4.88</td>
<td>2.88</td>
<td>2.67</td>
</tr>
</tbody>
</table>

F value (df, residual df) are given, with measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Different letters denote significant differences between treatment means (LSD test, P<0.05). Relative values with respect to nonmycorrhizal control plants are used. Values given in parentheses are absolute values for control plants.

TABLE 6-3
Growth, P content, and mycorrhiza development in maize, with respect to the species (SPEC) and to the soil tillage origin (T) of AMF isolates used for inoculation. Results of two-way ANOVA are shown.

<table>
<thead>
<tr>
<th></th>
<th>SPEC</th>
<th>T</th>
<th>SPEC x T</th>
<th>G.i.</th>
<th>G.m.</th>
<th>G.cl.</th>
<th>G.c.</th>
<th>Till</th>
<th>No-till</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F value (3,1,43)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant top dry weight</td>
<td>3.82</td>
<td>2.53</td>
<td>0.26</td>
<td>1.03</td>
<td>0.94</td>
<td>0.93</td>
<td>1.03</td>
<td>0.96</td>
<td>1.00</td>
</tr>
<tr>
<td>(g) “Rel”</td>
<td>*</td>
<td>ns</td>
<td></td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Root dry weight</td>
<td>11.92</td>
<td>2.57</td>
<td>3.09</td>
<td>0.74</td>
<td>0.75</td>
<td>1.00</td>
<td>1.08</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td>(g) “Rel”</td>
<td>***</td>
<td>ns</td>
<td>*</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Root: shoot ratio</td>
<td>12.10</td>
<td>5.44</td>
<td>2.06</td>
<td>0.12</td>
<td>0.13</td>
<td>0.18</td>
<td>0.18</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>P (mg/plant) “Rel”</td>
<td>12.66</td>
<td>0.58</td>
<td>1.35</td>
<td>1.21</td>
<td>1.07</td>
<td>0.93</td>
<td>1.01</td>
<td>1.04</td>
<td>1.07</td>
</tr>
<tr>
<td>AMF hyphae (% root length)</td>
<td>51.12</td>
<td>9.33</td>
<td>19.66</td>
<td>90.7</td>
<td>76.2</td>
<td>78.9</td>
<td>59.4</td>
<td>73.6</td>
<td>79.1</td>
</tr>
<tr>
<td>Arbuscule abundance</td>
<td>10.29</td>
<td>11.18</td>
<td>13.35</td>
<td>40.0</td>
<td>55.3</td>
<td>61.1</td>
<td>49.4</td>
<td>46.8</td>
<td>56.1</td>
</tr>
<tr>
<td>(% root length)</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>c</td>
<td>ab</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Vesicle abundance</td>
<td>357.61</td>
<td>12.61</td>
<td>11.01</td>
<td>42.3</td>
<td>0.0</td>
<td>2.9</td>
<td>1.4</td>
<td>13.5</td>
<td>9.8</td>
</tr>
<tr>
<td>(% root length)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Chitin content</td>
<td>21.85</td>
<td>1.53</td>
<td>4.31</td>
<td>5.9</td>
<td>4.7</td>
<td>2.6</td>
<td>2.8</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>“Rel”</td>
<td>5</td>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

F values (df1, df2, residual df) are given, with a measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Different letters denote significant differences between treatment means (LSD test, P<0.05). Variables, where values relative to the nonmycorrhizal control plants were compared, are labelled with suffix “Rel”.

Footnotes: 1 – *G. intraradices*, 2 – *G. mosseae*, 3 – *G. claroideum* type A, 4 – *G. claroideum* type B, 5 – mg chitin per g glucose in the roots, relative to control plants

FIGURE 6-3
Relationship between AMF root colonisation rates (% root length colonised) and root chitin contents, and the P content achieved by maize plants (tops+roots) growing in single pots and inoculated with different AMF isolates from tilled and no-tilled soils. Average values for each AMF-isolate treatment were used (six replicate pots per treatment).
6.4. DISCUSSION

In the first experiment, soil sterilisation and recolonization with AMF-free soil microflora lead to a better growth and an increased P uptake by the plants. This might be due to the fact besides removing the AMF, sterilisation eliminated pathogenic fungi and/or root-grazing nematodes that were not re-inoculated with the soil washing (Troelstra et al. 2001). Furthermore, soil sterilisation can result in an increase in soil P availability and other microbially bound nutrients (Oehl et al. 2001), which might in turn lead to an increased nutrient uptake by plant. P availability in sterilised and non-sterilised substrate was, however, not measured in this study. Another reason for the better plant performance observed in the sterilised soil might be that sterilisation and subsequent re-inoculation with soil washing, promoted the growth of plant-growth-promoting or nutrient-solubilising micro-organisms (Troelstra et al. 2001). The influence of the soil tillage treatment from which the soil was sampled, on the growth and P uptake of maize might be explained by the altered chemical properties of the topsoil. The 0-10 cm layer of the no-tilled and chiselled fields contained significantly more available P and K than the tilled topsoil (see also Table 3-1).

The decrease in Zn uptake by maize as the rate of P fertilisation increases has been known since a long time although the underlying processes have not been yet completely deciphered (Takkar et al. 1976, Marschner 1995). This would agree with the decrease that we observed in Zn uptake by maize as P fertilisation was increased in the AMF-containing CH soil. However this known antagonism does not agree with the increase in Zn acquisition observed in the AMF containing NT soil as P fertilisation increased. Furthermore no variation in Zn uptake by maize were observed between the three tillage treatments in the AMF-free soils. All these results suggest that the different microbial communities present in the AMF containing soils might have had different effects on Zn uptake as the P nutrition of the crop varied. The microbial community of the non-sterilised NT soils appeared to be particularly efficient in transferring Zn to the plant. As a major difference in the microbial communities of these soils (sterilised, non-sterilised)
was the presence of AMF, these results suggested that the AMF of the no-tilled soils could improve Zn transfer from the soil to the plant. This needs however to be further studied with pure AMF isolates (see chapter 8).

Development of AMF hyphae and arbuscules in the roots was suppressed by high P fertilisation in the CT and CH soil, but not in the NT soils. This shows that even when the soils had been mechanically disturbed during inoculum preparation for the experiment, the effects of the former tillage history on the AMF can still be observed. The results suggest an increased tolerance of the AMF from the NT soils as compared with the AMF from the CT soils, to a high soil P content. However, the hypothesis about AMF tolerance/adaptation to high soil P levels has to be further justified, because it is not clear whether the arbuscule incidence is the proper measure of the functional performance of the AMF. Although a bell-like curve for AMF colonisation (total of hyphae and arbuscules) in response to P addition has been frequently found (Sanders and Tinker 1973, Bolan et al. 1984), evidence for the same in the incidence of arbuscules is still contradictory (see Smith and Read 1997).

In contrast to the large difference observed in maize growth in sterilised and unsterilised soils, no difference in maize top biomass production was found in response to inoculation with several pure isolates of AMF from the same field site, as compared with the non-mycorrhizal control. This is in agreement with the results by Boucher et al. (1999) and Karasawa et al. (2001), who did not detect any growth improvement of maize grown in pots in response to inoculation with pure AMF strains. This further indicates that the growth reduction of maize in unsterilised soils as compared with those grown in sterilised substrate was not due to AMF, but to some other factor. The frequently observed growth depression in cereals caused by AMF has been ascribed to the increased consumption of plant carbon by the AMF (Graham and Abbott 2000). It was suggested that AMF would have no beneficial effects on plant growth and P uptake under P sufficient conditions, when plant needs for nutrient uptake are saturated by the uptake by roots (Graham and Eissenstat 1998).
Although pure isolates of AMF had no effect on the biomass of plant tops, some of them reduced the growth of maize roots. This is in agreement with the findings of Kothari et al. (1990), who reported a 16% reduction in maize root biomass following inoculation with AMF. They also did not observe any increase in shoot biomass production of maize by the AMF. This is due to the high carbon drain caused by the root-associated AMF structures in AMF-colonised plants compared with nonmycorrhizal plants (Jakobsen 1991). One of the AMF, which significantly reduced root biomass of maize in this study, was *Glomus intraradices*. This AMF species was also the only one significantly increasing plant P content in this experiment done with a low P substrate. *G. intraradices* has been previously reported to improve P nutrition of maize much more markedly than its biomass production (Nurlaeny et al. 1996), which is also in agreement with the results of this study.

Generally small effects of AMF on plant growth and P uptake, which were observed in the pot experiment, might have been also caused by the fact, that the experiments were performed in small containers, where high root density is frequently encountered (Joner and Leyval 2001). Root density was, however, not measured in this study. It was recognised that plant responsiveness to AMF increases with increasing pot size or with lowering root density (Koide 1991, Burslem et al. 1995), and therefore, it is necessary to confirm our previous findings on diversity of AMF effects on plant biomass production and nutrient uptake in another experimental system. Such a test must be done in larger pots, or performed in pots with root-free compartments, so that the extension of root depletion zone for nutrient uptake by AMF would be observable (Schweiger and Jakobsen 2000). The fact that *G. mossae* and *G. claroideum* could not increase the total P uptake by maize in this experimental set up suggest that these fungi accessed the same pools of P as maize roots confirming the results of Bolan (1991), Li et al. (1991b) and Smith et al. (2001). The higher P uptake mediated by an inoculation with *G. intraradices* on the contrary suggests that this AMF was able either to take up forms of P unavailable
to the plants, or to take up P from the soil solution at lower concentration than plant roots. Further research is needed to confirm this result.

Despite the difficulties in analytical procedure, chitin measurement seems to be a reliable marker for the mycorrhizal biomass, very similar to specific fatty acids (Olsson et al. 1995, Larsen et al. 1998) and more suitable than ergosterol measurements (Frey et al. 1994). We also estimated the ergosterol content in the roots, but we could not find differences between mycorrhizal and non-mycorrhizal treatments (data not shown). Plant P uptake could be related to the biomass of AMF in the roots as measured by root chitin content, but not to the AMF colonisation rate as measured by root staining method.

In this study, we observed different plant growth responses (top or root biomass production) and plant P uptake, which could be attributed to differences among AMF species and AMF isolates belonging to *Glomus* spp. We also observed some evidence for differences in activity between the AMF isolates caused by different soil tillage history, which needs further confirmation. The differences observed, however, did not systematically translate into a different P acquisition efficiency of the plants even in the relatively P poor conditions used in experiment 2. Similar absence of the effect of the fungal isolate origin was also observed by Enkhtuya et al. (2000), who compared indigenous and non-indigenous fungi from degraded ecosystems and man-made habitats. On the other hand, indications were given that indigenous AMF from polluted sites might cause different effects on plant uptake of heavy metals (Joner and Leyval 2001) as compared with non-indigenous AMF.
CONCLUSIONS

1. Inter- and intraspecific differences between monosporic isolates of *Glomus* spp. were observed in terms of maize top and root growth and P uptake, which could not be, however, unequivocally attributed to the differences in the tillage history of the soils, from which the AMF isolates were obtained. The functional properties of AMF such as their effects on plant root growth or plant P uptake, seem to be more variable on interspecific than on the intraspecific (= inter-isolate) level. The differences in activity of AMF from differently tilled soils (arbuscule vs. vesicle colonisation of roots) need further study. The overall differences between the mycorrhizal and the control treatments were rather small, probably because of the lack of optimal conditions to demonstrate mycorrhizal benefits on plant growth due to the small-size pots.

2. The effect of AMF communities from differently tilled soils on plant biomass production and P uptake was variable, and could not be unequivocally attributed to the AMF only, but rather to the chemical properties of soil from different tillage treatments. Our results suggest that the AMF present in non-tilled could however significantly increase Zn transfer to the plant. Since the interpretation of the results obtained in the experiment 1 is hampered by the presence of various other soil organisms in the non-sterilised and in the sterilised soils, these findings should be confirmed by using artificial AMF assemblages obtained by mixing relevant AMF isolates from differently tilled soils.
7. STRATEGIES OF SOIL EXPLORATION BY AMF FROM GENUS *Glomus*

**ABSTRACT**

The diversity in P uptake strategies among three species of *Glomus* isolated from a continuously tilled and a no-tilled soil was assessed. Two types of compartmented cultivation containers were used to measure the uptake by mycorrhizal mycelium of radioactively labelled soil phosphorus (P) at different distances from plant roots. Radioactive P was introduced either as a pre-labelled soil block that had to be colonised by AMF hyphae, or by injection of aqueous P solution in soil already colonised by the hyphae.

Three groups were observed among the *Glomus* spp. in terms of P transport. 1) *G. mosseae* was able to rapidly colonise and transport P from distances up to 14 cm from the plant roots. 2) *G. intraradices* also transported P from distances up to 14 cm from the plant roots but could transport larger quantities of P from an already colonised niche as compared with *G. mosseae*. 3) *G. claroideum* transported P from a maximal distance of 6 cm. Observed differences could be explained by species-specific pattern in mycelium development. The differences in functional properties among the isolates of *Glomus* spp. could be attributed to the species, but not to the tillage history of the AMF strains.

**KEYWORDS**

arbuscular mycorrhizal fungi, *Glomus*, functional diversity, *Zea mays* (maize), tillage, phosphorus (P) uptake and transport, root-free zone
7.1. INTRODUCTION

In this chapter we study the differences in P acquisition strategies among single-spore isolates of three species of *Glomus*, obtained from both continuously tilled and no-tilled soils and observed in the roots of field grown maize (Chapter 5). This was done to check whether some functional properties of a particular AMF species were influenced by soil tillage applied for several years or whether the functionality of the AMF community depended solely on its species composition.

The first hypothesis stated at the beginning of this dissertation was tested in this chapter:

1) **Soil tillage affects functional properties of the AMF species (hypothesis 1.3)**

This hypothesis was tested by addressing the following specific questions:

i) Are there any important differences in soil P uptake strategies among the AMF co-existing in the field tillage experiment?

ii) Are AMF isolated from the NT soil more efficient in taking up soil P as compared with the AMF from the CT soil?

iii) Is this due to 1) improved mycelium growth to further distance from the roots? or 2) higher P - uptake efficiency of their extraradical mycelium?

This study was performed in a glasshouse using compartmented cultivation containers, in which root-free compartments had been labelled with radioactive P. These experiments allowed to characterise the distance, from which AMF hyphae were capable of taking up and transporting P from the soil to the plants as well as the P-uptake efficiency of AMF extraradical mycelium when the radioactive P solution was injected in a soil compartment previously colonised by the AMF.
7.2. MATERIAL AND METHODS

7.2.1. Inoculum

Eight monosporic AMF isolates belonging to three species of *Glomus* (*G. intraradices*, *G. mosseae*, and *G. claroideum*) were isolated from CT and NT soils from the field tillage experiment described in section 3.3.1. For details on AMF isolation see Jansa et al. (2002). These AMF isolates were made available through INVAM and BEG (reference numbers are given in Table 6-2).

Inoculum for the experiment was produced on wheat (*Triticum aestivum* L. cv. Albis), grown for 3 months in a growth chamber in a soil-sand mixture (1:4 v: v), allowing for the production of 40-50 spores per gram of substrate. The inoculum was prepared immediately after harvesting the plant tops by chopping the roots to 1 cm pieces and mixing it homogeneously with the spore-containing growth substrate. Inoculum was stored for less than 8 weeks at 4°C before use.

7.2.2. Experimental plants

Maize seeds (*Zea mays* L. cv. Corso) were surface-sterilised with 5% calcium hypochlorite for 15 minutes and germinated on moist sand at 25°C in darkness for 2 days. Germinating maize seeds were planted into 50 mL pots containing the inoculum (as described above) mixed with expanded Montmorillonite clay (Oil Dri Chem-Sorb WR24/18, Brenntag, Vitrolles, France) in a ratio of 1:1 (v: v). Non-mycorrhizal controls were established by inoculation with substrate and root fragments of nonmycorrhizal wheat, grown in a sterilised soil-sand mixture.

Maize seedlings were then grown for 2 weeks in the growth chamber (300 µmol photon m⁻² s⁻¹, 16h photoperiod, 25/20°C day/night, 50/80% relative air humidity, resp.), and subsequently transferred with their adjacent substrate into the experimental containers (described bellow). The AMF colonisation development in the roots was
controlled in 2 weeks old plants by either Trypan-Blue staining (after Phillips and Hayman 1970) or by UV autofluorescence at excitation wavelength 460-490 nm and emission at 510 nm (Ames et al. 1982, see Fig. 7-1).

The plants were then grown for additional 3 weeks in the greenhouse under following conditions: 16h photoperiod, 25°C/20°C, resp., aerial humidity 40-50%. The minimum light intensity (combined of solar and artificial light - 400W DL/BH Lamps, Eye, Japan) was set at 400 μmol photons m⁻² s⁻¹. Then, radioactive (³³P) label was added to the containers, and the plants were grown for additional 19 days. Plants were fertilised with P-free Hoagland nutrient solution (Sylvia and Hubbell 1986) at the rate of 25 mL plant⁻¹ week⁻¹.

7.2.3. Experimental containers, substrate and labelling

The experiment was performed in two different types of compartmented systems, which allowed studying the extension of P depletion zone of the roots by AMF, and the transport of P by hyphal network of the AMF over different distances from the roots (Fig. 7-2). Both of the systems consisted of three compartments: a plant compartment, where the inoculated plants were grown, an intermediate segment of variable width, and a radioactive labelled compartment. Nylon screen (mesh size 20 μm) ensured spatial separation of different compartments, allowing the penetration of AMF hyphae but not of maize roots. Phosphorus uptake and transport by AMF was measured by the translocation of radioisotope (³³P) from a labelled soil compartment placed at different distances from the plant root compartment.

7.2.3.1. Cuvette system

The container construction type called “Cuvette” (Fig 7-2a) was constructed according to Schüepp et al. (1987b) and possessed a large contact area between the compartments (180 cm²). The plant compartment was 4 cm wide, and had a volume of 750 mL. The width of the intermediate compartment varied from 2 to 10 cm. Nylon screens separated the intermediate compartment from both the plant and the radioactive
compartments on its opposite sides. The plant and the intermediate compartments were filled with a substrate mixture consisting of mycorrhiza-free soil, quartz sand (0.7-1.2 mm), and expanded Montmorillonite clay in a ratio of 1:2:2 (v: v: v). The soil originated from an agricultural field in Eschikon (Switzerland) and its characterisation was given in chapter 6. The total P content of the soil (as measured after incineration and extraction with 5.6M HCl) was 745 mg P/kg. The amount of P isotopically exchangeable within 1 minute (E1min-P), which corresponds to the pool readily available for plant or fungal uptake (Fardeau 1996), was 15.4 mg/kg. Soil properties were similar to the properties of the soil in the tillage experiment in Tänikon, from which the AMF were obtained (see section 3.3.1. and the method section in chapter 6).

All components of the substrate mixture were first autoclaved separately, and the soil was then treated with an aqueous solution extracted from the original unsterile soil. This solution was prepared by filtering 10% soil suspension in tap water (w: v) 3 times through a paper filter Whatman No 1. This was done in order to reintroduce the original soil bacteria to the autoclaved soil. This soil was afterwards incubated for 6 weeks under greenhouse conditions before use. Total P content of the substrate mixture was 196 mg P/kg, and the readily available P pool (E1min-P value) in the mixture was 2.5 mg P/kg. This level of P is under the critical level of 5 mg P/kg soil, under which P becomes limiting for crop yield (Gallet 2001). Under P limiting conditions, establishment of functional AMF symbiosis in maize roots will be favoured (Hetrick et al. 1986).

The radioactive compartment was filled with 400 mL field soil (sterilised as described above), homogeneously labelled with 2.47 MBq 33PO₄ (carrier-free orthophosphate, Amersham Pharmacia Biotech, Piscataway, NJ, USA) nineteen days before harvest. The soil layer was under- and over-layered with the soil-sand-expanded Montmorillonite clay mixture described above. Five different sizes of the cuvette system with different width of the intermediate compartment (2, 4, 6, 8, and 10 cm) were built for each AMF isolate. Each cuvette was planted with three maize plants whose tops were separately analysed and considered as replicates. Roots and mycelium samples (sampled
with a cork-borer of 1cm diameter in the intermediate segment, in the substrate adjacent to the labelled compartment) were mixed for each cuvette and analysed together. DW, total P content and $^{33}$P radioactivity measurements from the roots (calculated per single plant) were added to the measurements from the tops.

**7.2.3.2. Starpot system**

The second construction type called “Starpot” (Fig 7-2b) consisted of six 750ml PVC pots in which individual maize plants were grown. These pots were inter-connected with tubes of different lengths (4, 6, 8, 10, 12, and 14 cm) to the central pot, into which the radioactively labelled soil was placed. The content of the tubes was separated from those of the pots by a nylon screen (mesh size 20 μm). The opening of the tubes was 3.14 cm$^2$. The tubes were filled with a substrate mixture consisting of soil, sand, and expanded Montmorillonite clay (5:2:3, v: v: v). Pots, in which the plants were grown, were filled with a substrate mixture of soil, sand, and expanded Montmorillonite clay mixed in a ratio 1:2:2 (v: v: v). The central pot was filled with 750 mL sterile field soil (prepared as described above), and was labelled by multiple (10) injections of a radioactive P solution (8.21 MBq $^{33}$PO$_4$ per starpot system in 10 mL of water) nineteen days before harvest. Three whole starpot systems each comprising 6 distant pots and one central pot (Fig 7-2b) were included in each treatment. This design provided three replicated plants growing at each of the six distances from the labelled compartment. Because of spatial limitations, the experiment was split into two parts, which were run subsequently. Each run contained a non-mycorrhizal control treatment to compare the effects of inoculation with different AMF (comparison of relative effects) among both series.

In both cuvettes and starpots, plant compartments were watered with deionized water by a time-controlled automatic watering facility, which maintained the substrate moisture at 60-80% of its water holding capacity. Watering of the intermediate compartments (in the cuvette system only) and of the labelled compartments was done by a tensiometer-controlled system (Blumat, Austria), which maintained the substrate moisture at 50-60% of its water holding capacity.
One of the differences between the cuvette and the starpot systems was the mode of introduction of labelled P. In the case of the cuvette system, the $^{33}$PO$_4$ was added as a labelled soil block, and the soil had to be first colonised by the AMF before they could take up any phosphorus from it. In the case of the starpot system, a radioactive solution was injected into the pre-established mycelium network. This system therefore allowed observing the P uptake and transport capabilities of an already established fungal extraradical mycelium.

**7.2.4. Plant and AMF analysis**

Shoot and root biomass was measured after drying the plant material at 105°C for 48 hours. Non-radioactive samples were milled and radioactively labelled samples were cut to pieces < 1 cm. Plant samples (roots and shoots) were then incinerated during 6 hours at 550°C, and the ashes were subsequently solubilised in 2 mL 5.6M HCl. The total P concentration in the solution was determined colorimetrically with the malachite green method (Ohno and Zibilske 1991) and the $^{33}$P concentration was measured after neutralisation of the extract with NaOH. The radioactivity was measured with a Packard-2500 TR counter (Packard BioScience, Meriden, CT, USA) using a Packard Ultima Gold scintillation cocktail mixed with the samples in ratio 5:1 (v: v). All the measurements were performed using both negative (background, water) and positive controls (aliquot of the labelling solution treated the same way as the samples). The absolute radioactivity of the samples (for estimation of the specific P activities) was deducted from the estimation of the counting efficiency with the original $^{33}$P-batch delivered by Amersham.

Mycorrhizal infectious structures in the roots were stained after clearing the roots in 1.8M KOH at 90°C for 1 hour, and neutralising the root samples in 0.5M HCl for 30 min. The staining with a mixture of Trypan- and Methylene Blue (each 0.05% in lactic acid: glycerol: water, 1:1:1 v: v: v) was performed following a procedure modified from Phillips and Hayman (1970). The percentage of root length colonisation with AMF was estimated by the grid-line intersects method (Giovannetti and Mosse 1980). The length of
the mycelium in the soil samples was estimated following the filtration-gridline method described by Sylvia (1992) using Millipore RAWG02500 membranes (Millipore, Bedford, Massachusetts, USA).

7.2.5. Statistics

Statistical evaluation was performed with Statgraphics® software version 3.1 (Manugistics 1997), employing the following techniques: analysis of variance (ANOVA), analysis of covariance (ANCOVA), regression analysis, and comparison of correlation coefficients. Using distance from plant to the labelled soil as a covariate in analysis of covariance was done in order to decide whether the distance between plant and the labelled compartment could account for some of the differences attributed to one of the main effects.

**FIGURE 7-1**
Mycorrhizal structures of *Glomus intraradices* in 2-weeks old maize plants, visualised either by Trypan-Blue staining (A-B) or by UV autofluorescence (C-D). A, C: mycorrhizal roots; B, D: control nonmycorrhizal roots.
FIGURE 7-2
Compartmented container design. (a) Cuvette system, (b) Starpot system. Both systems consist of 3 compartments: a root compartment, where the inoculated plant is grown (A), an hyphal intermediate compartment (B) and an hyphal compartment where the radioactive tracer is added (C).
7.3. RESULTS AND DISCUSSION

7.3.1. Dry matter production of maize plants

Inoculation with various AMF isolates had little effect on the dry matter production of maize plant tops as compared with the non-mycorrhizal control in both the cuvette and the starpot systems (analysis not shown, data for mycorrhizal treatments in Tables 7-1 and 7-2). Significant differences in maize growth were sometimes observed between different AMF treatments. Inoculation with *G. claroideum* type B resulted in better growth of plant tops in the starpot system, as compared with the plants inoculated with *G. intraradices* and *G. mosseae* (Table 7-2). No differences in plant biomass production with respect to the inoculation with different AMF were, however, observed in the cuvette experiment (Table 7-1). No effect of the isolate origin (i.e. CT or NT tillage history) was observed on the dry matter production of maize aerial parts (Table 7-1 and Table 7-2).

The little responsiveness of maize to the inoculation with AMF observed in this study is in agreement with the results of Boucher et al. (1999), Kaeppler et al. (2000), and Karasawa et al. (2001), who observed only limited effects of mycorrhizal inoculation on growth of maize. Many maize cultivars did not grow better in response to AMF inoculation under both P sufficient and deficient conditions. Even highly mycorrhiza-responsive maize cultivars, which showed a growth response to inoculation with AMF under P deficient conditions, did not respond to mycorrhiza under P sufficient conditions (Kaeppler et al. 2000).

7.3.2. P content in maize plants in the cuvette and starpot systems

The isolate of *Glomus intraradices* from CT soil increased the P content of plants in the cuvette systems by 43% (*P = 0.002*), and in the starpots by 20% (*P = 0.04*), as compared with the nonmycorrhizal control (Fig. 7-3, Fig. 7-4). The other AMF were less efficient in increasing plant P content (Table 7-1 and Table 7-2). Altogether, four (out of eight) AMF isolates significantly increased plant P content as compared with nonmycorrhizal control in the cuvette system, resulting in a P surplus of 29-43%. *Glomus*
mosseae decreased total P uptake by the plants in the cuvette system, as compared with other AMF (Table 7-1), but not with the nonmycorrhizal control plants (Fig. 7-3).

Three AMF isolates (G. mosseae from CT soil, G. claroideum type A and G. claroideum type B from NT soils) caused decrease in the total P uptake by the plants in the starpot system (Fig. 7-4). On the other hand, only one isolate of G. intraradices (from CT soil) significantly increased maize P content in the starpot system as compared with the nonmycorrhizal control plants (see above). Li et al. (1991b) found an increase of 70-80% of plant P content in AMF infected plants using a similar compartmented system compared with plants grown without AMF. The test plant was in their experiment white clover (Trifolium repens L.) growing in symbiosis with G. mosseae. However, the performance of AMF isolates belonging to G. mosseae could not be confirmed in our study. The contradiction resulting from this comparison might be explained by the concept of functional compatibility of a certain plant-fungus combination (Ravnskov and Jakobsen 1995). This would mean that some AMF isolate/species would behave as a symbiont only when colonising roots of a certain (“compatible”) plant species, while it would behave as a parasite when colonising roots of another (“incompatible”) plant species.

Higher P content in the plants inoculated with AMF, growing in a compartmented system, is due to the access of AMF to P sources spatially unavailable to the roots. This P is taken up by the hyphae and transported to the plants (George et al. 1995). Although we observed an increase in P content in maize inoculated with four AMF isolates as compared with nonmycorrhizal plants, this did not translate into any growth improvement of the plants. This is in agreement with the results of Karasawa et al. (2001), who also did not observe any increase in plant growth due to the improved P nutrition caused by AMF. These results support the hypothesis that a whole spectrum of plant-AMF relations can exist, ranging from mutualism to parasitism (Johnson et al. 1997).

No consistent effect of the origin of the AMF isolates (soil tillage history) was observed in terms of plant P content in the cuvette system (Table 7-1). Consistent effect
of tillage origin of the isolates (higher P uptake by plants colonised by AMF from CT soil) was observed in the starpot system (Table 7-2). The significance of the comparison of the P content of maize growing in the starpots (Table 7-2) with respect to the tillage origin of the AMF has been, however, biased by a highly significant (P<0.01) interaction between factors. This interaction was probably significant due to large inter-isolate differences within single species, and also due to the effects were different for different AMF species (types). Provided only eight AMF isolates were studied, this topic should be studied further using more isolates of AMF from the field experiment before drawing final conclusions.

**TABLE 7-1**

Growth, P uptake, and mycorrhiza development in maize growing in cuvette containers, with regard to the species and the soil tillage of origin of AMF used for inoculation. Results of ANCOVA (two factors and one covariate) are shown. Factors are species (S) and tillage treatment (T) of the AMF used for inoculation, the distance of radioactively labelled soil from root compartment is covariate (DIST).

<table>
<thead>
<tr>
<th>F value (3,1,111)</th>
<th>Fungal species</th>
<th>Fungal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G. i.¹</td>
<td>G. m.²</td>
</tr>
<tr>
<td>Plant top dry weight (g)</td>
<td>0.21 ns</td>
<td>0.14 ns</td>
</tr>
<tr>
<td></td>
<td>1.00 a</td>
<td>0.95 a</td>
</tr>
<tr>
<td>Till No-till</td>
<td>0.98 a</td>
<td>0.96 a</td>
</tr>
<tr>
<td>P (mg/plant)⁸</td>
<td>15.19 ***</td>
<td>0.01 ns</td>
</tr>
<tr>
<td></td>
<td>1.36 a</td>
<td>0.83 b</td>
</tr>
<tr>
<td></td>
<td>1.17 a</td>
<td>1.16 a</td>
</tr>
<tr>
<td>³²P/³¹P in the plant (kBq/mg P)⁹</td>
<td>264.9 ***</td>
<td>2.38 ns</td>
</tr>
<tr>
<td></td>
<td>6.61 b</td>
<td>10.82 a</td>
</tr>
<tr>
<td></td>
<td>4.9 a</td>
<td>5.4 a</td>
</tr>
<tr>
<td>Percent ³²P transported to the plant</td>
<td>82.29 ***</td>
<td>0.32 ns</td>
</tr>
<tr>
<td></td>
<td>8.49 a</td>
<td>8.73 a</td>
</tr>
<tr>
<td></td>
<td>4.77 a</td>
<td>5.04 a</td>
</tr>
<tr>
<td>AMF hyphae (% root length)</td>
<td>33.17 ***</td>
<td>0.09 ns</td>
</tr>
<tr>
<td></td>
<td>85.7 a</td>
<td>82.4 a</td>
</tr>
<tr>
<td></td>
<td>74.1 a</td>
<td>69.9 a</td>
</tr>
<tr>
<td>Arbuscules (% root length)</td>
<td>7.66 ***</td>
<td>1.41 ns</td>
</tr>
<tr>
<td></td>
<td>41.4 a</td>
<td>45.8 a</td>
</tr>
<tr>
<td></td>
<td>43.4 a</td>
<td>35.9 a</td>
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<tr>
<td>Vesicles (% root length)</td>
<td>25.88 ***</td>
<td>6.43 *</td>
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<tr>
<td></td>
<td>23.2 a</td>
<td>0.0 c</td>
</tr>
<tr>
<td></td>
<td>12.0 a</td>
<td>5.9 b</td>
</tr>
</tbody>
</table>

F values (df₁, df₂, df₃, residual df) are given, with measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Different letters denote significant differences between treatment means (LSD test, P<0.05). Values relative to the nonmycorrhizal control plants are shown for plant dry weight (average for control was 5.7 g) and for P content of the plant tops (average for the control treatment was 9.21 mg/plant).

Footnotes: 1 - G. intraradices, 2 – G. mosseae, 3 – G. claroideum type A, 4 – G. claroideum type B, 5 – P content per entire plant, 6 – specific P activity (SA-P)
### TABLE 7-2
Growth, P uptake, and mycorrhiza development in maize growing in starpots, with regard to the species and the origin of AMF isolates used for inoculation. Results of ANCOVA (two factors, and one covariate) are shown. Factors are species (SP) and tillage treatment (T) of the AMF used for inoculation, the distance of radioactively labelled soil from root compartment is covariate (DIST).

<table>
<thead>
<tr>
<th></th>
<th>F value (3,1,1,111)</th>
<th>Fungal species</th>
<th>Fungal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP T SPxT DIST</td>
<td>G. i.1</td>
<td>G.m.2</td>
</tr>
<tr>
<td>Plant top dry</td>
<td>2.48 (*)</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>weight (g)</td>
<td>0.33 ns</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>0.09 ns</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>0.88 b</td>
<td>0.87 ab</td>
<td>0.90 a</td>
</tr>
<tr>
<td></td>
<td>0.84 b</td>
<td>0.83 b</td>
<td>0.88 b</td>
</tr>
<tr>
<td></td>
<td>0.97 a</td>
<td>0.85 b</td>
<td></td>
</tr>
<tr>
<td>P (mg/plant)</td>
<td>10.69 ***</td>
<td>5.93</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>10.06 **</td>
<td>0.15 c</td>
<td>0.14 c</td>
</tr>
<tr>
<td></td>
<td>5.32 **</td>
<td>0.074 c</td>
<td>0.073 c</td>
</tr>
<tr>
<td></td>
<td>3.19 (*)</td>
<td>0.20 ns</td>
<td>0.03 ns</td>
</tr>
<tr>
<td></td>
<td>0.17 ns</td>
<td>0.03 ns</td>
<td>0.03 ns</td>
</tr>
<tr>
<td></td>
<td>0.32 ns</td>
<td>0.03 ns</td>
<td>0.03 ns</td>
</tr>
<tr>
<td></td>
<td>43.83 ***</td>
<td>0.03 ns</td>
<td>0.03 ns</td>
</tr>
<tr>
<td></td>
<td>10.01 a</td>
<td>5.93 b</td>
<td>0.15 c</td>
</tr>
<tr>
<td></td>
<td>5.93 b</td>
<td>0.15 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.10 ***</td>
<td>2.72 a</td>
<td>9.073 c</td>
</tr>
<tr>
<td></td>
<td>0.05 ns</td>
<td>1.10 b</td>
<td>0.074 c</td>
</tr>
<tr>
<td></td>
<td>0.34 ns</td>
<td>0.074 c</td>
<td>0.073 c</td>
</tr>
<tr>
<td></td>
<td>39.99 ***</td>
<td>0.96 a</td>
<td>1.03 a</td>
</tr>
<tr>
<td>Percent 33P</td>
<td>15.33 ***</td>
<td>2.72 a</td>
<td>9.073 c</td>
</tr>
<tr>
<td>transported to</td>
<td>0.05 ns</td>
<td>1.10 b</td>
<td>0.074 c</td>
</tr>
<tr>
<td>the plant</td>
<td>0.34 ns</td>
<td>0.074 c</td>
<td>0.073 c</td>
</tr>
<tr>
<td>AMF hyphae (%</td>
<td>91.19 ***</td>
<td>87.9 a</td>
<td>9.073 c</td>
</tr>
<tr>
<td>root length)</td>
<td>4.08 ***</td>
<td>63.2 b</td>
<td>4.92 c</td>
</tr>
<tr>
<td></td>
<td>11.97 ***</td>
<td>59.4 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.20 ns</td>
<td>49.2 c</td>
<td>63.2 b</td>
</tr>
<tr>
<td></td>
<td>87.9 a</td>
<td>66.2 a</td>
<td>63.2 b</td>
</tr>
<tr>
<td>Arbuscules (%</td>
<td>21.57 ***</td>
<td>27.5 b</td>
<td>30.6 b</td>
</tr>
<tr>
<td>root length)</td>
<td>6.61 ***</td>
<td>31.7 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.55 ***</td>
<td>44.7 b</td>
<td>35.1 a</td>
</tr>
<tr>
<td></td>
<td>4.25 **</td>
<td>27.5 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.7 b</td>
<td>27.5 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.55 ***</td>
<td>30.6 b</td>
<td></td>
</tr>
<tr>
<td>Vesicles (%</td>
<td>379.2 ***</td>
<td>39.0 a</td>
<td>10.7 a</td>
</tr>
<tr>
<td>root length)</td>
<td>0.12 ns</td>
<td>0.2 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.47 (*)</td>
<td>0.5 c</td>
<td>11.0 a</td>
</tr>
<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
<td></td>
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<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<td></td>
<td>0.03 ns</td>
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<td></td>
<td>0.03 ns</td>
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<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
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<td>0.03 ns</td>
<td>0.5 c</td>
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<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03 ns</td>
<td>0.5 c</td>
<td></td>
</tr>
</tbody>
</table>

F values (df1, df2, dfcov, residual df) are given, with measure of significance: n.s. not significant, (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. Different letters denote significant differences between treatment means (LSD test, P<0.05). Values relative to the nonmycorrhizal control plants are shown for plant dry weight (average for control was 8.39 g) and for P content of the plant tops (average for the control treatment was 15.35 mg/plant).

Footnotes: 1 - G. intraradices, 2 – G. mosseae, 3 – G. claroideum type A, 4 – G. claroideum type B, 5 – P content per entire plant, 6 – specific P activity (SA-P)

#### 7.3.3. 33P uptake and transport by AMF hyphae

The amount of 33P taken up by mycorrhizal hyphae in the root free zone and transported to the plants is shown for both cuvette and starpot systems in Fig. 7-5 and Fig. 7-6, respectively. No P transport from root-free zone was observed in nonmycorrhizal control plants (Fig. 7-5 and Fig. 7-6). The distance between the labelled and the plant compartments did significantly account for the explanation of the differences in P uptake by the AMF from the labelled compartment (Table 7-1, Table 7-2). In the cuvette system, the amounts of labelled phosphate taken up and transported by *Glomus mosseae* and *G. intraradices* did not markedly change as the size of the intermediate compartment, separating the host plant from the labelled soil, increased from...
2 to 10 cm (Fig. 7-5). Between 7.2% and 13.3% of the added radioactive isotope was taken up by these two fungi and transported to the plants within 19 days. *G. claroideum* isolates were less efficient in transporting radioactive P from the distant compartment (Table 7-1). These AMF could only take up 2.8% to 11.0% of the added isotope when the labelled soil was located 2 cm away from the host plant roots. With increasing distance, the uptake and transport efficiency of the both fungi rapidly decreased, and no measurable transport was detected from distances further than 8 cm (Fig. 7-5).

Hyphal growth and uniform soil P depletion at distances up to 11 cm from the roots of a host plant (*Trifolium repens* L.) have been observed for *Glomus mosseae* by Li et al. (1991a). A direct confirmation of the P transport through the extraradical mycelium of AMF from distances up to 7 cm from plant (*Trifolium subterraneum* L.) roots was provided by means of $^{32}$P-isotope tracing (Jakobsen et al. 1992b). In that study it was observed that *Acaulospora leavis* could transport P over distances of up to 7 cm, while *Glomus* sp. transported P only from distances shorter than 1 cm. Smith et al. (2000) recently demonstrated similar functional diversity between *Glomus caledonium* and *Scutellospora calospora*. In this work, *Scutellospora* was shown to exploit the P present in the root compartment, while *Glomus* could take up more phosphate from the root-free zone, separated from the roots by a nylon screen. Our observation in this study confirms a similar diversity of functions within the genus *Glomus*. Since the AMF used in this study were all isolated from the same field site and were proved to co-exist within the root system of field grown plants (chapter 5), we suggest that under field conditions various AMF may co-exist and provide plants with P from both close and from distant zones from the roots.

The P uptake and transport in the starpots decreased with increasing length of the intermediate segment in the treatment inoculated with *Glomus intraradices* or *G. mosseae* (Fig. 7-6). The proportion of added $^{33}$P recovered in the maize biomass reached a maximum of 9.2% with *G. intraradices*, and 5.6% with *G. mosseae* (both isolates from NT soil), when the labelled compartment was as close as 4 cm to the plant roots (Fig. 7-
6). Some $^{33}$P was still detectable in maize when the labelled compartment was placed at 14 cm from the root compartment with *G. intraradices* and at a distance of 10 cm with *G. mosseae*. No transport was observed in case of *G. claroideum* from any distance (Fig. 7-6). This was probably because of already the shortest distance used (4 cm) was at the farthest limit of those fungi. The comparison of $^{33}$P uptake by mycorrhizal maize in both of our experimental set-ups suggests that the decrease of P uptake with increasing distance in the starpot system was mainly due to limited mycelium growth caused by the small volume of connecting tube (Fig. 7-2) and not due to the competition among hyphal networks belonging to the separate plants.

The ratio of labelled phosphate to the whole P content of the plant ($^{33}$P/$^{31}$P specific activity, SA) differed in both experimental systems between *G. intraradices* and *G. mosseae* (Table 7-1, Table 7-2, Fig. 7-7, Fig. 7-8). The SA of maize was higher if plants were inoculated with *G. mosseae* than with *G. intraradices* in the cuvette set-up (Fig 7-7, Table 7-1). The opposite was observed in the starpot set-up (Fig. 7-8, Table 7-2). These results suggested that both AMF species had different strategies to exploit soil P resources. *G. mosseae* was capable of rapid colonisation of remote soil zones, such as $^{33}$P-labelled soil block, which was introduced into the cuvette system. The reason for this fast colonisation might be the rapid hyphal growth by this AMF species. *G. mosseae* might be thus qualified as a rapid expander, indicating adaptation to changing environment (R-strategy organism, according to the theory of Pianka, 1970). This is also supported by higher incidence of this fungus in arable (periodically disturbed) soils (Blaszkowski 1993, Helgason et al. 1998). This AMF species might, however, not be highly efficient in supporting plant P uptake since the plants inoculated with *G. mosseae* did not take up more P than the nonmycorrhizal control plants (Fig. 7-3).

The higher SA of maize in the starpot system inoculated with *G. intraradices* as compared with that inoculated with *G. mosseae* (Fig. 7-8, Table 7-2), together with the higher overall P content of plants inoculated with *G. intraradices* (Fig. 7-3), indicated that the hyphae of *G. intraradices* were highly efficient in taking up soil P and transporting it to the plants. Compared with *G. mosseae* and *G. intraradices*,
FIGURE 7-3
Total P content in the maize plants (tops + roots) growing in cuvette systems with different size of the root-free compartment. Relative effects of inoculation with eight different AMF isolates with respect to the nonmycorrhizal control are shown. Mean +SE of three replicate plants.

FIGURE 7-4
Total P content in the maize plants (tops + roots) growing in starpots with different size of the root-free compartment. Relative effects of inoculation with eight different AMF isolates with respect to the nonmycorrhizal control are shown. Mean +SE of three replicate plants.
G. claroideum isolates were less efficient in transporting P from larger distances. However, they might play an important role in P uptake from sites located in the vicinity of roots, as was shown for Scutellospora calospora (Smith et al. 2000), or they might be involved in some other aspects of soil-plant interactions (Newsham et al. 1995). Highly significant interaction (P < 0.001) between the effects of AMF species and the tillage origin of the AMF isolates was observed in the cuvette experiment for the P specific activity (ratio of $^{33}$P/$^{31}$P, Table 7-1). This means that for one AMF species, the isolate from NT was more effective in taking up P from the labelled compartment than the isolate from the CT, and that the contrary was true for some other AMF species. However, the AMF species identity explains most of the variability in P uptake efficiency of the AMF from the labelled compartment, as could be seen from the comparison of the F values (Table 7-1). Significance of covariate effect (the distance between the labelled and the plant compartments) on the specific activity of P in the plants growing in the starpot systems indicate that the distance explained some of the variability accounted to the main effects (Table 7-2). The effect of distance on the P uptake and transport by the AMF was therefore further studied using regression analysis.

Finally the effects of different factors such as the species, functional group, or tillage origin of the different AMF strains were compared with respect to the P uptake and transport capabilities of those fungi in the cuvette system (Table 7-3). The largest proportion (approximately 85-87%) of the variability of the distant P uptake and transport capabilities of the studied AMF isolates could be attributed to either the AMF species or the functional group of species (G. mosseae + G. intraradices vs. G. claroideum). The effect of distance on the uptake and transport of P through mycorrhizal hyphae to the plants was still significant, if all data for all fungi were pooled (P = 0.008), but it explained only about 15% of the variability (Table 7-3). No significant effect of the tillage history of the AMF isolates on their P uptake and transport capabilities was detected (Table 7-3). The differences were observed only on inter-specific level, with only minor variation among isolates belonging to the same species.
TABLE 7-3

Contribution of different factors to explain the effect of distance from roots, at which the labelled soil was introduced, on the mycorrhiza-mediated P uptake by the maize plants (fraction of the labelled phosphate introduced, which was found in the plant biomass at the end of the experiment).

<table>
<thead>
<tr>
<th>Data separation by</th>
<th>df</th>
<th>Residual df</th>
<th>F-value</th>
<th>Percentage of explained/total variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMF isolate</td>
<td>15</td>
<td>23</td>
<td>30.2 ***</td>
<td>91.9</td>
</tr>
<tr>
<td>AMF species</td>
<td>7</td>
<td>31</td>
<td>34.0 ***</td>
<td>85.5</td>
</tr>
<tr>
<td>AMF functional group²</td>
<td>3</td>
<td>35</td>
<td>84.72 ***</td>
<td>86.9</td>
</tr>
<tr>
<td>Tillage</td>
<td>3</td>
<td>35</td>
<td>2.37 n.s.</td>
<td>9.77</td>
</tr>
<tr>
<td>no separation³</td>
<td>1</td>
<td>37</td>
<td>7.87 **</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Log-linear regression model was used. Mean values for each distance-fungal isolate combination (5 distances and 8 fungal isolates) measured in the cuvette experiment were used for the analysis without including the non-mycorrhizal control. The tests were performed by comparison-of-regression-lines procedure (Statgraphics software).

Footnotes: 1 – R² adjusted for the df, 2 - *G. mosseae*+*G. intraradices* vs. *G. claroideum*, 3 - all data pooled

The detected differences among AMF species are probably due to differences in mycelium spreading capabilities, as already proposed for different genera of AMF by Li et al. (1991a), and confirmed by Jakobsen et al. (2001). The mycelium length of the different *Glomus* spp. isolates (Fig. 7-9) measured in our study confirmed this hypothesis.

A highly significant correlation was found (P < 0.0001, R² = 0.825), when the P uptake data and the mycelium length in substrate were compared (Fig. 7-10). Very similar results (R² = 0.87) were recently reported by Jakobsen et al. (2001), who showed a close relationship between the mycelium length in the soil and P the uptake capabilities of AMF communities under the field conditions.

7.3.4. AMF colonisation of roots

The root colonisation rates in all mycorrhizal treatments reached at least 50% of root length colonised by the AMF. This ensured that the above-described results on P uptake by AMF hyphal network are comparable among the different fungi and are relevant for the field situation, where high level of root colonisation is usually observed (Abbott and Robson 1981, Jakobsen et al. 2001, chapter 4). Differences in hyphal,
FIGURE 7-5
Transport of labelled phosphorus by AMF to the maize plants (tops and roots) from the root-free compartment placed at different distances from the plants. Experiment in a cuvette system, inoculated with eight different AMF isolates. Mean +SE of three replicate plants.

FIGURE 7-6
Transport of labelled phosphorus by AMF to the maize plants (tops and roots) from the root-free compartment placed at different distances from the plants. Experiment in a starpot system, inoculated with eight different AMF isolates. Mean +SE of three replicate plants.
arbuscule, and vesicle colonisation were observed among AMF isolates. Some of this variability can be explained by differences in AMF species (Tables 7-1 and 7-2), which is in agreement with previous studies (van der Heijden et al. 1998b). Higher incidence of hyphae and arbuscules in the roots colonised by the AMF from the NT soils was observed in the starpot containers, and higher incidence of vesicles in the roots colonised by the AMF from the CT soils was observed in the cuvettes (Tables 7-1 and 7-2). Whether this indicates higher activity of the AMF from the NT soils (similarly as suggested in the chapter 6) needs further study. However, there was a high inter-isolate variability,
FIGURE 7-9
AMF mycelium length in the growth substrate in the cuvette experiment. Substrate samples were taken from the intermediate compartment at the distance from the plant segment, at which the labelled soil has been inserted. Actual distance from the root compartment is shown on X-axis. Measurements for each distance-fungal isolate combination were done once and thus no measure of variability could be given.

FIGURE 7-10
P transport by AMF from different distances from plant roots, as a function of the length of mycelium in respective distances (in the cuvette system). Data were pooled for all fungi and distances. Data for control (non-inoculated) plants were not included in this analysis. Regression curve shown: $Y = -3.2 + 15.1 \times X^{0.5}$, $R^2=0.85$. 
resulting in a high significance of interaction between the factor of fungal species and the tillage origin of the isolate (Tables 7-1 and 7-2). This means that the effects observed might have been due to inter-isolate differences rather than due to tillage treatment of the original soil and it also means that for some AMF species, the isolate from NT was more efficient root coloniser compared with the isolate from CT, and for some other AMF species this was the opposite. This is in agreement with a common faith that there is a large intraspecific variability in infectivity and physiology of AMF. Almost no experimental data are available showing the range of differences in functional properties such as effects on plant growth and nutrient uptake under the AMF species level (Feldmann et al. 1998). Only recently, variability has been characterised in the groups of *G. mosseae*, *G. claroideum* and *G. geosporum* (Knudsen et al. 2001, Larsen et al. 2001).

For conclusions about the effects of soil tillage treatment on the functional properties of AMF we would need to study more isolates of the same species originated from the different tillage treatment as well as we would need some more information about genetical basis of the AMF functioning.

Both the single-pot trial (described in chapter 6) and the radioactively labelled experiment described in this chapter were performed under exactly the same conditions, using the same plants, the same inoculum batch and the same substrate. The same root colonisation developmental pattern of the AMF isolates was observed in both trials. This allowed a comparison of the results of the root colonisation development by the AMF isolates as assessed by different methods (chapter 6) with their functionality assessment (P uptake and transport capabilities from remote distances from host plant roots) as described in this chapter. Only the mean values of the root colonisation by AMF hyphae and of the chitin content of the roots were considered for each of the AMF isolates, both measured in the experiment described in chapter 6. These were correlated with the mean values of the percentage of introduced $^{33}\text{P}$ transported by the AMF isolates to the maize from the distance of 4 cm (in the cuvette set-up).
The P uptake and transport capability of the AMF used in this study was better explained by the chitin content of the roots \( R^2 = 0.892, P = 0.006 \), than by the root colonisation rate, assessed by root-staining method \( R^2 = 0.426, P = 0.079 \). This is probably because chitin provided a more adequate quantification of the AMF biomass (Frey et al. 1994), as compared with the measurements of the percentage of colonised root length (McGonigle et al. 1990b). However, the significance of correlation was in both cases lower than that of the correlation between P transport by the AMF and the mycelium development at the site where the soil P was taken up (Fig. 7-10).

7.4. CONCLUSIONS

1. Our results suggested the existence of three distinct functional groups (in terms of P uptake and translocation) within the genus *Glomus*. 1) *G. mosseae* was able to rapidly colonise nutrient rich zones and transport P to the plant from distances up to 14 cm. 2) *G. intraradices* also transported P from distances up to 14 cm from the plant roots but could transport larger quantities of P from an already colonised niche compared with *G. mosseae*. 3) all isolates of *G. claroideum* transported P from a maximal distance of 6 cm. We did not observe any consistent effect of the tillage management of the field soil, from which the AMF isolates were obtained, on the fungal performance in terms of P uptake and transport. It is thus concluded that the functionality of the AMF in terms of P uptake and transport was not significantly influenced by soil tillage.

2. P uptake and transport capabilities are closely linked to the AMF species-specific mycelium-spreading pattern, while plant growth reaction and mycorrhizal structures development in the roots might be variable on a lower, intraspecific level. The relationships between these two aspects of the AM symbiosis are unclear.
3. *Glomus* spp. having the three strategies for P uptake and transport were observed in the roots of maize grown in the differently tilled soils (chapter 5). This suggests that in the three tillage treatments a sufficient functional diversity might be present for an optimal use of the P resources. However not all AMF species identified in the roots of field-grown maize by molecular tools (chapter 5) were isolated as pure AMF cultures to the time-point of this study and therefore the P uptake tests could not be performed with all relevant AMF. Completing the functional tests using other AMF isolates belonging to *Gigaspora* spp., *Scutellospora* spp., and *Acaulospora* spp. from the studied field experiment (Jansa et al. 2002) would be highly desirable so as to decide whether the tillage-induced changes in AMF community composition could have important consequences for the functionality of the AMF communities present under different tillage treatment.
The uptake and transport of P and Zn by *Glomus intraradices* growing in symbiosis with maize (*Zea mays* L.) were studied during 25 days in two compartmented systems (cuvette, star pot) in a glasshouse. These systems were composed of three compartments: a plant root compartment, an intermediate compartment (which size varied from 4 to 20 cm), through which only hyphae could grow, and a last compartment labelled with $^{33}$PO$_4$ and free $^{65}$Zn that could also only be explored by the AMF hyphae. Total dry matter production, root colonisation, P and Zn (radioactive and stable) contents of mycorrhizal and nonmycorrhizal plants as well as hyphal length in the intermediate compartment were measured.

Significant amount of radioactive P and Zn isotopes were transported to the plants only when the mycorrhiza was present in the system. Transport of $^{33}$P and $^{65}$Zn by AMF hyphae to the plant decreased as the distance between the plant and the labelled compartments increased from 4 to 15 cm. No transport was observed from distances exceeding 15 cm. In a compartmented system with a relatively large contact zone between the compartments (the cuvette system), 27% and 9% of the added $^{33}$P and $^{65}$Zn
placed at 5 cm distance from the roots were transported to the plant within 25 days, respectively. In a compartmented system with a small contact zone between the compartments (the star pot system), 10% and 3.5% of the added $^{33}\text{P}$ and $^{65}\text{Zn}$ placed at 4 cm distance from the roots were transported to the plant within 25 days, respectively. The uptake patterns of both P and Zn from different distances, as assessed by radioactive tracers, were strongly correlated, which indicated that uptake of both of the elements could be used as indicators of AMF functional activity.

The uptake and transport of both P and Zn by AMF from distances up to 15 cm from the roots resulted in a significant increase of both total P and Zn contents in the plants. In mycorrhizal plants growing in the cuvette system, about three times higher contents of P and Zn were encountered than in the nonmycorrhizal control plants.

**KEYWORDS**

arbuscular mycorrhiza, *Glomus intraradices*, maize, phosphorus, zinc, uptake, transport, root-free zone, mycelium
8.1. INTRODUCTION

Previous results (chapter 4) suggest that different tillage practices affect Zn uptake by plants and that it could be related to changes in AMF activity in the differently tilled soils (chapter 6). In this chapter it was decided, using a single isolate of \textit{G. intraradices}, to compare the relative contribution of this AMF to the P and Zn nutrition of the maize. This was done so as to check whether plant Zn content could also be used as a functional marker of AMF activity in mycorrhizal plants.

This study was performed using the two systems employed in the previous chapter (cuvette and starpot, chapter 7). In this study, however, the soil labelling was performed identically with both systems by adding a soil block labelled with both $^{33}$PO$_4$ and free $^{65}$Zn in order to obtain comparable results in both of the systems.

8.2 MATERIAL AND METHODS

The uptake and transport of P and Zn was studied on a model association between the maize (\textit{Zea mays} L.) and \textit{Glomus intraradices}, because previously this AMF species was identified to be very efficient in transporting P to maize in a compartmented system (chapter 7). A monosporic isolate of the \textit{G. intraradices} isolated a continuously tilled soil from the Hausweid-Tänikon field experiment (INVAM: SW205) was used. The soil used for the experiment originated from an agricultural field in Eschikon (soil properties given in the method section of chapter 6). The soil was sterilised and recolonised by soil bacteria (see chapter 6), and contained 747 mg P/kg, estimated after incineration at 550°C followed by extraction with 5.6M HCl (modified from Saunders and Williams 1955 described in the chapter 4.2.1.3). Soil Zn content was 34.7 mg Zn/kg (estimated by incineration and extraction method described in the chapter 4.2.1.3). The availability of P and Zn in the sterilised soil was assessed by the method of isotopic exchange kinetics (described in the chapter 4.2.1.3) (Fardeau 1996, Sinaj et al. 1999). $E_{1\text{min}}$ was 15.4 mg
P/kg soil and 2.4 mg Zn/kg soil, while r1/R was 0.543 and 0.038 for P and Zn, respectively. The n value (i.e. the exponent of the power function, which describes the decrease of radioactivity in the solution after one minute of exchange) was 0.227 and 0.188 for P and Zn, respectively.

8.2.1. Inoculum

The AMF inoculum used in this experiment was produced on *Plantago lanceolata* L. growing in 750 mL pots filled with a soil – sand mixture 1:4 (v: v). The plants were grown in the greenhouse for 11 months, yielding a final spore density of about 100 spores per gram of substrate. The roots were afterwards cut with scissors to pieces not exceeding 1 cm and mixed with the spore containing substrate. The inoculum was then mixed with expanded Montmorillonite clay (Oil Dri Chem-Sorb WR24/18, Brenntag, Vitrolles, France) in a ratio of 1:1 (v: v) and filled into 50 mL containers, which were subsequently planted with pre-germinated maize seeds. The substrate for the nonmycorrhizal treatment was prepared by autoclaving the soil: sand: expanded Montmorillonite clay mixture (1:4:5, v: v: v) and by adding to it a water extract from the AMF inoculum (suspended in tap water 1:10 w: v, and 3 times filtrated through a Whatman No 1 paper filter).

8.2.2. Plants

Seeds of maize (*Zea mays* L. cv. Corso) were surface-sterilised with 5% calcium hypochlorite for 15 minutes and germinated on moist sand at 25°C in darkness for 2 days. Germinated seeds were planted into 50 mL inoculum containers (one seed per container), and grown for 2 weeks in a growth chamber (300 μmol photon m⁻² s⁻¹, 16h photoperiod, 25/20°C day/night, 50/80% relative air humidity, resp.). The rooted plantlets were then transferred with the adjacent substrate block into the experimental containers (described bellow) and grown for additional 3 weeks in the greenhouse under the following conditions: 16h photoperiod, 25°C/20°C day/night, and atmospheric humidity 40-50%. The minimum light intensity (combined of solar and artificial light - 400W DL/BH Lamps, Eye, Japan) was set at 400 μmol photons m⁻² s⁻¹. Plants were watered with deionised water as described in the next section. Every plant was weekly fertilised with
10 mL of 7-times concentrated P-free Hoagland nutrient solution (Sylvia and Hubbell 1986).

After 3 weeks of growth in the experimental containers, the radioactively labelled soil was added, and the plants were grown in the greenhouse for an additional 25 days before being harvested and analysed.

8.2.3. Experimental containers and labelling

Two types of compartmented containers (the cuvette and the starpot) were used, as described in chapter 7. In both systems, the spatial separation of different compartments was ensured by 20 μm nylon mesh screens, and the systems always consisted of 3 compartments: a plant compartment, where the inoculated plants were grown, an intermediate segment of variable length, and a radioactively labelled compartment. The intermediate and the labelled compartments were only accessible for the AMF hyphae, and the plant compartment was accessible for both AMF and the roots (see also Fig. 7-2).

The first container construction type (Cuvette) comprised a large contact area between the compartments (180 cm²). The plant and the intermediate compartments were filled with a substrate mixture consisting of autoclaved soil: expanded Montmorillonite clay: quartz sand (0.7 - 1.2 mm) mixed in a ratio of 1:2:2 (v: v: v). Previous to the experiment the autoclaved soil had been re-inoculated with soil bacteria by adding a soil water extract (prepared from aqueous soil suspension 1:10 (w: v) of the unsterile soil by 3 times filtering through Whatman No 1 filter paper) and incubated for 6 weeks under ambient temperature. The plant compartment (4 cm wide, volume 750 mL) was planted with 3 maize plants. The intermediate compartment had a variable width from 5 to 20 cm. The radioactive compartment was filled with 400 mL of autoclaved field soil (330 g dry weight) labelled with ³³P (5.55 MBq ³³P in the form of carrier-free orthophosphate, Amersham Pharmacia Biotech, Piscataway, NJ, USA) and with ⁶⁵Zn (835 kBq ⁶⁵Zn, NEN- Perkin-Elmer Life Sciences, Boston, MA, USA). The soil layer was under- and over-layered with non-radioactive soil-sand-expanded Montmorillonite clay mixture.
described above. The radioisotopes were dissolved in 0.25M HCl prior to labelling, and the soil was labelled with 1 mL of the radioactive solution, intensively mixed and incubated in darkness at ambient temperature for 48 h. Four different lengths of the intermediate compartment were considered: 5, 10, 15, and 20 cm. Three replicate cuvette units were built for each intermediate compartment size in the mycorrhizal treatment, while only two replicates per each distance were made for the control treatment (because of spatial limitations). Each of the maize plants was considered as a replicate. Roots from each cuvette container (containing 3 plants) were pooled and analysed together and an average per plant was calculated.

The starpot experimental set-up consisted of six 750 mL PVC pots where individual maize plants were grown. These pots were connected with tubes of different lengths (4, 6, 8, 10, 12, and 14 cm; 2 cm diameter, contact zone 3.14 cm²) to the central pot, which contained the radioactively labelled soil. The content of the tubes was separated from the pot body by a nylon membrane of 20 µm opening. The connecting tubes were filled with a sterile substrate mixture soil-sand-expanded Montmorillonite clay (5:2:3, v: v: v). Plant pots were filled with a substrate mixture soil-sand-expanded Montmorillonite clay (1:2:2, v: v: v) as described above. The central pot was filled with autoclaved soil, and a 100ml centrifugation bottle was inserted into the middle, which was later replaced with a block of 70 mL (58 g dry weight) of labelled soil. While labelling, 1 mL of 0.25M HCl solution containing a mixture of 9.17 MBq ³³P (Amersham, same specifications as above) and 835 kBq ⁶⁵Zn (NEN, same specifications as above) was thoroughly mixed with 70 mL of soil, which was then put into the middle of the central pot. Three replicates were included for both the mycorrhizal and the control treatments, yielding 3 replicated plants for each distance between the plant and of the labelled pot.

Watering of the plant compartments with deionized water was done in both systems by time-controlled automatic watering facility, maintaining the substrate humidity at 60-80% of its water holding capacity. Watering of the intermediate segment (in the cuvette system only) and of the labelled compartments was done by tensiometer-controlled
watering facility (Blumat, Austria), maintaining the substrate humidity at 50-60% of its water holding capacity.

8.2.4. Plant and substrate analysis

Shoot and root biomass was measured after drying the plant material at 105°C for 48 hours. The plants (shoot and roots analysed separately) were cut to pieces <1cm, incinerated (6 hours at 550°C), and the ashes were solubilised in 2 mL of 5.6M HCl. Total P concentration in the extracts was measured colorimetrically using the malachite green method (Ohno and Zibilske 1991). The concentration of ³²P and ⁶⁵Zn in the solution was measured by β-scintillation counting after neutralisation of extracts with NaOH, using energy separation (0-600-1500 keV) to distinguish between the two radioisotopes. Scintillation counting was performed with Packard (Packard BioScience, Meriden, CT, USA) 2500 TR counter using a Packard Ultima Gold scintillation cocktail mixed with the samples in ratio 5:1 (v: v). Measurements of plant samples was performed concomitantly with the measurement of the original labelling solution under the same conditions so as to compensate for counting-efficiency errors (as discussed in chapter 7).

The concentration of total Zn plant material was assessed in the acid extracts (described above) by ion-chromatography (Dionex DX500 system, DIONEX, Chicago, IL, USA). Samples of 100 μL were injected and separated on guard- and analytical columns Ionpac CG5 and Ionpac CS5. The eluent was 50 mM oxalic acid/LiOH (pH 4.80), and had a flow rate of 1.0 mL/min. Ions were detected by absorbance at 520 nm after post-column reaction with 4×10⁻⁴M 4-(2-pyridylazo)-resorcinol in 3.0 M HN₄OH / 1.0M CH₃COOH. The flow rate of the reagent was 0.5mL/min. Zn peak appeared under these conditions after approximately 12 min.

Mycorrhizal colonisation structures in the roots were stained after clearing the roots in 1.8M KOH (90°C, 1h) in a mixture of Trypan- and Methylene Blue (each 0.05% in lactic acid: glycerol: water, 1:1:1 v: v: v), following a procedure modified from Phillips
and Hayman (1970). The percentage of root length colonised by AMF was estimated by the grid-line intersect method (Giovannetti and Mosse 1980).

Mycelium length in the substrate was estimated following the filtration-gridline method described by Sylvia (1992) using Millipore RAWG02500 membranes (Millipore, Bedford, Massachusetts USA). Mycelium length was assessed in the cuvette system by taking soil cores (length 10 cm, diameter 1 cm) in three zones of the intermediate segment: close to the root compartment (region X), in the middle of the intermediate segment (region Y), and close to the labelled compartment (region Z). In the starpots, mycelium length was assessed only in bulk samples taken from the connecting tubes.

8.2.5. Nutrient uptake efficiency of AMF hyphae

The design of cuvette experiment allowed calculating the total uptake of P and Zn by the mycorrhizal plant from each of the three compartments.

Let:

- $P_a$ be the amount of P taken up by the mycorrhizal plant and derived from the plant compartment (mg P/plant)
- $P_b$ be the amount of P taken up by the mycorrhizal plant and derived from the intermediate compartment (mg P/plant)
- $P_c$ be the amount of P taken up by the mycorrhizal plant and derived from the soil (labelled) compartment (mg P/plant)
- $P_{\text{plant}}$ be the total amount of P taken up by the mycorrhizal plant during the experiment (mg P/plant)

Then

$$P_{\text{plant}} = P_a + P_b + P_c \quad (\text{Eq. 1})$$

And similarly

$$Zn_{\text{plant}} = Zn_a + Zn_b + Zn_c \quad (\text{Eq. 2})$$
Assuming that the uptake of P and Zn by the mycorrhizal plant from the plant compartment is not different from the uptake of P and Zn by non-mycorrhizal plant from the plant compartment, it is possible to estimate $P_a$ and $Zn_a$. Such an assumption was probably not fully fulfilled, but results obtained in the chapter 6 showed that the P and Zn uptake from the plant compartment were similar for both the non-mycorrhizal and mycorrhizal plants.

We assume that $G. \text{intraradices}$ takes up only PO$_4$ and Zn in forms that are available also for plant roots (Smith et al. 2001, Zhu et al. 2001), which means the isotopically exchangeable PO$_4$ and Zn (Frossard and Sinaj 1997). Thus it is possible, knowing the specific activity of PO$_4$ and free Zn in the soil solution measured in a batch experiment according to Fardeau (1996) to calculate the specific activity of these elements in the soil solution of the labelled compartment. Then we could calculate the total amount of P and Zn coming from this compartment and taken up by the plant.

Let

- $R'$ the total amount of radioactivity added as $^{33}$PO$_4$ or $^{65}$Zn into the labelled compartment and divided by the weight of labelled soil* (MBq/kg)
- $r'_\text{plant}$ the total amount of $^{33}$PO$_4$ or $^{65}$Zn recovered in the plant (MBq)
- $R$ the total amount of radioactivity added as $^{33}$PO$_4$ or $^{65}$Zn (MBq/mL) in a soil/water suspension at a 1:10 w:v ratio
- $r_{1\text{min}}$ the concentration of $^{33}$PO$_4$ or $^{65}$Zn (MBq/mL) remaining in the aqueous phase after 1 minute of isotopic exchange in a 1:10 soil/water suspension
- $r_t$ the concentration of $^{33}$PO$_4$ or $^{65}$Zn (MBq/mL) remaining in the aqueous phase after $t$ minute of isotopic exchange in a 1:10 soil/water suspension
- $t$ the duration of isotopic exchange (minute)
- $C_P$ the orthophosphate concentration in the soil solution (mg PO$_4$/l)
- $C_{Zn}$ the free Zn concentration in the soil solution (mg Zn/l)

* in this experiment, we used 0.33 kg dry weight soil per labelled compartment
Knowing that according to Fardeau (1996)
\[ r_t / R = (r_{t\text{min}} / R) \times t^{-n} \quad \text{(Eq. 3)} \]

The proportion of radioactivity added as $^{33}\text{PO}_4$ or as $^{65}\text{Zn}$ and remaining in the aqueous phase after 2 days of soil incubation and after 27 days (2 days of incubation and 25 days of plant growth) can be calculated using Equation 3.

Assuming that the $C_P$ and $C_{Zn}$ did not change significantly during plant growth because the soil kept releasing nutrient to the soil solution, the specific activities $SA$ (kg soil/mg P) of the soil solution for $\text{PO}_4$ and $\text{Zn}$ can be calculated using the following equations for 2 and 27 days of isotopic exchange.

\[ SA_P = (r_t / R) / (C_P \times 10) \quad \text{(Eq. 4)} \]
\[ SA_{Zn} = (r_t / R) / (C_{Zn} \times 10) \quad \text{(Eq. 5)} \]

Since the specific activity (SA) calculated after 2 and 27 days were very similar, values were averaged for P and for Zn yielding an average $SA_P$ value for the soil solution ($SA_{P-av}$) and an average $SA_{Zn}$ value for the soil solution ($SA_{Zn-av}$).

We hypothesise that the $\text{PO}_4$ and $\text{Zn}$ taken up by the plant and derived from the labelled compartment had specific activities identical to those of the $\text{PO}_4$ and $\text{Zn}$ located in the aqueous phase of the labelled compartment:

\[ (r'_{\text{plant}} / R') / P_c = SA_{P-av} \quad \text{(Eq. 6)} \]
\[ (r'_{\text{plant}} / R') / Zn_c = SA_{Zn-av} \quad \text{(Eq. 7)} \]

Then the total amount of $\text{PO}_4$ and $\text{Zn}$ taken up by the plant and derived from the labelled compartment can be calculated as follows:

\[ P_c = (r'_{\text{plant}} / R') / SA_{P-av} \quad \text{(Eq. 8)} \]
\[ Zn_c = (r'_{\text{plant}} / R') / SA_{Zn-av} \quad \text{(Eq. 9)} \]
And, given equation 1:

\[
P_b = P_{\text{plant}} - P_a - P_c \quad \text{(Eq. 10)}
\]

\[
Zn_b = Zn_{\text{plant}} - Zn_a - Zn_c \quad \text{(Eq. 11)}
\]

Although these calculations suffer from some incertitude we shall assume that they give a realistic estimation for total (stable + radioactive) P and Zn uptake by plant roots and AMF hyphae in the different compartments of the cuvette set-up.

8.2.6. Statistics

Analysis of variance (ANOVA), analysis of covariance (ANCOVA), and regression analysis were done using Statgraphics® software version 3.1 (Manugistics, 1997). The significance values for correlation originate from the lack-of-fit ANOVA for a specified regression model. Different letters denote significant differences between the treatments on a 5% probability level based on multiple range LSD-F-test. Heuristic search for the best fit of regression has been performed using TableCurve™ 2D software version 4 (SPSS, Chicago, IL, USA).

8.3. RESULTS

8.3.1. Cuvette experiment

Plant biomass production (top + roots) was not significantly affected by inoculation with *Glomus intraradices* in the cuvette containers (Table 8-1), although mycorrhizal plants tended to be smaller (Fig. 8-1). However, root: shoot biomass partitioning was significantly increased in the mycorrhizal plants (Table 8-1). This effect was more pronounced for the smallest length of the intermediate compartment (Table 8-1, Fig. 8-1). The P and Zn content was up to 3 times higher in the mycorrhizal plants (tops + roots) as compared with the control (Table 8-1, Fig. 8-2).
Significant amount of radioactive P and Zn isotopes were transported to the plants only when the mycorrhiza was present in the system (Table 8-1, Fig. 8-3). Transport of both $^{33}$P and $^{65}$Zn was strongly dependent on the length of the intermediate compartment, i.e. on the distance between the labelled soil and the roots (Table 8-1, Table 8-2). Transport patterns of $^{65}$Zn and $^{33}$P by AMF from different distances from the roots were strongly correlated to each other (linear regression, $R^2 = 0.87$, $P < 0.001$, Fig. 8-7), indicating a common underlying mechanism. Uptake and transport of $^{33}$P isotope to the plants through AMF from the distance of 5 cm from the roots was 26.8% of the introduced radioisotope within 25 days. As for $^{65}$Zn, 8.8% of the total amount of introduced isotope were translocated to the plants. When the labelled soil was located 15 cm away from the roots, only traces of the isotopes could be recovered in the plants, and at the distance of 20 cm no transport of radioactive tracers could be observed. Therefore the surplus of the total plant P in mycorrhizal plants (as compared with the non-mycorrhizal control) in the cuvettes with 20 cm long intermediate compartment was due to AMF-mediated uptake of P from both plant and intermediate compartments. The surplus of P in

**FIGURE 8-1**
Effect of mycorrhiza on the growth of maize plants in the cuvette containers with different width of the intermediate segment. Means +SE of means of six (control) or nine (mycorrhizal treatment) replicate plants are given.
**TABLE 8-1**

Growth and nutrient content of maize in the cuvette containers with regard to the mycorrhizal treatment. Results of analysis of covariance (ANCOVA) are given. Distance of labelled segment from the plants (=size of the cuvette) was used as covariate (DIST), and the contrast between mycorrhizal and control treatments (TREATMENT) was studied.

<table>
<thead>
<tr>
<th></th>
<th>F value (1,1,57)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TREATMENT</td>
<td>DIST</td>
<td>Nonmycorrhizal control</td>
<td>Mycorrhizal treatment</td>
<td></td>
</tr>
<tr>
<td>Total plant DW (g)</td>
<td>1.94</td>
<td>0.27 ns</td>
<td>8.69</td>
<td>8.04</td>
<td></td>
</tr>
<tr>
<td>Root / shoot biomass</td>
<td>23.94 ***</td>
<td>4.91 *</td>
<td>0.13 a</td>
<td>0.20 b</td>
<td></td>
</tr>
<tr>
<td>P (mg per plant)</td>
<td>40.13 ***</td>
<td>5.86 *</td>
<td>4.81 b</td>
<td>10.57 a</td>
<td></td>
</tr>
<tr>
<td>Zn (µg per plant)</td>
<td>97.82 ***</td>
<td>5.70 *</td>
<td>79.9 b</td>
<td>139.6 a</td>
<td></td>
</tr>
<tr>
<td>% introduced $^{33}$P</td>
<td>34.22 ***</td>
<td>44.37 ***</td>
<td>0.01 b</td>
<td>12.10 a</td>
<td></td>
</tr>
<tr>
<td>transported to plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% introduced $^{65}$Zn</td>
<td>31.86 ***</td>
<td>37.70 ***</td>
<td>0.01 b</td>
<td>4.33 a</td>
<td></td>
</tr>
<tr>
<td>transported to plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific P activity ($^{33}$P/$^{31}$P) in the plants (kBq/mg P)</td>
<td>40.63 ***</td>
<td>63.34 ***</td>
<td>0.0 b</td>
<td>41.6 a</td>
<td></td>
</tr>
<tr>
<td>Specific Zn activity ($^{65}$Zn/total Zn) in the plants (kBq/mg Zn)</td>
<td>27.23 ***</td>
<td>42.67 ***</td>
<td>0.1 b</td>
<td>141.2 a</td>
<td></td>
</tr>
</tbody>
</table>

F values (df, dfcov, residual df) are given, with measure of significance: ns not significant, (*)P<0.1, *P<0.05, **P<0.01, ***P<0.001. Different letters denote significant differences between treatment means (LSD multiple range test, P<0.05). Nine and six replicate plants were included into the mycorrhizal and control treatments at each distance, resp.

The mycorrhizal plants growing in cuvettes with shorter intermediate compartment was always the result of AMF uptake from all three compartments (plant, intermediate, and the labelled one) (Table 8-3).

Maize roots were highly colonised by the AMF in all inoculated containers. No influence of the length of the intermediate compartment on AMF colonisation was found (Table 8-2). No colonisation was found in the non-mycorrhizal treatment.

The pattern of $^{33}$P and $^{65}$Zn uptake through AMF extraradical mycelium to the plants was positively correlated with the length of mycelium in region Z, which was close to the labelled compartment (Table 8-2). However, if mycelium length was estimated in
the middle of the intermediate compartment (region Y), hardly any correlation with isotope transport could be found (correlation coefficients being -0.36, and –0.40, for $^{33}$P and $^{65}$Zn resp., P >0.1 in both cases). Moreover, if mycelium length was assessed at the proximity of the root segment (region X, Table 8-2), significant negative correlation with transport of $^{33}$P and $^{65}$Zn was found (correlation coefficient –0.78 for $^{33}$P and -0.79 for $^{65}$Zn transport, respectively, P < 0.01 in both cases). Calculated inflow for stable P and Zn into the mycelium of the intermediate compartment ranged between 10 and 47 fmol m$^{-1}$ s$^{-1}$ and between 0.05 and 0.18 fmol m$^{-1}$ s$^{-1}$, respectively (Table 8-3).

**FIGURE 8-2**
Total content of P and Zn in maize plants (tops + roots) grown in the cuvette containers. Mean +SE of means are given (six replicates in nonmycorrhizal control and nine replicates in mycorrhizal treatment).

**FIGURE 8-3**
Percentage of introduced $^{33}$P and $^{65}$Zn detected in plants (tops + roots) growing in the cuvette containers 25 days after labelling. The isotopes were transported to the plant roots by mycelium net through the root-free intermediate compartment from the labelled compartment.
TABLE 8-2

AMF colonisation of the roots, transport of $^{32}$P and $^{65}$Zn from different distances to the roots of maize, and the length of AMF mycelium in three regions of the intermediate compartment.

<table>
<thead>
<tr>
<th>Distance between the plant and labelled segments</th>
<th>F value (3,8)</th>
<th>Hyphal colonisation (% root length)</th>
<th>Arbuscular colonisation (% root length)</th>
<th>Vesicle colonisation (% root length)</th>
<th>Mycelium length (m/g substrate) (region X)</th>
<th>Mycelium length (m/g substrate) (region Y)</th>
<th>Mycelium length (m/g substrate) (region Z)</th>
<th>Percentage of introduced $^{32}$P transported to plants</th>
<th>Percentage of introduced $^{65}$Zn transported to plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 cm</td>
<td>98.0</td>
<td>98.0</td>
<td>93.3</td>
<td>94.0</td>
<td>6.14</td>
<td>6.4</td>
<td>27.01</td>
<td>174.11</td>
<td>37.70</td>
</tr>
<tr>
<td>10 cm</td>
<td>93.3</td>
<td>93.3</td>
<td>93.3</td>
<td>94.0</td>
<td>2.9</td>
<td>6.4</td>
<td>4.7</td>
<td>26.8</td>
<td>8.8</td>
</tr>
<tr>
<td>15 cm</td>
<td>94.0</td>
<td>94.0</td>
<td>94.0</td>
<td>94.0</td>
<td>4.1</td>
<td>6.4</td>
<td>1.1</td>
<td>20.8</td>
<td>8.1</td>
</tr>
<tr>
<td>20 cm</td>
<td>95.3</td>
<td>95.3</td>
<td>95.3</td>
<td>95.3</td>
<td>5.7</td>
<td>7.0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

To assess the development of AMF mycelium, 3 samples were collected from each intermediate compartment, one adjacent to the root segment (region X), second from the middle of the intersegment (region Y), and the third from the side adjacent to the labelled compartment (region Z). Results of analysis of variance (ANOVA) with F values (df, residual df) are given, measure of significance: ns not significant, (*) P<0.1, *P<0.05, **P<0.01, ***P<0.001. Different letters denote significant differences between treatment means (LSD multiple range test, P<0.05). Only data from mycorrhizal treatment were used. Data from three replicate cuvette units per each distance (each containing 3 pooled plant samples) were used. Transport of radioactive isotopes was calculated as the percentage of the isotope used for labelling detected in the plant biomass at harvest.

8.3.2. Starpot experiment

AMF reduced shoot biomass production in the starpots (Table 8-4) and caused increase in root biomass production of maize (Fig. 8-4). This resulted in significant shift in the root: shoot biomass ratio, similar to the cuvette experiment (Table 8-4, Fig. 8-4). This latter effect was more pronounced when the distance between the root and the labelled compartments was the smallest (Table 8-4).
TABLE 8-3
Nutrient uptake by mycorrhizal maize from different compartments in the cuvette experimental set-up, and the nutrient uptake efficiency of AMF hyphae in the intermediate compartment.

a) P uptake

<table>
<thead>
<tr>
<th>Intermediate compartment size</th>
<th>( P_a ) (mg)</th>
<th>( P_b ) (mg)</th>
<th>( P_c ) (mg)</th>
<th>AMF mycelium length (m)</th>
<th>P inflow (fmol P m(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5cm</td>
<td>13.34</td>
<td>10.21</td>
<td>7.89</td>
<td>3280</td>
<td>47</td>
</tr>
<tr>
<td>10cm</td>
<td>15.29</td>
<td>19.84</td>
<td>6.11</td>
<td>8200</td>
<td>37</td>
</tr>
<tr>
<td>15cm</td>
<td>16.95</td>
<td>8.57</td>
<td>0.23</td>
<td>9700</td>
<td>13</td>
</tr>
<tr>
<td>20cm</td>
<td>13.96</td>
<td>10.02</td>
<td>0.00</td>
<td>15280</td>
<td>10</td>
</tr>
</tbody>
</table>

b) Zn uptake

<table>
<thead>
<tr>
<th>Intermediate compartment size</th>
<th>( Zn_a ) (mg)</th>
<th>( Zn_b ) (mg)</th>
<th>( Zn_c ) (mg)</th>
<th>AMF mycelium length (m)</th>
<th>Zn inflow (fmol Zn m(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5cm</td>
<td>0.203</td>
<td>0.022</td>
<td>0.383</td>
<td>3280</td>
<td>0.05</td>
</tr>
<tr>
<td>10cm</td>
<td>0.249</td>
<td>0.131</td>
<td>0.353</td>
<td>8200</td>
<td>0.12</td>
</tr>
<tr>
<td>15cm</td>
<td>0.278</td>
<td>0.252</td>
<td>0.014</td>
<td>9700</td>
<td>0.18</td>
</tr>
<tr>
<td>20cm</td>
<td>0.241</td>
<td>0.201</td>
<td>0.001</td>
<td>15280</td>
<td>0.09</td>
</tr>
</tbody>
</table>

1 - length of the compartment between the plant and the labelled compartments, 2 – amount of nutrient in the plants originating from the plant compartment, 3 – amount of nutrient in the plants originating from the intermediate compartment, 4 – amount of nutrient in the plants originating from the labelled compartment, 5 – mycelium length in the intermediate compartment (average of the measurements given in Table 5-2), 6 – calculated P/Zn inflow for the unit mycelium length in the intermediate compartment. Average values per each of the intermediate compartment size were used. Values shown apply for the whole cuvette system, which consists of three separate plants.

AMF increased the P and Zn content in maize (Table 8-4, Fig. 8-5). A significant negative correlation was observed between the length of the intermediate compartment and the total P content of mycorrhizal plants (\( R^2 = 0.32, P = 0.014 \)), but not for the total Zn content of the plants (Fig. 8-5, see also the significance of the covariate in Table 8-4). \(^{33}\)P and \(^{65}\)Zn were transferred to the plants only if the plants were mycorrhizal (Table 8-4, Fig. 8-6). The uptake of both \(^{33}\)P and \(^{65}\)Zn by the mycorrhizal plants was negatively correlated to the length of the connecting tube separating the root and the labelled soil compartments (Fig. 8-6). The percentage of introduced \(^{65}\)Zn and \(^{33}\)P transported by the AMF towards the plants from different distances were strongly correlated to each other (linear regression, \( R^2 = 0.98, P < 0.001 \), Fig 8-7), as in the cuvette experiment.
Maize root colonisation by the AMF reached in all inoculated starpots 96 (±0.8)\% of the root length. The length of the connecting tube between the plant and the soil compartments did not affect the AMF colonisation rate in the plants (Table 8-5). No colonisation was found in control (nonmycorrhizal) treatment. Mycelium length in the intermediate compartment of nonmycorrhizal control pots (due to the presence of nonmycorrhizal soil fungi) was about 1\% of the mycelium length observed in the mycorrhizal treatment. The length of the intermediate compartment (connecting tube) did not affect the mycelium length in that compartment as measured in the mixed substrate samples of the connecting tube content (Table 8-5).

**Figure 8-4**

Effect of mycorrhiza and the distance between the plant and the labelled compartments on the growth of maize in the starpot containers. Means +SE of means of three replicate plants are given.
**TABLE 8-4**
Effect of AMF on the growth and nutrient content of maize plants in the starpots. Results of analysis of covariance (ANCOVA) are given. Distance of labelled segment from the plants (= size of the connecting tube) was used as covariate (DIST), contrast between mycorrhizal and control treatments (TREATMENT) was studied.

<table>
<thead>
<tr>
<th></th>
<th>F value (1, 1, 33)</th>
<th>TREATMENT</th>
<th>DIST</th>
<th>Nonmycorrhizal control</th>
<th>Mycorrhizal treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plant DW (g)</td>
<td>10.81 *</td>
<td>1.18 ns</td>
<td>9.08 a</td>
<td>8.31 b</td>
<td></td>
</tr>
<tr>
<td>Root / shoot biomass</td>
<td>59.85 ***</td>
<td>12.62 **</td>
<td>0.15 b</td>
<td>0.25 a</td>
<td></td>
</tr>
<tr>
<td>P (mg per plant)</td>
<td>21.44 ***</td>
<td>7.78 **</td>
<td>7.62 b</td>
<td>10.48 a</td>
<td></td>
</tr>
<tr>
<td>Zn (µg per plant)</td>
<td>19.07 ***</td>
<td>0.80 ns</td>
<td>114 b</td>
<td>163 a</td>
<td></td>
</tr>
<tr>
<td>% added 32P transported to plant (%)</td>
<td>12.13 **</td>
<td>15.16 ***</td>
<td>0.0 b</td>
<td>2.8 a</td>
<td></td>
</tr>
<tr>
<td>% added 65Zn transported to plant (%)</td>
<td>13.10 **</td>
<td>14.06 ***</td>
<td>0.0 b</td>
<td>1.0 a</td>
<td></td>
</tr>
<tr>
<td>Specific P activity (32P/31P) in the plants (kBq/mg P)</td>
<td>17.03 ***</td>
<td>19.69 ***</td>
<td>0.1 b</td>
<td>20.7 a</td>
<td></td>
</tr>
<tr>
<td>Specific Zn activity (65Zn/total Zn) in the plants (kBq/mg Zn)</td>
<td>19.18 ***</td>
<td>18.93 ***</td>
<td>0.0 b</td>
<td>82.4 a</td>
<td></td>
</tr>
</tbody>
</table>

F values (df, dfcov, residual df) are given, with measure of significance: ns not significant, (*)P<0.1, *P<0.05, **P<0.01, ***P<0.001. Different letters denote significant differences between treatment means (LSD multiple range test, P<0.05). Three replicate plants were included into the mycorrhizal and control treatments at each distance.

**FIGURE 8-5**
Content of P and Zn in maize plants (tops + roots) grown in the starpot containers. Means +SE of means are given of data from three replicate plants.
TABLE 8-5
AMF colonisation of maize roots and the transport of labelled P and Zn placed at different distances from the roots, by the AMF extraradical mycelium in the starpot system. Results of analysis of variance (ANOVA) are given.

<table>
<thead>
<tr>
<th>Distance between the plant and labelled segments</th>
<th>F value (5, 12)</th>
<th>hyphal colonisation (% root length)</th>
<th>arbuscular colonisation (% root length)</th>
<th>vesicle colonisation (% root length)</th>
<th>mycelium length (m/g) (sample of content of connecting tube)</th>
<th>Percentage of introduced $^{33}$P transported to plants</th>
<th>Percentage of introduced $^{65}$Zn transported to plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 cm</td>
<td>6 cm</td>
<td>8 cm</td>
<td>10 cm</td>
<td>12 cm</td>
<td>14 cm</td>
<td>4 cm</td>
<td>6 cm</td>
</tr>
<tr>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>11.52***</td>
<td>7.80**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F values (df, residual df) are given, with measure of significance: ns not significant, (*) P<0.1, *P<0.05, **P<0.01, ***P<0.001. Different letters denote significant differences between treatments (LSD multiple range test, P<0.05). Only data from mycorrhizal treatment (three replicated units per each distance) were used. Transport of radioactive isotopes was calculated as the percentage of the isotope used for labelling detected in the plant biomass at harvest.

FIGURE 8-6
Percentage of applied $^{33}$P and $^{65}$Zn detected in maize plants grown in the starpots 25 days after labelling. The isotopes were transported to the plant roots by mycelium net through the root-free compartment from the labelled compartment. Regression curves for mycorrhizal treatments are given ($^{33}$P: $R^2=0.82$, P<0.001; $^{65}$Zn: $R^2=0.76$, P<0.001).
FIGURE 8-7
Relationship between the uptake of $^{33}$P and $^{65}$Zn by the AMF hyphae from the labelled compartment. The percentage of the radioactive isotope added, which was detected in the plants, is shown. Data for separate plants growing in different distances from radioactively labelled soils either in the cuvettes of in the starpots experiment, and the regression curves for each of the experiments are shown. Both regressions are significant at the P<0.001 level.

8.4. DISCUSSION

8.4.1. Shoot and root biomass of maize

Inoculation of maize with *Glomus intraradices* increased the root biomass in both cuvette and starpot systems, but decreased the overall plant biomass in the starpot system and had no significant effect on plant biomass production in the cuvette system. The positive effect of AMF on plant root biomass production decreased as the distance between the root- and the labelled soil compartments increased.

The effects of mycorrhizal inoculation on plant growth were shown to vary from negative to positive responses, depending on plant host and on the fungal species, on the plant nutrient status, and on environmental conditions (Jun and Allen 1991, Weissenhorn et al. 1994, Weissenhorn et al. 1995, Smith and Read 1997). We observed in our previous experiments (Chapters 6 and 7) a reduction in plant biomass in the presence of *Glomus*...
intraradices, probably due to increased carbon drain to the mycorrhizal structures (Fitter 1991), but we did not observe an increase in root biomass as a response to mycorrhization previously. This discrepancy between the results from different experiments might be explained by the different environmental influences (the experiments were conducted at different times of the year), although greenhouse conditions including photoperiod were kept constant.

8.4.2. Uptake and transport of mineral elements by *Glomus intraradices*

*Glomus intraradices* significantly affected plant uptake of both P and Zn. In both of the cuvette and the starpot set-ups the uptake and transport of labelled P and Zn were significantly correlated, which indicated the existence of a common underlying mechanism for the uptake of the two elements. For similar size of the intermediate compartment the absolute transfer rates of $^{33}$P and $^{65}$Zn in the cuvette were higher as compared with those observed with the starpots. These differences among the two systems were due to different size of the contact zone between the compartments and to the different geometry of the intermediate compartment between the two different set-ups (soil block versus a narrow tube). It is, however, also to be mentioned that the curve shape describing the AMF uptake of elements from different distances from the roots (Fig. 8-6) might also be influenced by the competition among the separate plants growing in the same starpot system. This question should be targeted in the future experiments assessing the contribution of competition to the shape of the uptake curve.

Many AMF were shown to be able to exploit efficiently soil available phosphorus, and thus these fungi might contribute significantly to the P nutrition of the host plants (Kothari et al. 1991, Marschner and Dell 1994, George et al. 1995, Smith and Read 1997). Almost 27% of the added $^{33}$P were transported to the maize plants growing in the cuvette system by *Glomus intraradices* from the distance of 5 cm from the roots. Such a high transfer was probably due to the high available P content of the soil used, which reduced the transfer of the introduced isotope, into less rapidly exchangeable pools. Another possible explanation might be the extensive mycorrhiza development in the
experimental containers. The results obtained in this chapter further support the hypothesis made in the chapter 7 that *G. intraradices* is able of exploiting very efficiently niches that were already colonised by its hyphae since, in the cuvette system, 30 to 50% of the P taken up by maize was derived from the intermediate compartment, which contained a substrate with a low P availability.

*G. intraradices* was also efficient in taking up and transporting Zn, resulting in transfer of almost 9% of the added $^{65}$Zn to the plants from the distance of 5 cm within 25 days in the cuvette set-up. The transport of $^{65}$Zn by mycorrhizal hyphae as observed by Mehravaran et al. (2000) and others (Cooper and Tinker 1978, Bürkert and Robson 1994) was about two orders of magnitude lower than that observed in this study. This could be explained by the use of different host plants (clover vs. maize), different efficiency of the particular AMF symbionts used, or by the differences in Zn content and availability in the soil used for the experiments. For example, Liu et al. (2000) have shown that another isolate of *G. intraradices* played an important role in Zn uptake of maize under low Zn conditions but not under Zn sufficient conditions.

### 8.4.3. AMF mycelium growth and mycorrhiza-mediated nutrient uptake of maize

Mycelium development of the AMF was the common underlying factor elucidating the transport of both P and Zn from the root-free compartment (see also the results in chapter 7). This is in agreement with the results reported by Jakobsen et al. (1992 a, b), Schweiger et al. (1999), Smith et al. (2000), and Jakobsen et al. (2001). The P inflow per unit of hyphal length and per unit of time, as assessed in the cuvette experiment was in a good agreement with Jakobsen et al. (1992a), but about one order of magnitude lower than the values reported by Sanders and Tinker (1973), Cooper and Tinker (1978), and Li et al. (1991a,b). This points to the necessity of more detailed study of the mechanisms of the P uptake by AMF and their variability within AMF communities. The Zn inflow per unit of hyphal length and per unit of time, was about two orders of magnitude lower than that calculated for P, which is in agreement with the lower requirements of plants for Zn as compared with P (Smith and Read 1997). Sufficient concentration of Zn in maize
tissue is for example about 100 times lower than that of phosphorus (25 mg/kg Zn vs. 3000 mg/kg P, Bergmann 1988).

Mycelium spreading of the genotype of *Glomus intraradices* used in this study was limited to 15-20 cm from the plant roots in both the cuvettes and the starpots. This was shown either directly (by observing mycelium development in the substrate) or indirectly by P transport pattern from the different distances (Table 8-2, Fig. 8-3, Fig. 8-6). The effect of the distance between the root and the labelled compartments on mycelium length in the starpot system could not be accurately measured at each point along the length of the connecting tube, because of the sampling difficulties. We measured mycelium length in a mixed sample of the whole volume of the connection tube. Any differences were therefore missed due to the sampling approach (Table 8-5).

In the cuvette system, however, different patterns of AMF mycelium development depending on the length of the intermediate compartment could be observed. For example in case when the labelled soil compartment (nutrient rich patch) was too far from the roots to be accessed by AMF hyphae, then an increase in mycelium length was observed in the proximity of the roots (Table 8-2). This indicates that the dynamic of the mycelium development may depends on the actual environmental conditions. This is the first direct evidence for the ability of the same fungal isolate to modify its mycelium growth pattern in order to maximise the nutrient uptake from localised resources in the soil (as proposed by Cui and Caldwell 1996).

### 8.5. CONCLUSIONS

1. *Glomus intraradices* isolated from the continuously tilled soil of the Hausweid-Tänikon long-term field experiment is efficient in taking up and transporting Zn and P from soil zones located less than 15 cm away from the maize root under greenhouse
conditions. Therefore, Zn acquisition efficiency by the mycorrhizal plant could be used as a functional marker for AMF activity.

However, two points have to be clarified in further studies: i) do the AMF really play an important role in Zn acquisition of the field grown plants? ii) does the variation in Zn uptake efficiency among different AMF isolates/species/genera relates only to the length of mycelium produced by the AMF in the soil, or does it depend also on the isolate/species-specific density/affinity of Zn transporters on the surface of the hyphae?

2. The maximum distance from which *G. intraradices* could take up nutrients from the soil probably does not exceed 15cm from the roots. This seems also to be the maximum mycelium spreading distance from the roots. The maximum mycelium spreading distance and the mycelium architecture (see chapter 7) has, however, still to be investigated for other AMF.

3. The cuvette and the starpot system are comparable, although the nutrient uptake from different distances follows a different curve. This might be influenced by i) the geometry of the containers (size of the contact zone between the compartments), ii) competition between the plants accessing the same labelled compartment via associated AMF mycelium in the starpot system. To dissect the role of the container geometry and AMF competition in the shape of the nutrient uptake curves, some more experiments are needed.
9. GENERAL DISCUSSION AND CONCLUSIONS

9.1. GENERAL DISCUSSION

This general discussion is divided in three parts.

i) First, we discuss the effects of soil tillage on AMF development in the roots.

ii) Second, we address tillage effects on species composition of AMF communities.

iii) Third, we discuss the relationships between the tillage effects on AMF activity in the soil, and the growth and nutrient acquisition by the plants.

1. In the field tillage experiment, the activity of AMF communities under NT conditions was higher than in the CT soils. This was shown by slightly improved AMF infection potential of the soil and by faster AMF colonisation development in the roots early in the growth season (chapter 4). Higher activity of AMF communities from NT soils was also observed in the single-pot study in chapter 6. These results were in a good agreement with previous observations reported by Anken et al. (1997) and Mozafar et al. (2000) from the same field site. Our results indicating higher AMF infectivity under reduced tillage conditions were also in agreement with other studies performed under different soil and climatic conditions (McGonigle and Miller 1993a, Miller et al. 1995, Gavito and Miller 1998, Kabir et al. 1998, McGonigle et al. 1999, Galvez et al. 2001).

2. The composition of AMF communities was significantly altered by different tillage regimes: Glomus spp. was predominant in roots of plants growing in CT soils, while the roots in the NT soils were colonised also by Scutellospora sp., which was completely missing from the CT system. Other AMF such as Gigaspora sp. were not
significantly affected by soil tillage regimes (chapter 5). These results show for the first time in the field experiment that soil tillage does affect the composition of “functionally-relevant” communities of AMF interacting with the roots. Previously, differences in composition of AMF communities induced by tillage were shown only by using the AMF spores isolated from the soil (Douds et al. 1995, Jansa et al. 2002). This was however only of a limited relevance to the actual abundance of different AMF species in the soils, because of the different sporulation rates by different AMF and also because of the difficulty of identifying the field collected spores (Bever et al. 1996, Douds and Millner 1999). Among different possible reasons for the changes in AMF community composition in response to soil tillage, the most probable is the different tolerance by different AMF species to the disturbance of soil mycelium network. This was experimentally shown by Boddington and Dodd (2000b), who observed negative and positive effects of soil disturbance on the root colonisation by Gigaspora and Glomus, respectively.

3. Both the data from the field experiment (chapter 4) and from the pots, where unsterile field soil from different tillage treatments was used as a complex AMF inoculum (chapter 6), did not show any consistent effect of soil tillage on plant growth and P acquisition, which could be attributed to different AMF activity in differently tilled soils. This absence of a strong effect of changing AMF communities on P acquisition by the plants, observed in both P-rich (field) and P-poor (pots) systems is in contradiction with previous studies (Vivekanadan and Fixen 1991, Miller et al. 1995, McGonigle et al. 1999). It is, however, possible that the P limitation was not the only environmental constraint for the growth of the plants in the pots filled with P-poor substrate, and therefore the symbiotic benefits of the AMF could not be fully manifested. Limitations of plant growth by low level of available nitrogen or by a small pot size are also to be taken into account.

Zinc acquisition efficiency by plants was higher in the NT than in annually ploughed soil (chapter 4). An experiment conducted under controlled conditions in the
presence or absence of the AMF communities also suggest that AMF from the NT soil might be superior to AMF from the CT soil in terms of Zn uptake and transport to maize (chapter 6). Because at least some AMF can play an important role in plant acquisition by Zn (chapter 8), the change in plant Zn acquisition efficiency might indicate changes in the activity of the AMF communities. These results are in agreement with the recent report by Ryan and Angus (2001), who also found a correlation between improved AMF development and increased Zn uptake efficiency, but not P uptake efficiency of the crops growing under field conditions. In previous studies it was also noted that although improved uptake of mineral nutrients is frequently observed under NT management, it does not always translate to improved yield of the crops (McGonigle and Miller 1993a, McGonigle and Miller 1996b, Gavito and Miller 1998, Galvez et al. 2001), which was also the case of this study.

In this work, no evidence of a systematic shift of the functional properties of the AMF in terms of nutrient uptake and transport, depending on their tillage history, were found within a set of monosporic AMF isolates obtained from both CT and NT soils (chapters 6 and 7). On the other hand, a large portion of variability of the AMF effects on plant growth and nutrient uptake could have been attributed to the AMF species and/or functional groups, i.e. groups of AMF species showing similar effects on plant growth or nutrient uptake. For example, three different functional groups differing in their strategies of soil exploration for nutrients have been identified among *Glomus* spp. isolated from the field experimental site. These results support the hypothesis that it might be the composition of the AMF communities rather than specific properties of a certain AMF genotype, adapted to the particular environment, which would determine the functionality of the AMF communities. This remains, however, still an open question, as the adaptation of AMF to different environmental constraints has been frequently hypothesised, but different experiments yielded contradictory data (Weissenhorn et al. 1994, Weissenhorn and Leyval 1995, Dodd 2000, Enkhtuya et al. 2000). Some of the experimental contradiction could be explained by loss of specific properties of the AMF acquired through adaptation by exposing the AMF to conditions different from their original
environment. Better understanding of the adaptation of AMF to different environmental constraints is however closely related to our understanding of the genetic structure and evolutionary processes in the group of AMF (Rosendahl and Taylor 1997, Kuhn et al. 2001, Vandenkornhuyse et al. 2001).

9.2. CONCLUSIONS

At the very beginning we hypothesised that if the tillage changes the composition of AMF communities, it should also change the nutrient acquisition efficiency of the plants growing under different tillage regimes. We have shown that the composition of AMF community colonising the roots was significantly affected by the tillage. We have also shown that different AMF species isolated from the experimental field had different effects on plant growth and nutrient uptake, regardless on the tillage treatment of their original soil. However, the hypothesis that the AMF compositional shift would translate to differences in plant nutrient acquisition efficiency was only little supported by the experimental data. The hypothesis was true for Zn, but it was not valid for P, in contrast to many previous studies.

The lack of AMF-mediated effect on the P uptake by the crops, which was encountered in the experimental field can be explained by i) generally high level of available phosphorus in the field soil, and/or ii) by the fact that AMF with different strategies for mineral nutrient uptake were present in all soil tillage treatments. This might mean that the symbiotic function of AMF in terms of P uptake and transport is saturated (it provides maximum possible benefit) under all the tillage treatments studied and thus the diversity of functions within AMF community is to certain extent redundant under these conditions.
10. Future perspectives

Further studies are needed to dissect the role of AMF and of the changes in nutrient concentration, distribution, and availability in the soils, or in the root growth, on the performance and nutrient uptake by crops growing under different management practices in the fields.

The study on identification of active AMF communities in the roots and in the soil should be extended in order to cover the full spectra of different AMF species, which were identified from the study site. For example, specific molecular markers for the whole family of *Acaulosporaceae*, which were represented at least by two different species in the studied field (Jansa et al. 2002), were not developed during this study because of the lack of enough viable spores of these AMF. The time course of colonisation of crop roots by different AMF during the growth season should be tracked by the molecular tools, which were developed in this study. This would yield complementary data to the observation of the AMF colonisation in the roots during the growth season, as presented in chapter 4, and might also be relevant for assessing functionality of AMF symbiosis with time, as depends on its species composition. Similarly, it would be interesting to know whether the species composition of AMF in the roots changes with increasing depth.

The study on functional diversity of AMF at the studied field site should be completed by testing the AMF genera (*Gigaspora, Scutellospora*), whose cultures were not available during time course of this study because of the time needed for
establishment of their pure cultures. An establishment of a standardised functional test to assess the symbiotic efficiency of different AMF would also allow better comparability of results obtained in different laboratories. It is necessary to define which factors of the greenhouse experiments such as the size of the containers, root density, substrate composition and humidity, nutrient availability, model plant genotype, inoculum infectivity, inoculum processing (mechanical disturbance) etc. should be precisely controlled in order to reduce variability of the results.

The estimation of the role of AMF communities in plant growth and nutrient uptake under field conditions should be complemented by observation of labelled nutrient transfer by AMF hyphae from a root-free meshbag buried to the field soil. To properly estimate the effect of complex AMF communities on plant growth and nutrient uptake under laboratory conditions, it will be necessary to conduct experiments with artificial AMF communities established by mixing relevant AMF isolates previously obtained from the fields. To assess if the tillage-induced changes in AMF composition in the soil were also responsible for some of the changes in soil properties such as the aggregate stability, more studies on the variability of the effects of different AMF species/isolates on soil physical/chemical properties are desirable.

The role of AMF in nutrient uptake by different elements should be studied so as to assess the multifunctionality of the AMF in the complex soil system. It seems, based on the results of the chapters 4 and 8, that AMF might play substantial role in uptake of some micronutrients rather than in the uptake of P in nutrient-rich agricultural soils. Nowadays, the multitracer labelling technique allows studying a single mycelium network for the uptake and transport of different elements, and in this respect it reduces the experimental error introduced by observing physically different cultivation units.
Detailed study of the nutrient uptake by different AMF isolates/species obtained from a single field site, which could be conducted in a compartmented system (chapter 7) might in the future lead to development of models predicting the symbiotic efficiency of AMF communities in the fields. This might also result in identification of possible strategies to increase the benefit from the arbuscular mycorrhizal symbiosis by manipulating the composition of AMF communities in the fields. More information will be in this respect needed for the nutrient-limited systems. Therefore, similar studies linking the AMF community structure and functionality should be conducted in nutrient poor systems such as in the tropics.
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APPENDIX

Diversity and structure of AMF communities as affected by tillage in a temperate soil.

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Abstract. Arbuscular mycorrhizal fungi (AMF) were studied in differently tilled soils from a long-term field experiment in Switzerland. Diversity and structure of AMF communities were surveyed either directly on spores isolated from the field soil or on spores isolated from trap cultures, planted with different host plants. Single-spore cultures were established from the AMF spores obtained from trap cultures. Identification of the AMF was made by observation of spore morphology and confirmed by sequencing of ITS rDNA. At least 17 recognised AMF species were identified in samples from field and/or trap cultures, belonging to five genera of AMF - *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora*. Tillage had a significant influence on the sporulation of some species and non-*Glomus* AMF tended to be more abundant in the no-tilled soil. The community structure of AMF in the field soil was significantly affected by tillage treatment. However, no significant differences in AMF diversity were detected among different soil tillage treatments. AMF community composition in trap cultures was affected much more by the species of the trap plant than by the original tillage treatment of the field soil. The use of trap cultures for fungal diversity estimation in comparison with direct observation of field samples is discussed. Electronic supplementary material to this paper can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00572-002-0163-z.

Keywords. Arbuscular mycorrhiza - Tillage - Diversity - Identification - Cultivation

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Introduction. The importance of arbuscular mycorrhizal fungal (AMF) diversity for plant diversity, productivity and ecosystem processes has been recognised recently (van der Heijden et al. 1998). This work and other studies (Bever et al. 1996; Streitwolf-Engel et al. 1997) have shown that different AMF species originating from the same soil can have different effects on plant growth. As a consequence, it appears important to assess the effect of soil management practices on AMF community structure and its diversity.

Mostly, the diversity of AMF in field soils has been studied based on their spore populations. It has been suggested that the diversity of AMF in a temperate grassland soil is limited to ten species and in cultivated soils to even fewer (Johnson 1993). However, a detailed survey of a grassland ecosystem (including also study of AMF assemblages in pots planted with different plants) has shown that 23 species of AMF co-existed in a single field site (Bever et al. 1996) associated with 25 plant species. This high diversity of AMF communities observed under field conditions might not be a site-specific observation, but could just be a result of the analysis of multiple soil samples (Morton et al. 1995). However, observation of spore populations alone may not provide adequate information about AMF community structure, because of the differences in growth and sporulation among AMF species (Land and Schönbeck 1991).

Higher diversity of AMF communities in the woodland ecosystem than on arable land was suggested from analysis of AMF sequences amplified from roots sampled from those two ecosystems (Helgason et al. 1998). The difference in diversity observed between woodland and arable ecosystems was assigned to a complex selective pressure of agricultural practices, such as ploughing, fertilisation and fungicide application, on the AMF communities. Abbott and Robson (1977), Blaszkowski (1993) and Talukdar and Germida (1993) reported prevalence of *Glomus* spp. in agriculturally used soils, in contrast to rich AMF communities containing *Gigaspora* spp., *Scutellospora* spp. and *Acaulospora* spp. in uncultivated soils (Blaszkowski 1993). Johnson (1993) observed a decrease in AMF community diversity due to application of mineral fertilisers, associated with a loss of non-*Glomus* fungi and increase in population of *Glomus intraradices*. Low
infection potential together with lower incidence of some AMF in high-input agricultural soils was also reported by Douds et al. (1993). This indicates that agricultural use of the soil may be an important factor affecting AMF diversity.

It was suggested that no-tillage conditions stimulate mycorrhizal activity in soil and in that way also nutrient uptake by the plants (Miller et al. 1995; Dodd 2000; Mozafar et al. 2000). Soil disturbance has been shown to reduce the density of AMF spores, species richness and the length of extraradical mycelium of AMF relative to undisturbed soil (Boddington and Dodd 2000). The amount of immunoreactive glycoprotein glomalin, which is an exudation product of AMF hyphae and which affects soil aggregation, was 1.5 times higher in no-tilled than tilled soils (Wright et al. 1999). However, the effect of soil tillage on the diversity and structure of AMF communities has been studied rarely for a single field tillage experiment.

The aim of this present study was to investigate the effect of tillage intensity on the AMF diversity and community structure in a Swiss agricultural soil.

Materials and methods.

Study site. The AMF communities were studied in a long-term field tillage experiment established in 1987 at Hausweid, Tänikon, Switzerland (N 47°29’10.0”, E 8°55’10.1”, altitude 540 m). Average climatic conditions are as follows: annual precipitation 1,179 mm, annual air temperature 8.2°C, July temperature 17.4°C, January temperature 0.6°C. The experiment was established in a completely randomised block design with four replicates. Each experimental plot had a rectangular shape of 6×12 m. Four-year crop rotation consisted of a rapeseed (Brassica napus L.), winter wheat (Triticum aestivum L.), maize (Zea mays L.), and winter wheat, with one harvest every year. The soil is a dystric Gleysol (FAO Classification System, Fitzpatrick 1980). The surface horizon (0-25 cm) has the following characteristics: sand 49%, silt 32%, clay 16%, organic matter 2.9%, pH (H2O) 5.8, available P content (Olsen) 52 mg P kg⁻¹ soil. For a detailed description of
the field site, see Anken et al. (1997). The study was restricted to only three tillage treatments: conventional tillage (ploughed to the depth of 25 cm), chisel treatment (loosening soil with a wing share chisel to the depth of 25 cm without turning soil upside-down), and no tillage.

**Spore isolation from field.** Soil cores were sampled always under winter wheat crops from the uppermost 10 cm horizon using a soil borer (3 cm diameter) in January 1999 and in January 2001, following rapeseed and maize cropping seasons, respectively. Thirty individual soil cores were taken randomly from each experimental plot, pooled and mixed to obtain a representative sample. Subsamples (10 g fresh weight) were mixed with 1% Deconex (Borer-Chemie, Zuchwil, Switzerland) in the ratio 1:8 and shaken on a linear shaker for 1 h. The suspension was decanted five times through analytical sieves (500 and 40 µm) and the material collected on the 40-µm sieve was subjected to step gradient centrifugation (5 min, 1,000 g) in 2.5 M sucrose-water gradient. The centrifugation was repeated three times. Soil humidity was estimated after drying soil samples at 105°C for 24 h.

**Culture establishment.** Trap cultures were established from fresh field soil sampled in January 1999 from tilled and no-tilled soils. The soil from each plot was mixed with autoclaved quartz sand (grain size 0.7-1.2 mm) in a ratio of 1:4 (v: v) and filled in 300-mL pots. Pots were planted with one of the five host plants: (1) sugar maize (*Zea mays* L.) cv. "Tasty Sweet", (2) leeks (*Allium porrum* L.) cv. "Zefa Plus", (3) *Plantago lanceolata* L. (collected in Eschikon, Switzerland), (4) sunflower (*Helianthus annuus* (L.)Merill.) cv. "Gelber Knirps" or (5) soybean (*Glycine max* L.) cv. "Paradies". The factorial combinations of soils and plants were replicated 3 times. Traps from the chisel treatment were established only 3 months later and thus were not included in most of the statistical analyses.

Plants were grown in the greenhouse for 5 months, automatically drip-irrigated with deionised water and fertilised once a month with 20 mL of eightfold concentrated
Hoagland nutrient solution (Sylvia and Hubbell 1986) containing 1/100 of the original phosphorus concentration. The conditions in the greenhouse were as follows: day/night photoperiod of 16/8 h, 25/20°C, respectively, aerial humidity 40-50%. Illumination was provided at a minimum intensity of 400 µmol photons m⁻² s⁻¹.

Spores of AMF were isolated from the substrate by wet sieving and decanting (Daniels and Skipper 1982) and by step-gradient centrifugation as described above. Monosporic cultures were established by placing healthy and fresh single spores of AMF on the germination root of a sterile P. lanceolata seedling and growing the plants for 3 months in the greenhouse in sterilised substrate (soil-quartz sand mixture, 1:4 v: v). Both components were first separately autoclaved and the soil was treated afterwards with soil bacterial filtrate and incubated for 4 weeks. The cultures were irrigated with a tensiometer-type controlled watering system (Blumat, Austria) maintaining the substrate humidity at 50-60% of its water holding capacity.

Identification of AMF.

**Soil samples from the field.** Spores isolated from the field soils were examined microscopically and identified according to the taxonomic system proposed by Morton (1988) and Walker (1992). Original descriptions were consulted (Schenck and Perez 1990) and spore morphology was compared with an internet-published reference culture database established by Morton (http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm). Spores were compared also with freshly formed AMF spores from trap cultures originating from the same field site. Spores were observed and photographed with either a stereomicroscope (Olympus SZX12), using combined through- and reflected-light illumination provided by fibre optics, or on a compound microscope (Olympus AX70) using Nomarski contrast. The spores were mounted in water (for stereomicroscopy only) or in either polyvinyl-lacto-glycerol (PVLG) or PVLG + Melzer reagent (1:1 v:v). Spores mounted in Melzer reagent containing media were crushed in order to observe staining of different spore wall layers. At least five spores of each AMF species were mounted in both PVLG and PVLG + Melzer media to observe their
morphology. Only apparently viable spores were used for the identification with the exception of non-*Glomus* AMF, from which only damaged spores were recovered from the field soil. In this case, identification could be performed only to the genus level based on the spores from the field soil. The particular AMF were then identified using the AMF spores from the trap cultures (as described below).

Spores from soil sampled after a rapeseed cropping season (sampled 1999) were to a great extent damaged or dead and could not be identified easily by microscopy. Therefore, we only recorded numbers of spores in different size classes (spore diameter 0-100 μm, 100-150 μm, 150-200 μm, and spores larger than 200 μm). Spores from soil after a maize cropping season (sampled 2001) could be identified to the level of genera or species. Therefore, spore numbers per taxa were recorded for the samples collected in 2001 per soil weight aliquot. The abundances of separate classes within each season were compared between the different soil treatments.

**AMF from trap and pure cultures.** The morphology of the spores was examined under the microscope as described above. Additionally, the internal transcribed spacer (ITS) region of rDNA from selected spores was sequenced to cross-check the visual identification. DNA from single spores (three separate spores from each AMF species recovered from the trap pots or three spores from each AMF monosporic isolate) was extracted, amplified, and sequenced according to Sanders et al. (1995) with the following modifications: 1. A heating step (3 min, 95°C) was included in the DNA extraction procedure to improve efficiency of extraction. 2. PCR Purification Kit (Qiagen) was used to purify the PCR product, which was subsequently inserted into pGEM-T Easy vector (Promega). 3. Cycle sequencing (using BigDye Terminator) was performed on a Perkin-Elmer ABI 310 Capillary Sequencer. One sequence clone was sequenced for each of the monosporic AMF isolates (because only minor sequence variation was encountered while sequencing multiple sequence clones). One to five sequence clones were sequenced from each PCR reaction with the spores recovered from the trap cultures.
Sequences obtained in this study were submitted to GenBank (NIH genetic sequence database http://www.ncbi.nlm.nih.gov) under accession numbers AY035639-AY035666.

Presence/absence of spores of different AMF species was recorded in the substrate of individual trap pots. The sporulation rate of each AMF was estimated according to a semi-quantitative scale from 0 to 3 (0: absence, 1: sporadic, up to 1 spore per g of substrate, 2: abundant, up to 5 spores per g, 3: many spores, over 5 spores per g of substrate).

**Statistical analysis.** Shannon-Wiener and Simpson's indices of diversity (Begon et al. 1998) were calculated using the spore counts in the field soil samples. One-way ANOVAs were used to compare the AMF diversity indices among different soil tillage treatments. The effect of tillage treatment on the abundance of different AMF species (different spore size classes) was assessed by univariate ANOVA tests following multivariate analysis of variance (MANOVA) analysis. The effect of tillage intensity on the community structure of AMF in the field soil was estimated by redundancy analysis and discriminant analysis. The effects of different tillage practices and the species of the host plant in trap cultures on the abundance of AMF species were assessed by univariate ANOVAs following MANOVA analysis, and by two-way ANOVA. Redundancy analysis was employed to assess the contribution of the tillage treatment of the soil and the identity of the host plant on the community structure of AMF in the trap cultures.

MANOVAs and discriminant analysis were calculated employing Systat software version 9.0 (SPSS 1999), redundancy analysis was computed using CANOCO software version 4.0 (TerBraak 1988). ANOVAs were calculated using Statgraphics software version 3.1 (Manugistics 1997), and the P-values were calculated using an LSD-based F test. In the redundancy analysis, the P-values reported stand for the significance of results as tested by the Monte-Carlo permutation test (Jongman et al. 1987). In MANOVA, the P-value was derived from an F-ratio calculated from Hotelling-Lawley trace statistics. For purposes of redundancy analysis, abundance data of all AMF species in the field soil
were classified along the tillage gradient scale (0: no till, 1: chisel, 2: conventional tillage). To test the effect of host plant species on AMF community composition in trap pots using redundancy analysis, host plant data were organised in five independent columns with value 1 indicating presence and 0 indicating absence of a particular plant species.

**Phylogenetic analysis.** The 5.8S sequences excised from the full-length ITS-rDNA sequences (ITS1-5.8S-ITS2) were used for comparison of different AMF species within the group of Eumycota. Reference sequences were obtained from the internet-located dataset of Redecker et al. (1999). For more detailed intraspecific analysis, variable ITS2 region was used. Reference sequences of ITS2 region were obtained from the GenBank. Multiple alignment of sequences was processed using ClustalW software (Thompson et al. 1994). Phylogenetic relationships were inferred using the Phylip Program Package (Felsenstein 1993), employing neighbour joining. The Kimura distance model was used, in which the value ratio of transition/transversion was set to 2. Delete-half-jack-knife (obtained by 500 times repeated sampling) was used as a criterion for support of phylogenetic tree branching. The phylograms were rooted on an outgroup species (*Phaseolus vulgaris* L. for analysis of all fungal sequences, or *Glomus claroideum* and *Gigaspora margarita* for analysis of separate glomalean subgroups (*Gigasporaceae* and *Glomaceae*, respectively).

**Results and discussion.**

**Isolation and identification of AMF.** Morphological identification of AMF from the field soil samples (where 14 species were detected) was confirmed by observing the morphology of freshly formed AMF spores in trap cultures (where 16 species were detected). The identification was also supported by sequencing of rDNA from the spores recovered from the trap cultures and performing phylogenetic analyses (Fig. 1, Fig. 2, Electronic Supplementary Material Fig. S2). A list of all AMF species found within the experimental field is given in Table 1. Seventeen AMF species (belonging to 5 genera)
were detected in the no-till plots (see Electronic Supplementary Material Fig. S1). Almost all of the AMF detected in the no-tilled soils were also recorded in the tilled treatment, with the exception of *Scutellospora*, *Entrophospora*, and *Acaulospora*, which were missing. However, the identification of some AMF, especially if based on the spores obtained from the field soil only (as was the case for *G. aggregatum*-like AMF and *G. microaggregatum* in this study), is prone to error and will need confirmation in subsequently established pot cultures, where the particular AMF grow. The AMF species richness observed in the field soil in this study was similar to the AMF species richness reported by Franke-Snyder et al. (2001), who detected 15 AMF species in a field experiment under different farming managements in the United States. Our results are, however, contradictory to the findings of Helgason et al. (1998), who suggested low richness of AMF in arable soils, reaching down to a single species (*Glomus mosseae*).

Trap cultures allowed detection of *Acaulospora* sp. and confirmation of the morphological identification of *Gigaspora* and *Scutellospora* by DNA sequencing. *Gigaspora, Scutellospora, Acaulospora*, and *Entrophospora* could not be subcultivated to single-spore cultures (20 pot cultures were established for each of these AMF genera), but *Gigaspora* sp. and *Scutellospora* sp. were subcultivated recently using multisporic cultures in which 10-15 spores were used as starting inoculum. In contrast to our findings, Brundrett et al. (1999a) compared different isolation techniques to obtain pure AMF cultures and reported low recovery of *Scutellospora*, *Gigaspora* and *Acaulospora* by trap culturing. They also proposed starting pure cultures of these AMF genera directly from spores collected in the field in order to obtain the highest isolation efficiency. This was not applicable in our case, as we established our trap cultures from field soil after the rapeseed season, when most of the AMF spores were damaged or dead.

In the time frame of our study, only five species of AMF could be cultivated in single spore cultures, all belonging to *Glomus* spp. (altogether over 1,000 single-spore cultures were established and 96 of them were successful). Twelve single-spore cultures belonging to these five *Glomus* species (*G. intraradices*, *G. mosseae*, *G. clarum*-like, *G.
chlorideum, and G. caledonium) were deposited in INVAM (International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi, Morgantown, Fla., USA, http://invam.caf.wvu.edu) as accession numbers SW201-SW212 and also submitted for registration to La Banque Européenne des Glomales (BEG).

The results of molecular analysis based on the 5.8S rDNA (Fig. 1) show clearly that the group of AMF is delimited from other Asco- and Basidiomycetes. Several groups were identified among the AMF (Fig. 1). The distinct phylogenetic lineages of AMF were further analysed using sequences of the highly variable ITS2 region (Fig. 2, Electronic Supplementary Material Fig. S2). Using this approach, Gigaspora sp. and Scutellospora sp. from trap cultures were found to be similar to Gigaspora margarita and Scutellospora pellucida, respectively (Fig. 2). Our observation of Gigaspora margarita from Switzerland is the first confirmed report of the presence of this AMF species in Europe. Previously this genus was thought to be absent from the European continent (Walker 1992). Gigaspora gigantea and some unrecognised Gigaspora sp. have been reported already from Poland (Blaszkowski 1993), but neither photographic records nor DNA analyses of those fungi have been reported and they were not isolated into culture.

Our sequences from Glomus mosseae fitted well into the highly variable group of G. mosseae, G. coronatum, and G. dimorphicum. The sequences of G. caledonium from both trap and pure cultures clustered into a separated branch from G. mosseae, together with G. geosporum. Unfortunately, there is as yet no ITS2 sequence of G. caledonium in the GenBank for comparison. In addition, Glomus caledonium and G. mosseae could be well matched with their reference cultures by means of isoenzyme analysis (Electronic Supplementary Material Fig. S3).

A large phylogenetic distance was observed between the groups of Glomus intraradices and G. clarideum in the phylogenetic analysis based on the ITS2 region. The AMF isolates identified as Glomus clarum-like (identification kindly confirmed by Dr. J. Morton) could not be separated from G. clarideum based on the variable ITS2
sequences (Electronic Supplementary Material Fig. S2), but the isoenzyme banding pattern supported the separation of \textit{G. clarum}-like and \textit{G. claroideum} (Electronic Supplementary Material Fig. S3). It was shown previously that different isolates of \textit{G. clarum} had different phylogenetic affinities, although they were all morphologically similar to \textit{G. clarum} (Schüssler et al. 2001).

Most of our sequences obtained from \textit{Entrophospora} sp. spores clustered with asco- or basidiomycetous fungi; one of them was linked distantly to the group \textit{Glomus occultum} - \textit{G. brasilianum} (Fig. 1). Therefore, it is probable that most of the amplified DNA did not originate from AMF but probably from intrasporal contaminations. Similar findings have been reported by Redecker et al. (1999), who noted that the sequences T1 and T3 (shown in Fig. 1) originating from \textit{Scutellospora} spores clustered together with the Ascomycete fungus \textit{Leptosphaeria} sp. Some of our sequences from \textit{Entrophospora} spores also clustered very close to the \textit{Leptosphaeria} - \textit{Phoma} complex. PCR amplification of rDNA from some other AMF (e.g. \textit{G. fasciculatum}, \textit{G. diaphanum}) from the trap cultures was not successful and, therefore, the sequences could not be obtained. In the context of molecular identification of AMF, it has to be mentioned that a large degree of molecular variability exists even within a single individual AMF (a single spore), as discussed, for example, by Kuhn et al. (2001). This points to the danger of identification of AMF from one or a few sequences and, thus, the results presented here must be interpreted with caution.

**Effect of soil tillage on the AMF community in the field.** Significantly more AMF spores were observed in soil from no-tilled wheat field than from the tilled field following the rapeseed season (P=0.05, Fig. 3A). However, the total spore count was not significantly affected by soil tillage following the maize season (Fig. 3B). Higher spore counts in the no-tilled soils after rapeseed may be due to increased presence of mycorrhizal weed plants (e.g. from the family \textit{Asteraceae}) in the no-tilled plots (Streit et al. 2000). These weeds may have supported AMF development in their roots and also caused some spore formation under the rapeseed crop, which is a non-mycorrhizal plant.
In the tilled plots, ploughing eliminated the majority of the weed plants (Streit et al. 2000) and, therefore, AMF development during the rapeseed season would be negligible. Generally, higher numbers of AMF spores were observed in the soil following the maize season than the rapeseed season. This is because maize is a very good AMF host and supports AMF development and spore formation, which in turn leads to increased AMF infectivity of the soils (Gavito and Varela 1993). We observed high absolute spore counts in the field soils than those reported from a range of different environments (Blaszkowski 1993; Allen et al. 1998). This could be due to an improved method for recovery of spores from soil, as well as to the fact that we studied agricultural soils, where the development of at least certain types of AMF is greatly enhanced. Up to 300 spores per gram of soil in fields used intensively for agriculture have been reported in the United States (Kurle and Pfleger 1996). The high spore counts reported in this present study do not, however, represent the actual numbers of infective propagules in the soil, which were about three times lower than the actual spore counts (data not shown). This is probably because some of the spores included in the counts were not viable, or were present in clusters which would function as one (unseparable) infective propagule.

The differences among total numbers of AMF spores in the tilled and no-tilled soils were mostly due to spores in the smallest size class (under 100 µm). Both the small-spore fraction and the total number of spores were affected by different tillage treatments (P<0.1, Fig. 3A). The differences in other size fractions of spores were not significant. Spore counts in season 2001 were not significantly different between the soil treatments, because the largest contributor to spore counts (G. intraradices) did not show significant differences with respect to soil tillage treatments (Fig. 3B). However, a significant effect of tillage on abundance of G. invermaium and G. microaggregatum was observed. An apparent increase in the incidence of Gigaspora, Scutellospora, and Entrophospora was noted in the no-tilled soil, but the results were not significant, probably because very few spores of the non-Glomus AMF were sampled. The increased presence of non-Glomus fungi (Gigaspora and Scutellospora) in uncultivated soils was shown previously based on both spore observation (Blaszkowski 1993) and root analyses (Helgason et al. 1998).
However, the occurrence of *Entrophospora infrequens* was not influenced by agricultural use of the soil in a previous study (Blaszkowski 1993). The low sporulation rate of some AMF may be an inherent character (Bever et al. 1996). Thus, the spore counts might not give representative values for the actual abundance of the AMF in the soil (Clapp et al. 1995). To approach a reliable quantification of the AMF in the rhizosphere by targeting soil mycelium network or intraradical colonisation structures, other methods, such as specific primer quantitative PCR or specific primer PCR coupled with multiple sampling, are needed (Edwards et al. 1997; Jacquot-Plumey et al. 2001).

Abundance of some large-spored *Glomus* spp. in the field in 2001 was slightly increased in conventionally tilled soil (*G. mosseae*, *P*<0.12), which points to the possible adaptation of different AMF species to different levels of soil disturbance. Similar observations were already reported by Abbott and Robson (1977) and Blaszkowski (1993), who observed *G. deserticola* and *G. mosseae*, respectively, to be more or less associated with cultivated soils. Prevalence of *Glomus* spp., especially *G. mosseae* and *G. clarum*, over *Gigaspora* spp. and *Acaulospora* spp. was also reported from cropped soils in Canada (Talukdar and Germida 1993).

Comparison of spore size classes and species of AMF may be difficult because sizes of different AMF species could overlap (Schenck and Perez 1990). Thus, only a very broad comparison was made. The small size fraction (<100 µm) in 1999 could be attributed to *G. intraradices* and other small-spores cluster forming AMF. The less numerous larger spore fractions (>100 µm) determined in the soils in 1999 would represent the whole range of AMF species, which could only be identified based on the apparently viable spores in 2001.

Soil tillage had no significant effect on the diversity of the AMF community, as assessed by the diversity indices (*P*=0.62 and *P*=0.74 for comparison of Simpson and Shannon-Wiener diversity indices, respectively). However, these indices may not reflect properly the ecosystem structure, especially if the differences in abundance between different
species are too large (for example, if there is pronounced dominance by one or a few species; Jongman et al. 1987), as was the case in this study (Fig. 3).

MANOVA confirmed significant differences in abundance of some AMF with respect to soil tillage treatment (Hotelling-Lawley trace statistics P=0.018). This also means that the effects of tillage on the abundance of the different AMF species were not the same. A subsequently applied discriminant analysis identified five AMF whose abundance explained 79.3% of the variability of the community structure shift along the tillage intensity gradient: Gigaspora sp., Scutellospora sp., G. invermaium, G. mosseae, and G. microaggregatum. Redundancy analysis was then used to assess how the community composition was affected by different tillage treatments (Fig. 4). The first canonical axis (corresponding to the tillage intensity gradient) explained 23.9% variability of the dataset. A Monte Carlo permutation test confirmed the community structure of the AMF to be significantly affected by different tillage management (P=0.005). Contrary to our results, Franke-Snyder et al. (2001) observed no significant change in composition of AMF community after 15 consecutive years of low-input, as compared with a conventional farming system.

**Effect of soil origin and host plant species on the AMF community in trap cultures.**

Analysis of the composition of spore assemblages in trap pots by MANOVA (Table 2) revealed a large effect of host plant species on the composition of AMF communities in the pots, while the effect of tillage treatment of the original soil was not significant. The abundance of three *Glomus* spp. in trap pots was significantly affected by the presence of a specific host plant (Table 3), while the host influence on another fungus (*G. mosseae*) was only marginally significant (ANOVA, F-test, P=0.05). On the other hand, the abundance of only a single AMF species (*G. caledonium*) was significantly affected (P=0.03) by the tillage treatment of the original soil. All non-*Glomus* AMF (*Gigaspora, Scutellospora, Acaulospora, Entrophospora*) and *G. fasciculatum* were detected exclusively in trap cultures from no-tilled soils (Table 1), but usually only in very low numbers; this might also be due to the disturbance of the original soil samples while
establishing the trap cultures. ANOVAs for abundance data of these AMF species were not significant.

Redundancy analysis quantified the effect of the two factors (tillage, host plant species) on AMF community composition. Tillage treatment of the original soil used for establishment of trap cultures accounted for 1.2% of the variability of AMF community composition and was not significant, as assessed by the Monte-Carlo permutation test (Fig. 5A, P=0.18). On the other hand, the effect of the host plant species accounted for 7.7% of the variability and was highly significant (Fig. 5B, P=0.01). This is consistent with previous reports showing a strong influence of the host plant species on fungal development in trap cultures (Brundrett et al. 1999b). It seems that the most important factor limiting growth of certain AMF taxa is the degree of their host specificity, as proposed by Bever et al. (1996). The highest level of diversity in the AMF community in trap pots was found when soybean (or sunflower) was used as a host plant (Fig. 5B). On the other hand, P. lanceolata supported development of Glomus spp. only. This is in contradiction to Bever et al. (1996), who found high sporulation of Scutellospora calospora on P. lanceolata, but it is in agreement with the results of Sanders and Fitter (1992), who reported that Scutellospora sp. did not sporulate well in association with P. lanceolata.

Conclusions. We found a trend of increase in the incidence of certain AMF, especially those not belonging to Glomus spp., in intensively used agricultural soil under long-term (13 years) reduced tillage management (chisel, no till). In conventionally tilled soils, almost exclusively AMF belonging to Glomus spp. were present (G. mosseae, G. claroideum, G. caledonium, G. constrictum, G. clarum-like). Five genera (Glomus, Gigaspora, Scutellospora, Acaulospora, and Entrophospora), and about 17 AMF species were recorded from a relatively small-scale field experiment (0.6 ha). It seems that the most efficient approach to observing the influence of environmental factors on AMF communities is to study the fungi directly from field samples (despite the difficulties with their identification) and simultaneously to cultivate them in pot cultures, where the spores
can be identified reasonably well. To eliminate selective effects of trap plant species on the AMF community structure, it is recommended to use several host plant species for establishment of trap cultures. Although morphological identification of AMF is the most widespread method and in many cases still unavoidable (it is fast, relatively cheap and easy to perform, though rather subjective), it should be carried out concomitantly with other (e.g. molecular) methods for mutual confirmation.

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Table 1. AMF species detected (based on the spore morphology) in different tillage treatments of the long-term field tillage experiment at Tänikon (Switzerland). F and T denote detection of the spores in the field soil samples and in trap cultures, respectively.

<table>
<thead>
<tr>
<th>AMF species</th>
<th>Detected in soil tillage treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conv. tillage</td>
</tr>
<tr>
<td>Acaulospora paulineae (?) Blaszkowski</td>
<td></td>
</tr>
<tr>
<td>Entrophospora infrequens Ames &amp; Schneider</td>
<td></td>
</tr>
<tr>
<td>Gigaspora margarita Becker &amp; Hall</td>
<td>F</td>
</tr>
<tr>
<td>Glomus aggregatum a Schenck &amp; Smith emend. Koske</td>
<td>F</td>
</tr>
<tr>
<td>Glomus caledonium Trappe &amp; Gerdemann</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus claroideum Schenck &amp; Smith</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus clarum-like b Nicolson &amp; Schenck</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus constrictum Trappe</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus diaphanum Morton &amp; Walker</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus etunicatum Becker &amp; Gerdemann</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus fasciculatum Gerd. &amp; Trappe emend. Walker &amp; Koske</td>
<td></td>
</tr>
<tr>
<td>Glomus geosporum Walker</td>
<td>F</td>
</tr>
<tr>
<td>Glomus intraradices Schenck &amp; Smith</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus invermaium Hall</td>
<td>F, T</td>
</tr>
<tr>
<td>Glomus microaggregatum Koske, Gemma &amp; Olexia</td>
<td>F</td>
</tr>
<tr>
<td>Glomus mosseaе Gerdemann &amp; Trappe</td>
<td>F, T</td>
</tr>
<tr>
<td>Scutellospora calospora Walker &amp; Sanders</td>
<td></td>
</tr>
<tr>
<td>Scutellospora pellucida Walker &amp; Sanders</td>
<td></td>
</tr>
</tbody>
</table>

a – The morphology of this AMF was distinct from *G. intraradices* in the field soil samples (the spores formed more compact clusters than *G. intraradices* and the outer wall layer was significantly thicker than the inner wall layer). Both *G. aggregatum* and *G. intraradices*, however, probably belong to the same species, as a continuum of morphologies was recognised between these two species (JC Dodd, pers. comm.). *G. aggregatum* morphotype was not confirmed in the trap cultures.

b – The morphology of these AMF was closely resembling *G. clarum*, although some of the monosporic isolates identified as *G. clarum*-like were later assigned to *G. luteum*. However, all the monosporic isolates, which were assigned to these two species (*G. clarum*-like and *G. luteum*) based on their spore morphology, were all closely related and probably represented a single AMF species (see isoenzyme data in electronical supplement Fig. S3) with transient morphology between *G. clarum* and *G. claroideum-G. luteum*. 
Table 2. Results of multivariate analysis of variance (MANOVA) on abundance of 12 different AMF species in trap pots, which were established from the field soil sampled from the plots subjected to two different levels of tillage intensity (conventional and zero tillage). The trap cultures were planted with 5 different host plants. Hotelling-Lawley (HTL) trace statistic was used to calculate approximate F ratio with accompanying numerator (num.) and denominator (den.) degrees of freedom.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>HTL</th>
<th>F-ratio</th>
<th>Num. df</th>
<th>Den. df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tillage</td>
<td>1</td>
<td>0.187</td>
<td>1.543</td>
<td>12</td>
<td>99</td>
<td>0.122</td>
</tr>
<tr>
<td>Host plant</td>
<td>4</td>
<td>1.055</td>
<td>2.143</td>
<td>48</td>
<td>390</td>
<td>0.000</td>
</tr>
<tr>
<td>Tillage x Host plants</td>
<td>4</td>
<td>0.397</td>
<td>0.807</td>
<td>48</td>
<td>390</td>
<td>0.818</td>
</tr>
</tbody>
</table>

Table 3. Effect of the tillage treatment and trap host plant species on abundance of different AMF in trap cultures (only data for conventional and no-tillage treatments are shown). F-values from univariate F-tests (df, numerator, residual df as denominator) following MANOVA are given. Statistical significance of results is shown: n.s. not significant (P≥0.1); (*) P< 0.1; * P<0.05; ** P<0.01.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Tillage (A) F_{1,110} =</th>
<th>Host plant (B) F_{4,110} =</th>
<th>Interaction A×B F_{4,110} =</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gigaspora</em> sp.</td>
<td>3.194 (*)</td>
<td>1.419 n.s.</td>
<td>1.419 n.s.</td>
</tr>
<tr>
<td><em>Scutellospora</em> sp.</td>
<td>1.000 n.s.</td>
<td>1.000 n.s.</td>
<td>1.000 n.s.</td>
</tr>
<tr>
<td><em>Entrophospora</em> sp.</td>
<td>1.000 n.s.</td>
<td>1.000 n.s.</td>
<td>1.000 n.s.</td>
</tr>
<tr>
<td><em>Acaulospora</em> sp.</td>
<td>2.000 n.s.</td>
<td>0.750 n.s.</td>
<td>0.750 n.s.</td>
</tr>
<tr>
<td><em>G. fasciculatum</em></td>
<td>1.960 n.s.</td>
<td>0.760 n.s.</td>
<td>0.760 n.s.</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>0.831 n.s.</td>
<td>2.424 (*)</td>
<td>1.928 n.s.</td>
</tr>
<tr>
<td><em>G. caledonium</em></td>
<td>4.851 *</td>
<td>0.742 n.s.</td>
<td>0.317 n.s.</td>
</tr>
<tr>
<td><em>G. claroid-like</em></td>
<td>0.640 n.s.</td>
<td>0.355 n.s.</td>
<td>0.088 n.s.</td>
</tr>
<tr>
<td><em>G. claroideum</em></td>
<td>0.776 n.s.</td>
<td>3.057 *</td>
<td>0.561 n.s.</td>
</tr>
<tr>
<td><em>G. etunicatum</em></td>
<td>0.046 n.s.</td>
<td>4.730 **</td>
<td>0.634 n.s.</td>
</tr>
<tr>
<td><em>G. intraradices</em></td>
<td>0.221 n.s.</td>
<td>0.939 n.s.</td>
<td>1.222 n.s.</td>
</tr>
<tr>
<td><em>G. diaphanum</em></td>
<td>0.824 n.s.</td>
<td>4.888 **</td>
<td>0.394 n.s.</td>
</tr>
</tbody>
</table>
Fig 1. Molecular identification of AMF based on phylogenetical inference analysis of 5.8S rDNA sequence data. Neighbour-joining phylogram with delete-half jack-knife values higher than 50 (500 times re-sampled) is shown. DNA was obtained from AMF spores isolated from trap cultures (labelled with “trap” and replicate number in parentheses), or from pure isolate cultures (labelled with isolate number and replicate number in parentheses). *Phaseolus vulgaris* sequence was used as outgroup. All newly reported sequences are contrasted by grey background. Several different sequences (mainly of ascomycetous and basidiomycetous origin) were found in *Entrophospora* spores (labelled “S.E.C. sequence) from trap cultures. Fungal DNA sequences used for comparison were obtained from the dataset by Redecker et al. (1999).
Fig 2. Molecular identification of *Gigaspora* sp. and *Scutellospora* sp. from trap pots based on phylogenetical inference analysis of sequence data of ITS2-region of rDNA. Neighbour-joining phylogram with delete-half jack-knife values higher than 50 (500 times re-sampled) is shown. DNA was obtained from AMF spores isolated from trap cultures (replicate number are given in parentheses). *Glomus claroideum* sequence was used as outgroup. All newly reported sequences are contrasted by grey background. Sequences are compared with previously published fungal sequences (obtained from GenBank), which are marked with their accession numbers (combination of letter and number coding).

Fig 3. Spore counts in field soils after rapeseed season (1999), when only two tillage treatments were compared (A), and spore counts in soils following maize season (2001), where three tillage treatments were compared (B). F-values following ANOVAs are given. Statistical significance of results is shown (n.s. not significant (P ≥ 0.1); (*) P < 0.1; * P<0.05; ** P<0.01)
Fig 4. Effect of soil tillage intensity on the community structure of AMF. Results of redundancy analysis are shown using the spore abundance data of the AMF species in the field soils in 2001, following the maize season. Size and orientation of the vectors represent correlation among them and with the axes. The smaller the angle between the vectors (or a vector and an axis) and the longer the vectors are, the more correlated are the variables represented by the vectors. Axis 1 represents the effect of the tillage intensity, axis 2 is the most explanatory axis pooling all other environmental effects on population structure of AMF. Abundance data of the AMF species in the field soil after maize crop (2001) were used.
Fig 5. Effect of tillage treatment of the original soil used for trap culture establishment, and the plant species, on the composition of AMF communities growing in the trap cultures. Redundancy analysis of mycorrhizal spore abundance data along a tillage intensity gradient (A), and redundancy analysis of AMF species abundance as affected by the species of a host plant (B), are shown. Vectors, which represent factors (tillage intensity or plant species), are dashed; vectors representing fungal species abundance are shown as full lines. Size and orientation of the vectors represents correlation among them and with the axes.
Fig S1. AMF spores from field soil and from pot cultures, which were established with field soil from a long-term field tillage experiment in Tänikon (Switzerland). Supposed identities of the AMF spores are given. Respective tillage treatment of the soil and trap plant species is given in parentheses. Spores were mounted in water (pictures Nr 13, 17, 21, 22, 25, 27, 29), and photographed under stereomicroscope, or mounted in PVLG (pictures 3, 5, 9, 14, 18, 30, 35, 37, 39, 41, 43) or PVLG + Melzer reagens (pictures 1, 2, 4, 6 – 8, 15, 16, 19, 20, 23, 24, 26, 28, 31 – 34, 36, 38, 40, 42, 44) and photographed under a compound microscope using Nomarski contrast optics (see method section).


Fig S2. Molecular identification of AMF based on the ITS2-rDNA sequences. Neighbour-joining phylograms are given with delete-half-jack-knife values (500-times re-sampling) higher than 50 are shown for the respective tree branching nodes. (A) Identification of *Glomus mosseae* and *Glomus caledonium* from both trap pots and pure monosporic cultures. Sequences which were obtained from spores from trap cultures are marked “trap” and replicate number and spores from pure cultures are marked with 3-digit number (243, 964, 658). Newly reported sequences are contrasted in grey. *Gigaspora margarita* sequence was used as outgroup. (B) Identification of *Glomus claroideum*, *G. clarum*-like (referred as *G. clarum* 610, *G. luteum* 132, and *G. luteum* 770) and *G. intraradices* from pure monosporic cultures. They are marked with 3-digit numbers of the pure culture (360, 672, 133). The reference sequences are marked with database accession numbers (combined letter + numbers marking). Newly reported sequences are contrasted in grey. *Gigaspora margarita* sequence was used as outgroup.
Fig S3. Isoenzyme (A: malate dehydrogenase, E.C. 1.1.1.37. and B: esterase, E.C. 3.1.1.1.) profiles of spore protein extracts from single-spore isolates. 100-200 spores per each sample were used. AMF isolate samples from the experimental field were loaded in the lanes 1-12 (lane 1, 7, 11 - *Glomus clarum*-like, isolated from till, no-till, chisel, resp.; lane 2,6,9 - *G. claroideum* from till, no-till, chisel, resp.; lane 3,5,10 – *G. intraradices* from till, no-till, chisel, resp; lane 4,12 – *G. mosseae* from till, no-till, resp.; lane 8 - *G. caledonium* from the chisel treatment), reference isolates were loaded in lanes 13-18 (lane 13 – *G. clarum* from Dr. Siqueira, Brazil; lane 14 - *G. claroideum* BEG23; lane 15 - *G. mosseae* BEG76; lane 16 - *G. caledonium* BEG20; lane 17 - *G. intraradices* BEG75; lane 18- *G. geosporum* BEG11).

The analysis was performed according to Rosendahl and Sen (1992: Isozyme Analysis of Mycorrhizal Fungi and Their Mycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the study of mycorrhiza, Methods in Microbiology, vol 24, Academic Press, London, pp 169-194), with following modifications: (1) The analysis was performed on vertical electrophoresis system (Biometra Maxigel G48, Göttingen, Germany), (2) Bromophenol Blue was added only to one sample per each series, because we have previously noted a drop in enzyme activity due to presence of this compound. (3) For staining of esterases we used a modified buffer system (0.1M phosphate buffer, pH 6.4). All staining compounds (α- and β-naphtyl acetate, Fast Blue RR salt) were dissolved in 4 mL DMSO (dimethylsulfoxide) prior to mixing into the buffer.