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EFFECTS OF ARBUSCULAR MYCORRHIZA AND NITROGEN NUTRITION ON GROWTH AND NUTRIENT UPTAKE OF CHICKPEA AND BARLEY

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Preface

Chickpea is a traditional low-input crop in the farming systems of the Indian subcontinent and the Near East where it is an integral part of the daily diet of the people. The crop is also popular in the Ethiopian Highlands and in Central and South America. Because of its adaptability to a wide range of environments, it is being promoted even in countries such as Australia, Canada and USA.

Research on the chickpea crop was neglected for many years and only recently due attention has been paid to it. The amount of work published on chickpea research during the past decade may well equal all that had appeared in the several preceding decades.

Very important crop plants, i.e., legumes species, can obtain a significant portion of their N requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium* strains. In crop rotations they improve the N nutrition and yield of subsequent cereals. Chickpea (*Cicer arietinum* L.) as a legume holds a unique position among pulse crops due to its seed protein content and wide adaptability in ecologically diverse environments. It has been shown that an intensive root colonization of chickpea by arbuscular mycorrhizal fungi (AMF) fosters plant growth and dry matter production. The positive fungal effect on plant P uptake is beneficial for the functioning of the nitrogenase enzyme of the rhizobial symbiont leading to a higher N_2 fixation. Mycorrhizal and rhizobial symbioses often act synergistically on infection rate, mineral nutrition and plant growth.

In this respect, it is of vital importance to promote the research and usage of underutilized crops worldwide and in Austria. The following work shall make a contribution to enhance knowledge and usage of chickpea.

Abstract

There is increasing evidence for the promoting effects of arbuscular mycorrhiza fungi (AMF) on the growth of practically important crops which is generally attributed to the improved uptake of nutrients. The present study evaluates the effects of inoculating AMF on growth and nutrient acquisition of chickpea and barley based on a series of pot experiments during 2 years. A range of soil biological and chemical conditions was used to test the AMF treatment in interaction with indigenous microbes (sterilized vs. non-sterilized soil), application of additional fertilizer N or co-inoculation of chickpea with nitrogen-fixing rhizobia. The effect of treatments on colonization by AMF, rhizobial nodule number and weight, plant dry matter and soil mineral N, nutrient concentration and uptake were determined in randomized complete block designs with five replications using a chernozem topsoil of silty loam in a 1:1 mixture with sand as basic substrate. Inoculated plants were effectively colonized by AMF and attained more dry matter than control plants in both sterilized and non-sterilized soil, but colonization levels varied substantially between years. Both, chickpea and barley showed growth enhancement, though the AMF colonization level was lower with barley than with chickpea. The level of soil mineral N did not affect AMF performance. The non-sterilized soil contained no natural rhizobia strains suitable for chickpea infection, but with rhizobia inoculation nodules developed. When they were present their number often exceeded 10 per plant, but most of them were of small size and apparently ineffective. We found hardly any consistent effect of nitrogen nutrition on chickpea growth, neither due to fertilizer N nor to rhizobial infection, and with rhizobia inoculation no effect on N uptake was obtained. Interactions between AMF inoculation and nitrogen nutrition were only rare and cannot be generalized. This suggests that compatible, effective rhizobia were not present in the inoculum product or their environmental demands were not fulfilled and presumably nitrogen also was no growth limiting factor in our experiments. At the maturity of chickpea and barley plants, there were significant effects of AMF inoculation on the N, P, K, Ca, Mg, Fe, Mn, Zn and Cu uptake in all our experiments. Only N uptake of chickpea in sterilized soil and Mn in barley was not significantly affected. Additional N supply was again of minor importance for these effects. From the results obtained in the present study, it can be concluded that the presence of indigenous AMF did not preclude a positive response to AM inoculation in nutrient uptake and dry matter production. AMF inoculation caused a better response in chickpea than in barley when looking at the nutrient concentration, while mycorrhizal inoculation of barley improved nutrient uptake in parallel with enhanced growth. Our study confirms the enhancement of growth and nutrient acquisition due to AMF inoculation on both chickpea and barley.

Key words: arbuscular mycorrhiza fungi (AMF), chickpea, barley, nitrogen nutrition, crop growth, nutrient concentration and uptake

Kurzfassung

Die Wachstumsförderung infolge der Besiedelung mit arbuskulärer Mykorrhiza (AM) bei vielen Nutzpflanzenarten ist zunehmend anerkannt. Als Ursache wird vor allem eine verbesserte Nährstoffaufnahme genannt. Die vorliegende Arbeit untersucht anhand einer Serie von Gefäßversuchen über 2 Jahre den Einfluss einer AM-Inokulation auf Wachstum und Nährstoffaufnahme von Kichererbse und Gerste. Eine Reihe bodenbiologischer und -chemischer Umweltbedingungen wurden hergestellt, um die AM-Behandlung in Wechselwirkung mit natürlich vorhandenen Mikroorganismen (Boden sterilisiert vs. nicht-sterilisiert), der Zugabe von mineralischer N-Düngung oder der Ko-Inokulation der Kichererbsen mit N_2-fixierenden Rhizobien zu testen. Die Effekte der Versuchsfaktoren auf AM-Besiedelung, Wurzelknöllchen-Zahl und -Gewicht, Pflanzen-Trockenmasse, N_{min} im Boden sowie Konzentration und Aufnahme von Makro- und Mikronährstoffen wurden in Blockanlagen mit 5 Wiederholungen erhoben. Das Substrat war ein Tschernosem-Oberboden aus schluffigem Lehm 1:1 gemischt mit Quarzsand. Die AM-Inokulation war erfolgreich und führte zu erhöhter Trockenmassebildung gegenüber den Kontrollpflanzen, sowohl in sterilisiertem als auch in nicht-sterilisiertem Boden. Die Kolonisierungsgrade waren in beiden Jahren allerdings unterschiedlich hoch. Kichererbse und Gerste zeigten ähnliche Wachstumsverbesserungen, obwohl der Kolonisierungsgrad bei Gerste niedriger ausfiel. Der N_{min}-Gehalt hatte keinen Einfluss auf die AM-Wirkung. Der nicht-sterilisierte Boden enthielt offenbar keine Rhizobien-Stämme, die Kichererbsen infizieren konnten, aber nach Rhizobium-Inokulation wurden Knöllchen entwickelt. An solchen Pflanzen waren es häufig mehr als 10, die allerdings klein blieben und anscheinend nicht biologisch aktiv waren. Die N-Ernährung (mineralisch oder über die Symbiose) ergab bei Kichererbsen überhaupt keine konsistenten Effekte, und die Rhizobien-Infektion erhöhte auch nicht die N-Aufnahme. Wechselwirkungen zwischen AM und N-Ernährung traten gelegentlich auf, erlauben aber keine Verallgemeinerung. Das Rhizobien-Inokulum enthielt offenbar keine wirksamen Rhizobium-Stämme oder deren Umweltansprüche wurden nicht erfüllt. Zudem war in den Versuchen N offenbar nicht wachstumsbegrenzend. Zur Reife von Kichererbsen und Gerste ergab die AM-Inokulation nachweisbare Effekte auf die Aufnahme von N, P, K, Ca, Mg, Fe, Mn, Zn und Cu. Lediglich die N-Aufnahme von Kichererbsen im sterilisierten Boden sowie die Mn-Aufnahme von Gerste blieben unbeeinflusst. Die N-Versorgung war auch hierfür nicht bedeutend. Die Ergebnisse erlauben die Schlussfolgerung, dass natürlich im Substrat vorhandene AM-Pilze den positiven Effekt einer zusätzlichen AM-Inokulation auf Wachstum und Nährstoffaufnahme nicht beeinträchtigten. Kichererbsen reagierten auf AM besonders positiv in den Nährstoffkonzentrationen, während sich bei Gerste die Trockenmassebildung und parallel dazu die Nährstoffaufnahme verbesserte. Insgesamt bestätigt die Arbeit, dass AM-Inokulation Wachstum und Nährstoffversorgung bei Kichererbsen und Gerste zu verbessern vermag.

Schlüsselworte: arbuskuläre Mykorrhiza (AM), Kichererbse, Gerste, Stickstoff-Ernährung, Wachstum, Nähstoffkonzentration und –aufnahme

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Introduction

Chickpea (*Cicer arietinum* L.) holds a unique position among pulse crops due to its seed protein content and wide adaptability in ecologically diverse environments. It plays a significant role in farming systems as a substitute for fallow in cereal rotations, where it contributes to the sustainability of production and reduces the need for N fertilization through fixing atmospheric nitrogen and breaking gramineous crop disease cycles (Jodha and Subba Rao, 1987; Herridge et al., 1995; Singh, 1997). Spring sown barley (*Hordeum vulgare* L.) is a typical non-legume alternative to chickpea in grain crop rotations due to similar vegetation periods. It is often used as a reference crop for estimating N fixation of grain legumes (Doughton et al., 1995; Carranca et al., 1999). Recently, it has been shown that an intensive root colonization of barley (Chaurasia and Khare, 2005) and chickpea (Saini et al., 2004) by arbuscular mycorrhizal fungi (AMF) fosters plant growth and dry matter production.

1.1 Chickpea

Chickpea (*Cicer arietinum* L.) belongs to the genus *Cicer*, tribe *Cicereae*, family *Fabaceae*, and subfamily *Papilionaceae* (Singh and Diwakar, 1995). Chickpea is a self-pollinating crop with a negligible percentage of outcrossing (Singh, 1997) and is generally considered to be a quantitative long-day plant. So far no day-neutrality or qualitative long-day response have been reported (Soltani et al., 2004). Flowering of many genotypes of chickpea is moderated by photoperiod (Summerfield et al., 1994) but thermal time gave a better relationship with development rate (Verghis et al., 1999).

It is a major pulse crop in South Asia, the Middle East, East Africa, western Mediterranean, Australia and Mexico. Chickpea acreage has increased steadily in Canada and parts of the USA in recent years. Chickpea and other pulse crops such as lentil (*Lens culinaris* Medik.), dry pea (*Pisum sativum* L.) and dry bean (*Phaseolus vulgaris* L.) are a major source of protein in human diets, particularly in low-income countries.

Two different chickpea types are distinguished: kabuli and desi. Kabulis have white flowers and beige seed, no anthocyanin in the aerial plant parts and relatively large seeds with a thin testa. Desis usually have purple flowers, anthocyanin pigmentation in the stem and leaves, and relatively small, colored, wrinkled seeds with thick seed coats (Malhotra et al., 1987; Rheenen, 1991).

1.1.1 Origin of chickpea

Chickpea was first cultivated at least 9500 years ago in the Fertile Crescent, in an area of present-day south-eastern Turkey and adjoining Syria and Iran, at the beginning of agriculture. The oldest chickpea excavation records date from 7500 B.C. (Smithson et al., 1985; van Maesen, 1987; Redden and Berger, 2007).

It is believed that the Hellenes took the crop westwards from Turkey to the Mediterranean region and eastwards to West Asia and the Indian subcontinent. By the Iron Age, chickpea consolidated its distribution in South and West Asia and appeared in Ethiopia for the first time. The New World saw the crop introduced by Spaniards and Portuguese merchants, while Asian settlers added new varieties later, for instance in the West Indies (Rheenen, 1991). A spectacular expansion of chickpea production took place in Australia, where from 1983 to 2007 the area increased from 3,100 to 306,000 ha, according to the Food and Agriculture Organization (FAO) Production Statistics (http://faostat.fao.org).

1.1.2 Cultivation areas, yield levels and trade

Only 18 out of 51 chickpea growing countries plant more than 20,000 hectares. Asia with 18 chickpea producing countries accounts for the bulk of the area (90%) and production (87%) in the world and involves highest (East Asia) and lowest (south Asia) yields of chickpea. The countries with high level of area harvested have an average yield of 743 kg ha^{-1}; since these countries account for such a large proportion of chickpea area (75%), the world average yield of 792 kg ha^{-1} is similar. In fact, average chickpea yield is very low compared with yields of cereals and also some pulses such as peas (1,500 kg ha^{-1}) and lentils (965 kg ha^{-1}). However, amongst pulse crops chickpea has consistently maintained a significant status, ranking third in production (14.6%) after bean (30.5%) and pea (22%). Average production data from 2006-2007 indicate that 76% of chickpea came from South Asia, 13% from West Asia, North Africa and East Africa, 3% from North America. Australia, Myanmar (South-East Asia) and Mexico (Central America) contributed 3%, 2.5% and 2% of global production, respectively. Any country of Europe, South America, East and Central Asia produced < 1% (FAO, 2007).

Chickpea dominates international markets relative to other legume crops and its trading is more than $ 8 billions annually (Yadav et al., 2007). According to FAO statistics (average 2005-2006) the important chickpea exporting countries are Australia, Turkey, Ethiopia and Mexico. The countries meeting a substantial part of their domestic demands of chickpea through imports are India, Bangladesh, Pakistan, Spain and Algeria.

1.1.3 Chemical components and nutritional benefits

Due to its high nutritional value, chickpea is an integral part of the daily dietary system for millions of people and, when combined with cereals, provides a nutritionally balanced amino acid-calorie composition with a ratio close to the ideal for humans. Most legumes have high nitrogen contents, due to their ability to fix atmospheric nitrogen through a symbiotic association with soil microbes. Frequent consumption of pulses is now recommended by most health organizations. Chickpea is a good source of energy, protein, minerals, vitamins, fibre, and also contains potentially health-beneficial minerals and vitamins. The nutritional value of chickpea has been documented in numerous publications (Norton et al., 1985; Williams and Singh, 1987; Huisman and Poel, 1994; Wood and Grusak, 2007); typical ranges were as follows: protein from 12.5 to 31.5%, carbohydrates from 51 to 71%, fiber from 1 to 13%, lipid from 3 to 10%, ash from 2.5 to 4%. The literature covering the chemical composition of chickpea seed is summarized in Table 1.1.

Table 1.1: General composition of chickpea seeds (%)

References	Protein	Carbohydrate	Fat	Dietary Fiber	Ash
Norton et al., 1985	20.6	50.0	5.7	15.0	n.d.
Williams and Singh, 1987	23.0	63.5	5.3	19.0	3.2
Hulse, 1994	23.3	61.5	5.0	8.5	n.d.
Huisman and Poel, 1994	22.3	61.0	n.d.	n.d.	n.d.
Wood and Grusak, 2007	22.2	60.2	5.6	28.0	3.0

n.d. not determined

The presence of many different types of proteins and other smaller molecules, including alkaloids, isoflavones, polyphenolics and a variety of oligosaccharides, make pulse seeds unique. Experimental evidence exists for the beneficial activity of pulse components in the prevention and treatment of various diseases. These results strongly support the claim that a diet with a regular intake of pulses, including chickpea, is one of the ways to maintain and improve health (Wood and Grusak, 2007).

1.1.4 Environmental conditions and crop husbandry

There are wide variations in the agroclimatic conditions under which chickpea is grown around the world. These environments differ in photoperiod, temperature and precipitation. Due to the variation in altitude, climate and mechanization, the crop is planted at different times of the year. Also agricultural practices such as irrigation, fertilization and pest control vary from one region to another. Excessive soil moisture, high humidity and cloudy weather limit flower production and fruit set and also increase the severity of common diseases, particularly ascochyta blight (Saxena, 1986). The highest mean yield is in North and Central America, reflecting significant irrigated production in Mexico, comparatively favorable rainfed environments in the USA and Canada and a high-input, mechanized agriculture; the lowest yield is in West Asia where the principal constraints are short growing seasons, diseases (especially ascochyta blight), low inputs and sometimes poor husbandry. Hence the advantages and disadvantages of chickpea-based cropping systems are often location-specific (Berrada et al., 2007).

It is likely that chickpea's poor performance under cold and chilling stress due to abortion of early flowers and pods, as well as its relative tolerance of high temperatures, are outcomes of the crop's unique evolution from a Mediterranean winter-annual life cycle, to post-rainy season spring sowing and subsequent dissemination to warmer, summer dominant rainfall regions. There is genetic variation available in the response to sub-optimal temperature (Sedgley et al., 1990).

Chickpea is grown on different types of soils ranging from sands (dunes in the 'Thal' of Pakistan) to sandy loams (northern India) to deep black cotton soils (central India, West Asia and the Ethiopian highlands). It is also cultivated on calciferous soils with a subsoil layer of $CaCO_3$ in West Asia. Chickpea requires good soil aeration. Therefore, heavy soils require care in seedbed preparation to ensure adequate aeration. The best soils for chickpea growth are deep loams or silty clay loams devoid of soluble salts with a pH range of 5.7 to 7.2. Salinity has an adverse effect on dry matter production and uptake of phosphorous, zinc and iron. Increased salinity (chloride or sulfate) leads to a decrease in nodule weight, leghemoglobin content, number and weight of pods per plant and seed. Acid soils with a pH of 4.6 seem to increase the problem of *Fusarium* wilt in chickpea (Saxena, 1986).

A viable chickpea seed with an initial moisture content of 10% may germinate at an optimum temperature (28-33°C) in about 5-6 days when it has imbibed sufficient water to reach a moisture level of more than 80%. The seeds generally are placed at a depth ranging from few centimeters to as deep as 10-15 cm (Singh and Diwakar, 1995). Deeper sowing may improve crop establishments where moisture from summer and autumn rainfall is stored in the subsoil below 5 cm (Siddique and Loss, 1999). These authors further suggested that deeper sowing improves the survival of rhizobia inoculation as soil temperature decreases and moisture availability increases with increasing depth. Various factors influencing optimum seeding depth include soil texture, date of sowing, climatic conditions and seed size (Sekhon and Singh, 2007).

Increasing chickpea plant density consistently increased seed yield per unit area (Miguélez Frade and Valenciano, 2005), despite more disease on plants at higher plant density (Gan et al., 2007) and a reduction in the number of pods per plant and the 1000-seed weight (Miguélez Frade and Valenciano, 2005; Valimohammadi et al., 2007). Decreasing chickpea plant density is a cultural practice that helps ensure the largest seed possible under given growing conditions

(Gan et al., 2003), however, decreasing plant density of an already poor competitor like chickpea makes losses due to weeds even worse (Yenish, 2007). Identifying optimum plant populations for groups of cultivars with similar plant architecture should be a component in an integrated strategy to minimize ascochyta blight and to control weeds in chickpea. They will depend upon the genotype and the environmental conditions under which chickpea is grown (Singh and Diwakar, 1995).

Chickpea is predominantly a rainfed crop, grown mainly on residual moisture and in drought-prone environments; only a small area (<10%) is irrigated. Chickpea has been successfully cultivated both in irrigated conditions and with conserved moisture under rainfed conditions (Saxena, 1986).

Both organic and inorganic sources of nutrients have been found to be useful for chickpea growth and yield. The response to nutrient application in chickpea depends on the nutrient status of the soil, agroclimatic conditions and the genotype (Ahlawat, 1990; Alloush et al., 2000).

1.1.4.1 N nutrition

Being a legume, chickpea obtains its nitrogen preferably through nitrogen fixation. Initial soil NO_3–N concentration and other factors like crop growth also affect the amount of N_2 fixed. An application of 15-25 kg N ha^{-1} has been found to be useful for stimulating growth and yield of chickpea nodulated by *Rhizobium* spp. (Singh and Diwakar, 1995). Increased fertilizer applications resulted in increased total and non-protein nitrogen in the seeds (Hulse, 1994) and a small effect on seed yield, but decreased nodule numbers by about 21% (Rupela and Beck, 1990; Berrada et al., 2007).

Chickpea is generally infected by *Rhizobium leguminosarum* ssp. *ciceri* (Kantar et al., 2003), *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* strains (Ben Romdhane et al., 2008, Table A1). The effect of artificial rhizobia inoculation on chickpea yield depends on the native rhizobial status as rhizobia species producing nodules in chickpea are specific only to this species. Fields in which well-nodulated chickpea was grown previously do not require inoculation. However, where chickpea is being grown after paddy or chickpea is being introduced for the first time, inoculation is advisable (Singh and Diwakar, 1995).

Inoculation of chickpea with effective bacterial strains significantly increases nodule dry weight, proportion of N derived from N_2 fixation (%Ndfa), seed yield and total biomass yield. Chickpea and rhizobia in association can annually fix up to 176 kg N ha^{-1} (Rupela and Saxena, 1987) and increase all yield parameters depending on cultivar, bacterial strain and environmental factors (Beck, 1992; Carranca et al., 1999; El Hadi and Elsheikh, 1999; Kantar et al., 2003; Valverde et al., 2006; Ben Romdhane et al., 2008).

1.2 Arbuscular mycorrhiza

In 1885 Albert Bernard Frank (Frank, 1885 cited by Siddiqui et al. (2008)), in his study of soil microbial-plant relationships, introduced the Greek term ‘mycorrhiza’, which literally means ‘fungus roots’. Mycorrhizal fungi play an essential role in plant growth, nutrient uptake, disease protection and overall soil fertility. They form symbiotic relationships with over 80% of all vascular plants. Of the seven types of mycorrhizae described (arbuscular, ecto-, ectendo-, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread (Siddiqui et al., 2008). .AM fungi (AMF) are obligate mutualistic symbionts belonging to the phylum *Glomeromycota* and have a ubiquitous distribution in global ecosystems (Sharif and Moawad, 2006). Although arbuscular mycorrhizas have often been ignored by foresters, they are characteristic of such valuable trees as *Acer*, *Araucaria*, *Podocarpus* and *Agathis*, as well as all the *Cupressaceae*, *Taxodiaceae*, *Taxaceae*, *Cephalotaxaceae* and the majority of tropical hardwoods (Smith and Read, 2008).

This chapter presents an overview of current knowledge of mycorrhizal functions and potential benefits. More detailed recent information about AMF can be obtained from comprehensive monographs by Smith and Read (2008) and Siddiqui et al. (2008), who have compiled the many reviews of this area. We did not mention the complete references here to avoid a redundant references list.

The name ‘arbuscular’ is derived from characteristic structures, the arbuscules which occur within the cortical cells of many plant roots together with storage vesicles located within or between the cells. These structures have been considered diagnostic for AM symbioses. The term vesicular-arbuscular mycorrhiza (VAM), which was in use for many decades, has been dropped in recognition that vesicles are formed by only 80% of AM fungi, but the name ‘arbuscular’ is currently retained, regardless of the structural diversity which is more and more widely appreciated. An arbuscular mycorrhiza has three important components: the root itself, an extraradical mycelium in the soil and the fungal structures within and between the cells of the root (Giasson et al., 2008; Smith and Read, 2008).

Colonization of roots by AM fungi can arise from three main sources of inoculum in soil: spores, infected root fragments and hyphae, collectively termed propagules. AM fungi show varying abilities to colonize roots from different sources of inoculum. In many habitats, persistent hyphal networks in soil, together with root fragments, are the main means by which plants become colonized even when significant spore populations are also present. Hyphal contact with the root is usually followed by adhesion of the hypha to the root surface and after about 2–3 days the formation of swollen appressoria. Penetration of plant cell walls is always associated with narrowing of the hyphal diameter to form a peg, followed by expansion as the hypha enters the lumen of the cell and formation of arbuscules around 2 days later. After colonization of the root cortex, extraradical and symbiotic mycelium grows and production of viable and infective spores completes the fungal life cycle. The formation of appressoria on the surface of the root shows that the fungus has recognized the presence of a potential host plant. However, changes in the middle lamella structure when intercellular spaces are colonized by hyphae indicate the involvement of fungal enzymes such as pectinases. Activities of other hydrolytic enzymes, such as cellulases and xyloglucanases, are also elevated in AM roots (Smith and Read, 2008).

Two different types of symbiotic interfaces can be found, depending on whether the fungus grows inter- or intracellularly in the root system. Intercellular interfaces are created when the fungal hyphae grow within the intercellular spaces of the root cortex, whereas intracellular interfaces are developed when the fungal hyphae penetrate the wall of the root cells. Intracellular structures include coils of fungal hyphae (in Paris-type mycorrhizas) and arbuscules (in Arum-type mycorrhizas) (Ferrol et al., 2002). Arbuscules are short-lived structures and begin to senesce after 4–10 days of activity (Sawers et al., 2008).

AMF plants have two potential pathways of nutrient uptake: (i) directly from the soil via roots or (ii) via the mycorrhizal symbiont. The AM pathway delivers nutrients to plant by three essential processes: uptake of the nutrients by the fungal mycelium followed by their translocation to the intraradical fungal structures (hyphae, arbuscules and coils) and transfer to the plant cells across the arbuscular interfaces. The fungal mycelium in soil can absorb nutrients beyond the zone depleted through root uptake, thus increasing the effectiveness with which the soil volume is exploited (Toro et al., 1997; Ruiz-Lozano et al., 2001; Bi et al., 2003; Rohyadi et al., 2004; Smith and Read, 2008).

1.2.1 Influence of agricultural practices on mycorrhiza

Modern agricultural practices have put new pressures on the plant mycorrhizal symbiosis. Tillage practices physically disrupt soil aggregates and AM hyphal networks resulting in declining soil structure, fertility and nutrient-cycling ability, and forcing more C allocation towards AM fungi to reestablish these networks rather than to glomalin formation, a glycoprotein produced by AM

fungi which helps in stabilizing soil aggregates. No tillage practices along with continuous cropping systems, using mycorrhizal host crops and reducing synthetic inputs, especially P, enhance the plant-mycorrhizal symbiotic relationship (Nichols, 2008; Panwar et. al., 2008).

1.2.1.1 Mineral fertilizers

One specific effect of high nitrogen inputs may be a reduction in the amount or the activity of the AM colonization (Azcón et al., 2003; Jia et al., 2004), but in other studies nitrogen has caused no obvious or only small response (Vaast and Zasoski, 1992; Vázquez et al., 2001). There is evidence that mycorrhizal colonization varies with N-form: plants supplied with NO_3 were found to have higher AM colonization than those fertilized either with NH_4 or a NO_3/NH_4 mixture (Hawkins and George, 2001; Ortas and Rowell, 2004). Studies of plant performance and N acquisition have identified variation in the capacity of mycorrhizal plants to benefit from fertilizer N depending on both N availability from soil (Jia et al., 2004; Azcón et al., 2008) and N-forms (Hawkins and George, 2001). For example Vázquez et al. (2001) showed that the beneficial mycorrhizal effect on plant protein content and dry matter of alfalfa is reduced under large quantities of N fertilizer. In addition, there is evidence that genetic variability in the fungal partner also influences N acquisition and that different plant-fungal combinations alter the degree of benefit derived by the host from fertilizer N (Vázquez et al., 2001). Due to the application of N fertilizer, either ammonium or nitrate, variations in the nutrient movement between AMF and associated plants have been identified (Ortas and Rowell, 2004). Cornejo et al. (2008) showed in an acid soil with Al and Mn phytotoxicity that the use of nitrate fertilizer resulted in reduction of the Mn and Al content in mycorrhizal wheat while addition of ammonium fertilizer increased P and Zn content, but so far underlying mechanisms were not revealed in detail. While NH_4 application decreases soil pH, NO_3 has an increasing effect (Bago et al., 1996; Ortas and Rowell, 2004). Lowering rhizosphere pH improves the solubility of P in the soil and consequently its availability to plants. Thus, plant growth and nutrient uptake can be influenced by both rhizosphere pH and mycorrhizal infection as affected by N source (Ortas and Rowell, 2004). These findings confirm other studies on the effects of nitrogen supply and mycorrhiza with different crops (Bago et al., 1996; Ortas et al., 1996).

Effects of P on AMF operate at several steps in the colonization process. High P levels have been shown to reduce the growth of germ tubes, the length or biomass of hyphae, the production of root exudates that stimulate branching of hyphae approaching roots, as well as growth of the extraradical mycelium, with consequences for both primary and secondary colonization. It is hypothesized that high concentration of soil P inhibits the expression of phosphate transporter genes (*GvPT*, *GiPT*, *GmosPT*), belonging to the Pht1 family, in the external mycelium and the gene encoding acid phosphatase. This indicates that AM colonization of root and AM root function are down-regulated by a P feedback mechanism, but the effects are not sufficiently well established for generalizations to be made (Smith and Read, 2008).

1.2.1.2 Organic amendments

The use of organic mulches, manure or slurries can have marked effects on the AM symbiosis and should be managed with caution. Both positive and negative effects have been noted. Inhibitory effects on mycorrhizal development after addition of sewage sludge were considered to be due to the effects of heavy metals in the sludge delaying the onset of infection. The effects of green manures are much more variable, and both negative and positive effects have been observed within the same experimental setup (Barea and Jeffries, 1995).

Reduced AM colonization of chickpea roots has been reported following the single addition of farmyard manure (FYM) while the use of FYM further positively affected the chickpea yield and P and N content and increased AM colonization where multi-inoculation (*Rhizobium*, *Bacillus*

and *Glomus*) were supplemented. This could be attributed to the microbial activity as key for mineralization of organic P in soils (Saini et al., 2004). Mycorrhizal hyphae intercept inorganic P released during mineralization of organic matter by microorganisms (Smith and Read, 2008).

Alloush et al. (2000) found enhanced AMF root colonization after addition of cattle manure to chickpea plants grown in acidic soil. However, stronger colonization did not result in enhanced shoot dry matter even though higher shoot P and K concentrations were noted. AM roots might be able to exploit sources of P in soil not normally available to plants. These include relatively insoluble forms of inorganic P, such as rock phosphate and Fe and Al phosphates, as well as sources of organic P such as phytate (Bolan, 1991). The mechanisms underlying increased uptake might depend upon hyphal exploitation of the soil volume, the possible excretion by hyphae of H^+ that lowers pH or organic anions with chelating ability and synergistic action between AM fungi and P-solubilizing microorganisms (Jakobsen, 1995; Smith and Read, 2008).

1.2.2 Mycorrhizal function

AMF are able to promote plant growth and to improve uptake of nutrients. AMF colonized plants are also known to be more tolerant than non-AMF plants to several biotic and abiotic stresses such as toxic metals, root pathogens, drought, high soil temperature, saline soils, adverse soil pH and transplanting (Paraskevopoulou-Paroussi et al., 1997; Ruiz-Lozano et al., 2001; Rabie and Almadini, 2005; Smith and Read, 2008; Turkmen et al., 2008). A literature review of the effects of mycorrhiza on nutrient uptake and concentrations with respect to species relevant for the present study together with characteristics of the soil conditions is given in Table 1.2.

1.2.2.1 Plant growth

More than 80% of all land plants establish an arbuscular mycorrhizal (AM) symbiosis. In this interaction, arbuscular mycorrhizal fungi (AMF) colonize roots of host plants and promote plant growth (Smith and Read, 2008). It is known from previous studies that inoculation of chickpea with *Glomus* sp. significantly increased growth over the control treatment (Singh and Tilak, 1989; Alloush et al., 2000; Zaidi et al., 2003; Akhtar and Siddiqui, 2007). Similar effects were observed with barley, using indigenous or selected AMF species (Owusu-Bennoah and Mosse, 1979; Clarke and Mosse, 1981; Powell, 1981).

Increased growth of plants after colonization with AMF may be attributed to the improved uptake of nutrients. This has been reported for a wide range of plant species including many crop plants (Jensen, 1982; Barea et al., 1987, 1996; Hirata et al., 1988; Weber et al., 1993; Al-Karaki and Clark 1999; Biró et al., 2000; Jia et al., 2004) and trees (Habte and Aziz, 1985; Manjunath and Habte, 1988; Okon et al., 1996).

In addition to their known effect on nutrient acquisition, mycorrhizal symbionts can positively act on host plant growth through a selective effect on microbial communities involved in soil functioning and soil fertility. Significant alterations in root physiology occur when plants become mycorrhizal and this association also alters root exudation both quantitatively and qualitatively (Posta et al., 1994; Giasson et al., 2008), as AMF catabolise some of the root exudates and modify root metabolic functions thus having a selective effect on soil microorganisms (Duponnois et al., 2008; Saldajeno et al., 2008).

Table 1.2: Review of the effects of mycorrhiza on nutrient uptake and concentrations in different legume and cereal crops. For symbols see footnote

Crop species	pH	soil	trait	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	P in soil (mg kg^{-1})	Added P to soil (mg kg^{-1})	N in soil (mg kg^{-1})	Added N to soil (mg kg^{-1})	References
Chickpea	n.d	sandy loam	uptake	n.d.									n.d.	n.d.	n.d.	n.d.	Akhtar and Siddiqui, 2007
			conc.	=	+	+	n.d.										
Chickpea	9.0	sandy clay loam	uptake	+	+	n.d.							16	26.2 as rock phosphate	200[1]	n.d.	Zaidi et al., 2003
			conc.	n.d.													
Chickpea	5.8	loamy	uptake	n.d.									4	n.d.	n.d.	133 as NO_3NH_4	Alloush et al., 2000
			conc.	n.d.	=	+	=	+	–	–	+	–					
Soybean	6.1	clay	uptake	n.d.	+		n.d.		=	+		n.d.	16	30 as triple super phosphate	n.d.	n.d.	Nogueira et al., 2007
			conc.	n.d.	+		n.d.		–	=		n.d.					
Soybean	8.1	loamy	uptake	=	=	n.d.							6.2	0.05 (-AM treatment) as K_2HPO_4	2.5[2]	1.2 (-AM treatment) or 1.8 (+AM treatment) as NO_3NH_4	Ruiz-Lozano et al., 2001
			conc.	n.d.													
Broad bean	7.0	sandy	uptake	+	+	n.d.							n.d.	0.03 as NaH_2PO	n.d.	10 as KNO_3	Jia et al., 2004
			conc.	+	+	n.d.											
Broad bean	7.0	sandy	uptake	=	+	n.d.							n.d.	0.03 as NaH_2PO	n.d.	250 as KNO_3	Jia et al., 2004
			conc.	=	+	n.d.											

Table 1.2 (continued)

Alfalfa	7.5	calcareous loamy chernozem	uptake	+	+	+	=	=	=	n.d.	=	=	70	n.d.	60[2]	n.d.	Biró et al., 2000
			conc.	n.d.													
Sesbania grandiflora	6.2	silty clay loam	uptake	+	+	+	n.d.	+	+	+	+	+	n.d.	n.d.	n.d.	n.d.	Habte and Aziz, 1985
			conc.	n.d.													
Cowpea	5.2	sandy loam and sand	uptake	n.d.	+		n.d.		+	+	+	n.d.	n.d.	36	n.d.	60 as NH_4; 178 as NO_3	Rohyadi et al., 2004
			conc.	n.d.	+		n.d.		–	=	–	n.d.					
Cowpea	5.6	sandy siliceous	uptake	n.d.	+	+	+	+	n.d.		+	n.d.	4.1	10 as KH_2PO_4	n.d.	150 as NH_4NO_3	Bagayoko et al., 2000
			conc.	n.d.	–	–	–	–	n.d.		–	n.d.					
Peanut	7.3	calcareous	uptake	n.d.									3.8	50 (-AM treatment) or 20 (+AM treatment) as $Ca(H_2PO_4)_2$	n.d.	100 as NH_4NO_3	Caris et al., 1998
			conc.	+			n.d.		=		n.d.						
Barley	7.8	clay loam	uptake	n.d.	+	=	n.d.		+	=	+	=	6.5	n.d.	n.d.	125 as ammonium Sulfate	Mohammad et al., 2003
			conc.	n.d.	+	=		n.d.									
Barley	8.0	silty clay	uptake	n.d.	+		n.d.			+	+	+	8	n.d.	n.d.	30 as NH_4NO_3	Al-Karaki and Clark 1999
			conc.	n.d.	+		n.d.			+	+	+					
Barley	7.1	sandy	uptake	n.d.	+			n.d.			+	+	7.2	n.d.	n.d.	0.45	Jensen 1982
			conc.	n.d.	=			n.d.			=	+					
Wheat	8.1	silty clay	uptake	n.d.	+		n.d.		+	+	+	+	4	n.d.	n.d.	n.d.	Al-Karaki and Al-Raddad 1997
			conc.	n.d.													

Maize	8.1	calcareous loam	uptake	n.d.	+	+	+	+	n.d.	+	+	+	67	20 as KH_2PO_4	n.d.	300 as NH_4NO_3	Bi et al., 2003
			conc.	n.d.	+	+	=	=	n.d.	=	=	=					
Maize	6.5	sandy loam	uptake	n.d.					+	=	+	+	60	n.d.	n.d.	80 as NH_4NO_3	Liu et al., 2000
			conc.	n.d.													
Millet	5.6	sandy	uptake	n.d.	=	–	=	=	n.d.		–	n.d.	4.1	10 as KH_2PO_4	n.d.	150 as NH_4NO_3	Bagayoko et al., 2000
			conc.	n.d.	–	–	=	–	n.d.		–	n.d.					
Sorghum	5.6	sandy	uptake	n.d.	+	+	+	+	n.d.		+	n.d.	4.1	10 (as KH_2PO_4)	n.d.	150 as NH_4NO_3	Bagayoko et al., 2000
			conc.	n.d.	–	–	–	–	n.d.		–	n.d.					
Sorghum	7.3	calcareous	uptake	n.d.									3.8	50 (-AM treatment) or 20 (+AM treatment) as $Ca(H_2PO_4)_2$	n.d.	100 as NH_4NO_3	Caris et al., 1998
			conc.	+			n.d.		+		n.d.						

Symbols: + increased uptake or concentration of elements, – decreased uptake or concentration of elements, = no effect, n.d. not determined

[1] is soil total N, [2] is soil mineral N

1.2.2.2 Water and nutrient uptake

Root colonization with AMF may enhance plant growth (Al-Karaki and Al-Raddad, 1997) and nutrient uptake (Al-Karaki and Clark, 1999) and protect nodules against senescence (Ruiz-Lozano et al., 2001) under drought stress. Although there is no direct evidence of hyphal water uptake in these studies, the results can be indirectly attributed to the involvement of fungal hyphae in water transport to roots. The effects of AM on water uptake via root can be explained on the basis of increased transpirational flux and stomatal conductance (Smith and Read, 2008) that were occasionally accompanied by higher xylem pressure potentials (Osonubi et al., 1991). AMF colonization can decrease hydraulic resistance to water transport in the below-ground system. This effect could be mediated by increases in the size or branching of the root system without changes in root biomass (Quilambo et al., 2005; Smith and Read, 2008).

Furthermore, AMF can enhance soil structure by secreting a slimy glycoprotein called glomalin. It plays a role in the formation of stable soil aggregates and may also create larger pores for better growth of hyphae, which allows for easier penetration of water and air and helps to prevent erosion (Piotrowski et al., 2004; Nichols, 2008).

The extent of uptake increase of individual elements differs depending on the experimental conditions used and is markedly influenced by the nutrient status of the soil, the plant species and cultivar and/or strains of AMF (Marschner and Dell, 1994; Bagayoko et al., 2000; Clark and Zeto, 2000; Liu et al., 2000; Bi et al., 2003; Ortas, 2003; Rohyadi et al., 2004).

Dependencies of legumes and cereal species on the mycorrhizal symbiosis varied dependent on soil fertility (Gamper et al., 2004). Differences in nutrients acquisition by AMF between legumes and cereal species may be due to a different response to nutrient deficiencies (Caris et al., 1998; Bagayoko et al., 2000; cf. Tab. 1.2; Gunes and Inal, 2008). Gunes and Inal (2008) found that chickpea cultivars are more P-efficient than wheat cultivars. P-efficiency of a cultivar is defined as the ability to produce a high yield in soil that is limited in P supply. They observed that the root growth of relatively P-inefficient wheat was significantly increased by high P, while it was decreased in comparatively P-efficient cultivars of chickpea in spite higher P concentration in shoot than those of P-inefficient wheat cultivars.

AM hyphae may play a considerable role in legumes' N nutrition (Azcón and El-Atrash, 1997; Redecker et al., 1997), and the uptake of both poorly mobile NH_4 (Chalot et al., 2006) and highly mobile NO_3 (Bago et al., 1996) can be elevated in mycorrhizal plants. Additional N uptake in nodulated legumes due to AMF colonization has also been observed (Barea et al., 1987; Biró et al., 2000). This can result from increased rhizobial N_2 fixation, as expected because of the better phosphorus supply by AMF (Jia et al., 2004), or from increased N uptake from soil by the AM hyphal network (Subba Rao et al., 1986). There are scarce data on the effects of N fertilization on chickpea in absence of rhizobia. A few existing studies show inconsistent results regarding growth, yield and N_2 fixation responses of chickpea to N fertilization in natural production environments and rotation systems (e.g. El-Ghandour and Galal, 2002; Walley et al., 2005; Gan et al., 2008). The results of different experiments (cf. Tab. 1.2) and suggested underlying mechanism of N uptake (Oliver et al., 1983; Faure et al., 1998) were not consistent, but uptake of N by the hyphae did not seem to play an important role in net plant N nutrition with high soil N while increased N uptake and concentrations were observed only with low soil N (Jia et al., 2004; cf. Tab. 1.2).

Acquisition of soil P by different crops increases due to mycorrhizal colonization in fertile as well as in marginal soil, but P availability is more enhanced in marginal soil conditions (Sharif and Moawad, 2006). Uptake of P by extraradical hyphae is followed by the synthesis of large amounts of polyphosphate (Ferrol et al., 2002). Mycorrhizal plants have been shown to increase the uptake from poorly soluble P sources, such as Fe- and Al-phosphate and rock phosphates, presumably due to solubilization by the release of organic acids and phosphatase enzymes. In

some cases this also increased their abilities to utilize calcium from rock phosphates (Bolan, 1991).

Some studies indicate increases in K uptake and/or concentration in AM plants, which might be expected considering the relative immobility of this ion in soil, whereas in other investigations K was found to be at the same or even lower uptake and/or concentration levels compared with non-mycorrhizal plants (cf. Tab. 1.2). Most of these experiments were carried out in P deficient soils to demonstrate the role of AMF in P acquisition. These results are inconsistent maybe because accumulation of K is strongly influenced by availability of N, P, Na and other elements (Mohammad et al., 2003; Smith and Read, 2008).

Not much is known about the role of mycorrhiza in uptake of Ca and Mg. Clark and Zeto (2000) reported that the acquisition of K, Ca and Mg was considerably more enhanced in AMF maize plants grown in acidic than in alkaline soil, while Bi et al. (2003) found enhanced acquisition of K, Ca and Mg by AM plants also under alkaline (pH 8.1) conditions. The AM effect on the uptake of K, Ca and Mg also seems to depend on plant species (Bagayoko et al., 2000; cf. Tab. 1.2).

Micronutrients are necessary for plants but required at very low amounts. With view to Fe, uptake and/or concentration increased after AM inoculation of cereals, but mycorrhizal inoculation had no significant influence on the uptake and/or concentration of Fe in legumes (cf. Tab. 1.2). Excessive uptake of Zn, Cu and Fe can lead to heavy metal toxicity (Marschner and Dell, 1994; Smith and Read, 2008). It was observed in some cases that mycorrhiza protected its host plant from excessive uptake of Mn and Fe even though extractable metal concentrations increased in the soil (Liu et al., 2000; Nogueira et al., 2007; cf. Tab. 1.2). Retention of metals in root systems can also be attributed to surface bound metals with cystein-containing ligands of fungal (including AMF) proteins (Christie et al., 2004). In some cases, changes in both rhizosphere microbial populations (decreased number of Mn reducers, especially fluorescent *Pseudomonas*) (Nogueira et al., 2007) and less root exudation of Mn-solubilizing compounds were probably responsible for the lower acquisition of Mn by mycorrhizal plants (Posta et al., 1994). Although Mn is more soluble in acidic compared to alkaline conditions (Clark and Zeto, 2000), enhanced Mn acquisition due to AMF has also been reported for plants grown in alkaline soils (cf. Tab. 1.2).

There is some evidence for increased uptake of Zn, which is also poorly mobile and deficient in some soils, and of Cu and Mn. However, this relationship does not always hold and an inverse or no relationship between AMF colonization and Zn, Mn and Cu uptake and concentrations in plants have been observed (cf. Tab. 1.2). The translocation of Zn in AMF also may be coupled to that of P, as Zn acts as a counter ion to polyphosphate in AMF (Christie et al., 2004). Some genes involved in the Zn molecular transport have been identified in both the AMF and plant components of the systems (Cavagnaro, 2008). When high rates of P are added to the soil, reduction in the Zn content of plants colonized by AMF may occur (Bi et al., 2003; Gunes and Inal, 2008).

1.3 Rhizobia

The ability to fix atmospheric N_2 is restricted to prokaryotic organisms. Within this group the ability occurs in many different species. These include cyanobacteria and actinomycetes, as well as eubacteria, including heterotrophic (e.g. *Azotobacter*), autotrophic (*Thiobacillus*), aerobic (*Bacillus*), anaerobic (*Clostridium*) and photosynthetic (*Rhodospirillum*) species. N_2-fixing organisms can live free in nature (e.g. *Azotobacter*), enter loose (associative) symbiosis with plants or establish longer-term relationships within specialized structures (nodule) provided by their host (*Rhizobium*) (Graham, 2001).

Rhizobia are the collective name for bacteria which form a well documented symbiosis with legumes. For the sake of simplicity, unless a specific organism is mentioned, these genera will be given the general term rhizobium. Infection of legumes by rhizobia results in the formation of

nodules, usually on roots. However, some rhizobia, for example *Azorhizobium caulinodans* and the photosynthetic *Photorhizobium thompsonianum*, form stem nodules on certain plants. One non-legume plant, *Parasponia*, is also known to become infected by rhizobium (Gordon et al., 2001).

Biological nitrogen (N_2) fixation is the reduction of atmospheric nitrogen gas to ammonia, according to the equation:

$$N_2 + 10\ H^+ + 8\ e^- + 16\ ATP \rightarrow 2\ NH_4^+ + H_2 + 16\ ADP + 16\ P_i \quad (1.1)$$

The reaction is mediated by the oxygen-sensitive enzyme nitrogenase and requires energy, as indicated by the consumption of adenosine triphosphate (ATP). This conversion of inert N_2 gas into a form utilized by most organisms is the second most important biological process on earth after photosynthesis (Graham, 2001).

Rhizobia, which can survive in the soil as saprophytes, are attracted, and their growth is stimulated by various compounds excreted into the rhizosphere of legumes. The establishment of the N_2-fixing symbiosis is the result of three major events: (i) intracellular infection of the host cells by the microsymbiont; (ii) nodule organogenesis; and (iii) the N_2-fixation process. The first two occur simultaneously, whereas N_2 fixation starts only after nodule organogenesis is complete and if bacterial infection is successful. A molecular dialogue between the symbiotic partners initiates the symbiosis. Specific flavonoids, exuded by the legume roots, are perceived by rhizobia in the rhizosphere via their putative receptors, the NodD proteins, which are the transcriptional activators of the nodulation genes (*nod*, *noe*, or *nol* genes). Phenolic compounds, especially flavonoids, are the only well-documented chemical signals that originate from plants and affect symbiotic bacteria. In the rhizosphere, however, the recognition of host-derived flavonoids by rhizobia is a complex process. Diverse classes of flavonoids up-regulate nod-gene expression including anthocyanidins, chalcones, coumestans, flavanones, flavones, flavonols and isoflavonoids. They are derived from phenylpropanoids that enter the flavonoid pathway through chalcone synthase. Generally, NodDs of broad host-range rhizobia respond to a wider range of flavonoids, while NodD proteins from restricted host-range rhizobia have more specific requirements for flavonoids (Kobayashi and Broughton, 2008).

Most nodulation genes (*nod*, *noe* and *nol*) are involved in the synthesis of host-specific lipo-chitooligosaccharides, called Nod factors, which are essential for the initial infection of root hairs. Nod factors induce root hair curling, formation of nodule primordia, expression of early nodulin (ENOD) genes in the plant, and allow the bacteria to enter the root-hairs. Rhizobia that are incapable of synthesising Nod factors are unable to penetrate root-hairs (Sadowsky, 2005).

Host plants have specific receptors for the Nod factors of their compatible symbiotic partners, a molecular key-lock system fundamental to the host-specificity of the legume-rhizobium symbiosis. The recognition of Nod factors by plant receptors opens the door for infection. This involves the formation of tubular infection threads, which guide the rhizobia inside the plant tissues. At the same time, Nod factors activate the cortical cells opposite the site of infection, leading to their dedifferentiation and division, and the formation of the nodule primordium. When the growing infection threads reach the primordium cells, bacteria are released into their cytoplasm. These intracellular bacteria are referred to as “bacteroids”. The infected plant cells stop dividing but instead start to differentiate, in conjunction with their microsymbiont, into N_2-fixing symbiotic cells (Maunoury et al., 2008).

Interaction of host and rhizobia is also accompanied by the expression of nodule-specific proteins or nodulins. Nodulin expression can vary temporally and spatially. Early nodulins are involved in infection or nodule development and may be expressed within six hours of inoculation. Later nodulins are involved in nodule function, carbon and nitrogen metabolism, or in O_2 transport. Nodule hemoglobin is an obvious example of the latter group (Reddy et al., 2002).

The nodule provides an environment with a low O_2 content, which is vital because nitrogenase, the enzyme that catalyzes biological nitrogen fixation, is denatured by O_2. Some O_2, however,

must be provided so that the bacteria can respire and produce the energy required for both survival and to drive nitrogen fixation. A special O_2 transporting protein, called leghemoglobin supplies this carefully controlled amount of O_2. The area of active N_2 fixation is either pink or red in color due to the presence of hemoglobin. In most legumes nodules are visible within six to ten days of inoculation; N_2 fixation as evidenced by improved plant growth and coloration of the nodules can occur within three weeks (Fisher and Newton, 2002).

1.3.1 Rhizobial biodiversity

The genus *Rhizobium* was initially defined by the ability of these organisms to induce nodule formation in legumes. Currently recognized genera and species are shown in Table A1, but owing to novel methodologies, additions to this list of bacteria occur continuously. Studies on rhizobial diversity in soil, explored initially through serology and enzyme-electrophoresis, have blossomed with the advent of polymerase chain reaction (PCR) and DNA sequence-based methodologies (Graham, 2008).

The symbiotic interaction - as described above - is highly specific, a given rhizobium strain being able to form nodules on a restricted number of plants, which constitute the host range of this strain. This host range can be quite narrow or much wider, as for *Rhizobium* sp., which can nodulate not only plants originating from more than 110 different genera of tropical legumes, but also the non-legume *Parasponia* (Gordon et al., 2001).

Chickpea are nodulated only by a few species. In early studies this led to the concept of cross-inoculation, with legumes grouped according to the different rhizobia with whichever they formed nodules. More than 20 cross-inoculation groups were identified, with the bacteria from the clover, medic, bean, lupin, pea and soybean groups named as separate species within the single genus *Rhizobium* (O'hara et al., 2002). Rhizobium strains that nodulate chickpea are specific and do not show inoculation affinity with any members of the known cross-inoculation groups with the possible exception of *Sesbania* (Gaur and Sen, 1979; Rupela and Saxena, 1987). Two species of the genus *Mesorhizobium*, *M. mediterraneum* and *M. ciceri* (Aouani et al., 2001; L'taief et al., 2007; cf. Tab. A1), and *Rhizobium leguminosarum* ssp. *ciceri* (El Hadi and Elsheikh, 1999; El-Ghandour and Galal, 2002; Kantar et al., 2003; cf. Tab. A1) are presently recognized as specific symbionts of *C. arietinum*. These three species do not encompass all the genetic diversity described so far for rhizobia nodulating chickpea, and there are a number of strains which still remain unclassified (Rivas et al., 2007). Other species and genomic groups, phylogenetically related to *Mesorhizobium* and *Sinorhizobium*, have been detected in several studies (Nour et al., 1994, 1995; Aouani et al., 2001; Maatallah et al., 2002; Ben Romdhane et al., 2007). In Spain and Portugal e.g., some strains belonging to the phylogenetic group of *Mesorhizobium tianshanense* and *M. amorphae* have been detected able to effectively nodulate *C. arietinum* (Rivas et al., 2007). The specificity of their interaction on chickpea cultivation needs to be investigated further, considering the inoculation patterns, crop and varietal history in the region, agricultural practices and soil conditions (O'hara et al., 2002).

1.3.2 Ecology of N_2-fixing bacteria

A variety of stresses may limit legume nitrogen fixation and productivity in world agriculture. These may be summarized as extremes of temperature, water and nutrient availability, and toxic soil factors such as sodicity and extreme pH (Tab. 1.3). Some strains of rhizobium display a better capacity to withstand stress than others. In fact, optimal N_2-fixing strains that are unable to adapt to the soil environment are eventually out-competed by other resident adapted strains, which often display inferior N_2-fixing phenotypes with a selected legume host (Sadowsky, 2005; Poole et al., 2008).

Table 1.3: Cell stressors relevant to root-nodule bacteria

Type	Description
Biological	Host reaction against an invading microbe or phage
Chemical	Insecticides
	Fungicides
	Heavy metals
	Osmotic (hyper- or hypo-osmotic conditions)
	Oxygen radical toxicity and anaerobiosis
	pH (acid or alkaline or pH shift)
	Starvation (carbon, nitrogen or phosphorus limitation)
Physical	Ultraviolet (UV) light
	Desiccation
	Thermal (high or low temperature)

1.3.2.1 Effect of elements in soil

Nodulation and N_2 fixation in this symbiosis require that host and microorganism are compatible, but also that the soil environment be appropriate for the exchange of signals preceding infection (Graham, 2008). Mineral nutrients may limit nitrogen fixation in legumes through direct effects upon host-plant growth and rhizobia, or through indirect effects upon the symbiosis (O'hara et al., 2002). Until quite recently, relatively little has been done to determine the ways in which rhizobia obtain, use, and respond to nutrients, either in the free-living or the symbiotic states.

1.3.2.1.1 Phosphorus

Despite not much information about specific requirements of some nutrients (B, Ca, Mg, Mn, Co, Cu, Fe, K, Mo, Ni, Se and Zn) for symbiotic development in legumes (Johnston et al., 2001; O'hara et al., 2002; Poole et al., 2008), experimental evidence exists that P can significantly limit the productivity of symbiotic legumes (Luyindula and Haque, 1992; Yang, 1995). The response of symbiotic N_2 fixation to altered phosphorus supply is a function of both indirect effects on host-plant growth (Robson et al., 1981; Jakobsen, 1985; Yahiya and Samiullah Fatma 1995) and direct effects on the metabolic function of nodules (Israel, 1987 and 1993; Wall et al., 2000; Miao et al., 2007). Severe phosphorus deficiency markedly impaired both host plant growth and symbiotic N_2 fixation (Thomson et al., 1992).

1.3.2.1.2 Nitrogen

While low levels of N fertilization or soil mineral N have been reported to enhance N_2 fixation in some legumes (Walley et al., 2005; Gan et al., 2008), high levels generally inhibit nodulation and N_2 fixation in most legumes (Evans et al., 1989; Luyindula and Haque, 1992; Doughton et al., 1993; Horn et al., 1996; Voisin et al., 2002). In certain field soils, mineralization of organic N and nitrification may provide levels of NO_3; which at the same time satisfy the N requirements of the young legume plants but inhibit nodulation. Subsequently NO_3 is exhausted by plant uptake, leaching and/or denitrification, thus plants may enter an N-deficient phase until enough nodules are being formed to compensate for the lack of soil N with an adequate supply of symbiotic N.

Sometimes this phase is so prolonged that yield is substantially reduced, as observed with soybeans (Herridge et al., 1984).

Several soil factors such as soil P level influence nodulation and nitrogen fixation tolerance to various levels of inorganic N (Wall et al., 2000), and large genetic variation has been observed in many legume species (Awonaike et al., 1990; Guo et al., 1992).

1.3.2.2 Other environmental factors

An important consideration for optimizing N_2 fixation in the legume-rhizobial symbiosis is the response of the microsymbiont and the nodule to limiting environmental factors. Overall, populations of rhizobia have been shown to vary in their tolerance to major environmental factors. This may be an adaptation to the preferred habitats of their respective host legumes.

- Temperature

In the tropics, soil temperatures may exceed 50 ºC at 1 cm depth.Effects of this stress upon nitrogen fixation directly, as well as upon nodulation and plant growth, are well documented. In Mediterranean-type climates, high temperature (above 30 ºC, often associated with water deficit) during spring and early summer is the primary determinant of the termination of the growing season and hence nitrogen fixation in annual legumes. In contrast, in Mediterranean and temperate agriculture, low temperatures during autumn and winter are a substantial impediment to legume growth and nodulation (Hungria et al., 2005).

- pH

The main effect of soil pH appears to be through a sensitivity of the rhizobia to form adequate nodulation, while plant growth must not be influenced severely by soil pH. For example in peas and *Medicago* spp., soil acidity reduced nodulation via its effect on rhizobial survival, while the growth of the host plants appeared less affected by soil pH. Conversely with lupins the necessary rhizobia survived even better at low pH (O'hara et al., 2002).

- Competition

Growth, survival and the establishment of rhizobial symbiosis is extremely influenced by competition among indigenous strains, other eventually introduced rhizobia and antagonism from other organisms (Thies et al., 1991; Kahindi et al., 1997).

- Pesticides and heavy metals

There is evidence that at least some of the pesticides used in agriculture can have adverse effects on the survival of rhizobia or on nodulation of legumes (Singh and Wright, 2002). Pollution of agricultural soils caused by the addition of heavy metal contaminated sewage sludges has been shown to completely suppress N_2 fixation in legumes due to the toxicity of heavy metals to rhizobium (Brussaard et al., 2007). Enhanced formulations, e.g. granular inoculants, and seed coating techniques that protect the bacteria from environmental stress or physically separate them from toxic chemicals, such as fungicides applied to seed, offer new research directions.

In general, farmers require inoculant strains to survive in sufficient numbers to provide a population able to nodulate under environmental constraints such as pH, temperature and competition from less effective indigenous and naturalized strains. Competition is significant in many areas, not the least in soils of the tropics and sub-tropics.

1.3.3 Rhizobial function

Root nodule bacteria fix atmospheric nitrogen thus improving plant growth. The establishment of nodulating bacteria on or around the legume root may also adversely affect establishment of some pathogens or reduce the damage they caused (Akhtar and Siddiqui, 2007).

1.3.3.1 Plant growth

The performance of plants and yield are low on soil with deficit N in the absence of inoculation or fertilization, especially when chickpea has not been grown before. Inoculation with a selected rhizobium strain or native rhizobial populations can cause a significant increment in nodule number per plant, nodule dry weight, plant yield and nitrogen content of chickpea over a non-inoculated control (Beck, 1992; El Hadi and Elsheikh, 1999; Kantar et al., 2003). The amount of increase in plant productivity with rhizobia varies between rhizobial strains, their combinations (Içgen et al., 2002) and co-inoculation with other microorganisms (Dashti et al., 1997; Rudresh et al., 2005; Wani et al., 2007). Co-inoculation of legumes with rhizobia and *Pseudomonas* sp. frequently caused a significant increase in dry weight of legume plants due to nodule promotion by *Pseudomonas* (Bolton et al., 1990; Goel et al., 2002; Valverde et al., 2006).

1.3.3.2 Nutrient uptake

Rhizobia inoculation stimulates plant dry matter and grain yield by affecting some plant physiological processes such as photosynthesis, nodulation and N_2 fixation in legumes (Dashti et al., 1997; Kantar et al., 2003). Mean values for N derived from the atmosphere by chickpea differed significantly among chickpea cultivars, rhizobial strains (Beck, 1992), combination of rhizobia with other beneficial microorganism such as phosphate solubilizing bacteria and mycorhizae (Subba Rao et al., 1986; Saini et al., 2004) and environmental conditions (Carranca et al., 1999). Effects of soil pH and of varying concentrations of some minerals on the outcome of symbiosis were also reported (Carranca et al., 1999; Içgen et al., 2002). Zaidi et al. (2003) indicated that chickpea P and N uptake were significantly enhanced as a result of inoculation with *Rhizobium* sp. If favorably interacting rhizotrophic microorganisms are used as microbial co-inoculants, nodulation can be improved as well as P, K, Ca, Mg and N uptake and hence also yields are increased (Peix et al., 2001; Saini et al., 2004).

1.4 Interaction of soil microorganisms

AM fungi are known to play key roles in plant nutrition and health and in soil quality, whereas N_2-fixing symbiotic bacteria, by cycling N from the atmosphere to the biosphere, represent a key input of fixed N into plant productivity (Barea et al., 2005). Very important crops, i.e. legume species, are able to form dual symbiosis with both AMF and rhizobia.

1.4.1 Mycorrhiza and rhizobia

Mycorrhiza formation is known to enhance nodulation and N_2 fixation by legumes (Subba Rao et al., 1986; Singh and Tilak 1989). Mycorrhizal and rhizobial symbioses often act synergistically on infection rate, mineral nutrition and plant growth (Gueye, 1992; El-Ghandour et al., 1996; El-Ghandour and Galal, 2002; Jia et al., 2004; Saini et al., 2004; Chalke et al., 2006). The positive fungal effect on plant P uptake is beneficial for the functioning of the nitrogenase enzyme of the rhizobial symbiont leading to a higher N_2 fixation (Ibijbijen et al., 1996; Duponnois et al., 2008).

As Akhtar and Siddiqui (2007) found, inoculation of both rhizobium and mycorrhiza symbionts together were more beneficial for plant growth and reducing root-rot index than either of them. At the same time, root colonization and root nodulation was increased when both symbionts were inoculated together. Negative effects of salinity on both nodulation and N_2 fixation can also be compensated for by AM (Rabie and Almadini, 2005).

A positive effect of the interactions between AM fungi and nodulating rhizobia under drought conditions was found with soybean, not only to protect plants against the detrimental effects of drought, but also to help them cope with the premature nodule senescense induced by drought stress. Alleviation of oxidative damage could be involved in AM protection against nodule senescense (Ruiz-Lozano et al., 2001).

Because of the relatively high P demand for nodule formation, it is obvious that a major benefit of AM on the symbiotic role of rhizobium must be the P supplied by the fungus (Robson et al., 1981; Jakobsen, 1985). However, nutrients other than P, such as Zn, Cu, Mo, Ca etc. can affect both the infectivity and the symbiotic effectiveness of rhizobium. Therefore, enhanced uptake of these elements by the AM symbiosis may also be involved in the interactions. Conversely, there is a high requirement for N by the AM fungi to synthesize chitin, the main constituent of its cell walls (Duponnois et al., 2008). Therefore, nodulation and AM formation appear to be mutually supportive (Xie et al., 1995). In natural conditions, AMF and rhizobium colonize the root almost simultaneously but the two endophytes do not seem to compete for infection sites. In certain cases, previous inoculation with one of the endophytes can depress the development of the other (Duponnois et al., 2008). This has been mainly attributed to competition for carbohydrates when host photosynthesis is limited (Bethlenfalvay et al., 1982). When this occurs, AM fungi usually show a competitive advantage for carbohydrates over rhizobium (Duponnois et al., 2008).

In addition to AMF, soils also contain various antagonistic and beneficial bacteria such as root pathogens or plant growth promoting rhizobacteria (Khan, 2006) that can affect mycorrhiza and rhizobia interactively. Associative (*Azospirillum brasilense*) and/or symbiotically (*Rhizobium meliloti*) nitrogen-fixing bacteria plus AMF (*Glomus fasciculatum*) resulted in an enhanced effect on plant growth, though *Azospirillum* in the absence of *Rhizobium* was antagonistic to AMF (Tsimilli-Michael et al., 2000). Zaidi et al. (2003) showed that plant yield and nutrient uptake were further augmented by the addition of the AM fungus *Glomus fasciculatum* combined with *Rhizobium* sp. and phosphate solubilising microorganisms (PSM) of *Pseudomonas striata*. In contrast a negative effect occurred on all the considered parameters when *G. fasciculatum* was added to the combination of *Rhizobium* sp. and another PSM, *Penicillium variable*.

These results indicate that selective and specific functional compatibility relationships exist among the microbial inoculants with respect to plant responses (Barea et al., 2005).

1.4.2 Effects of soil sterilization

Additional inoculation with selected AMF strains, which are available as commercial products, often yields better growth promotion than indigenous AMF populations (Da Silveira and Lima, 1996; Salami et al., 2005). It has been found that inoculation with AMF (*G. fasciculatum*) was effective in increasing N, P and K content of alfalfa shoots as long as indigenous AMF were excluded (Biró et al., 2000). Interactive effects of AMF and other rhizotrophic microorganisms on nutrient acquisition have been repeatedly observed (e.g. Zaidi et al., 2003). The effects of different co-inoculations can be much more pronounced in sterilized soil compared to non-sterilized controls. This reveals a buffering capacity of the control soils arising from the presence of indigenous microflora (Biró et al., 2000). Obviously mycorrhiza colonization can affect plant growth and nutrient status in both sterilized and non-sterilized soils (Habte and Aziz, 1985; Ikombo et al., 1991; Ortas, 2003; Chalk et al., 2006) and any difference depends on the type of present microorganisms and their interaction (Jensen, 1982; Bi et al., 2003; Rohyadi et al.,

2004). However, competition between mycorrhiza and other soil microorganism for available carbohydrates supplied by the host plant, as well as competition for limited available P between plant roots and microorganisms may reduce mycorrhiza effects on plant growth in non-sterilized soil (Baas, 1990). Some studies showed that AM inoculation significantly increased dry matter accumulation and nutrient uptake of some legumes (Okon et al., 1996; Biró et al., 2000) or barley (Owusu-Bennoah and Mosse, 1979; Clarke and Mosse, 1981; Powell, 1981) compared with the indigenous inoculums. Consequently, the growth promoting effect of AMF inoculation depends also on the effectiveness and infectivity of indigenous microbes and on the interactions between the main community members, i.e. between indigenous microbes, inoculated AMF and host genotype.

Rhizobia are generally considered excellent soil saprophytes and able to persist in soil for quite long periods in the absence of a suitable host. But what determines strain persistence ability in soil is still poorly defined. It has been showed that populations of indigenous rhizobia in soil can affect the fraction of nodules produced by inoculant strains. Most inoculant strains produced substantially more nodules where the soil was essentially free of indigenous rhizobia than where the population of indigenous rhizobia was larger (O'hara et al., 2002), and the probability of enhancing yield with existing inoculation technology decreases dramatically with increasing numbers of indigenous rhizobia (Thies et al., 1991).

2 Objective of the study

The study focused on exploring three subject areas:

The first set of objectives was (i) to evaluate the effects of inoculation of AMF on growth of chickpea in absence or presence of rhizobia due to soil sterilization and subsequent inoculation, (ii) to identify the interactions of indigenous soil microorganisms with AMF and rhizobia without soil sterilization and (iii) to compare the effects of AMF on chickpea and barley with and without soil sterilization.

Secondly, the study aimed at comparing N concentration and total N uptake in chickpea and barley depending on mycrorrhizal colonization and nitrogen nutrition either based on rhizobia inoculation or on the application of additional mineral fertilizer N.

Thirdly, another set of objectives of the experiments was (i) to evaluate the effect of AM inoculation on macro and micro nutrient concentrations and uptakes of chickpea in sterile soil, (ii) to identify the interactions of indigenous soil microorganisms with AMF on these traits without soil sterilization and (iii) to compare the effects of AMF on chickpea and barley nutrient status.

In our pot experiments, AMF inoculation was always tested in combination with or without application of additional mineral fertilizer N.

The experimental questions to be answered are:

1. Which levels of AMF colonization of chickpea and barley can be attained by inoculating mycorrhiza (Symbivit®) at different levels of nitrogen nutrition with and without soil sterilization?

2. How does additional nitrogen supply of chickpea and barley with either mineral fertilizer or by inoculating rhizobia (Radicin®, only chickpea) affect soil mineral N concentrations and nodule formation on chickpea in sterilized and non-sterilized soil?

3. How do chickpea and barley respond to mycorrhizal colonization with respect to plant growth, yield and nutrient uptake at different nitrogen supply levels with and without soil sterilization?

4. Are there any interactions between mycorrhizal colonization, rhizobial infection and soil mineral N level on plant growth, yield and nutrient uptake of chickpea and barley?

The following chapters (chapters 4.1 to 4.3) cover results of the addressed subject areas. Chapters 5 to 7 include discussion, general conclusions and an outlook.

3 Materials and methods

3.1 Treatments and experimental design

Experiments in Mitscherlich pots (6.3 l soil volume) were carried out during spring and summer 2006 and 2007 under sheltered conditions in a glasshouse in the city of Vienna, Austria, with the possibility to transfer the pots into ambient conditions of a fence house under favorable weather conditions. The experiments were in a randomized complete block design with five replications.

3.1.1 Pot experiment conditions

Chickpea (commercial seeds of unidentified Kabuli genotype) and barley (cv. Xanadu) seeds were sown in pots filled with a mixture (1:1) of sterilized soil and sand. The soil was a chernozem topsoil of silty loam taken from the experimental farm Gross-Enzersdorf of BOKU University. The soil-sand substrate was analyzed for some chemical indicators (cf. Tab. 3.1) and subsequently sterilized (105°C, 24 h) before sowing. Additionally, in 2007 we studied the effects of soil sterilization (with or without sterilization). In all treatments, one week after emergence, chickpea or barley seedlings were manually thinned out to three or ten seedlings per pot, respectively. Tap water (4 mg NO_3 l^{-1}) was supplied during the vegetation period daily if necessary to avoid any drought stress.

Table 3.1: Characteristics of pot experiments

Growing season	2006	2007
pH value ($CaCl_2$)	7.5	7.5
$N0_3$-N	5.6 mg kg^{-1} (25 kg N ha^{-1})	10.7 mg kg^{-1} (48 kg N ha^{-1})
Plant available P (CAL)	106 mg kg^{-1}	138 mg kg^{-1}
Plant available K (CAL)	191 mg kg^{-1}	248 mg kg^{-1}
Plant available Mg ($CaCl_2$)	94 mg kg^{-1}	115 mg kg^{-1}
Crop	chickpea	chickpea barley (only with non-sterilized soil)
Date of sowing	25 and 26 April	23 and 24 April
Harvest date at flowering	28 June	20 June
Harvest date at maturity	25-27 July	20 July (barley) and 7 August (chickpea)

Inoculation of the AMF treatments was done by adding the AMF product “Symbivit®” (Symbio-m, s.r.o., Lanškroun, CZ) to the pots at planting. It was placed below seeds at a rate of approximately 5 g for a group of seeds which were later thinned to one seedling. The inoculum consisted of an inert carrier (a mix of slate, zeolite and clay) that contained reproductive particles (spores, mycelium and colonised root fragments) from six different strains of *Glomus* spp. (*G. intraradices* BEG 98, *G. mosseae* BEG 99, *G. claroideum* BEG 93, *G. microagregatum* BEG 56, *G. caledonium* BEG 97, *G. etunicatum* BEG 92; BEG = La Banque Européenne des Glomales; International Institute of Biotechnology; Kent; GB; http://www.kent.ac.uk/bio/beg) isolated from a range of soils from various ecosystems. For inoculation with rhizobia (R+) we used the water

suspension “Radicin®” (Jost GmbH, Iserlohn, D) one week after emergence. Pots with N application (N+) received an amount of 314 mg N per pot equivalent to 100 kg N ha^{-1} as a calcium ammonium nitrate (27% N) solution one week after emergence.

3.1.2 Factorial design

The factorial design included the following factors:

- Year (2006 or 2007)
- Crop species (chickpea or barley)
- AMF inoculation (M+ or M-, i.e. with or without inoculum “Symbivit®”)
- Nitrogen nutrition (N- R-, N+ R- or N- R+, i.e. only soil supply, with mineral fertilizer at 314 mg N per pot (equivalent to100 kg N ha^{-1}) or with inoculation of rhizobia, the latter not for barley)
- Soil sterilization (with or without sterilization of soil, the latter only in 2007)
- Harvest date (H1 or H2, i.e. at flowering or physiological maturity)

Combinations of the factors resulted in four orthogonal subsets of data, subsequently indicated as experimental units I, II, IIIa and IIIb that were separately submitted to analyses of variance as described in chapter 4.

The effects of mycorrhiza inoculation on chickpea in sterilized soil were studied in unit I. Additionally units II and III included the effects of non-sterilized soil and the comparison chickpea with barley, respectively. All units comprised two levels of N fertilization.

3.2 Sampling and measurements

3.2.1 Plant and soil sampling procedures

Plants were harvested at flowering stage (H1) or at physiological maturity (H2) by removing them completely from the pots. Plants were divided into fractions. For the later evaluation of mycorrhizal colonization the roots were stored in an alcoholic solution (50% ethanol).

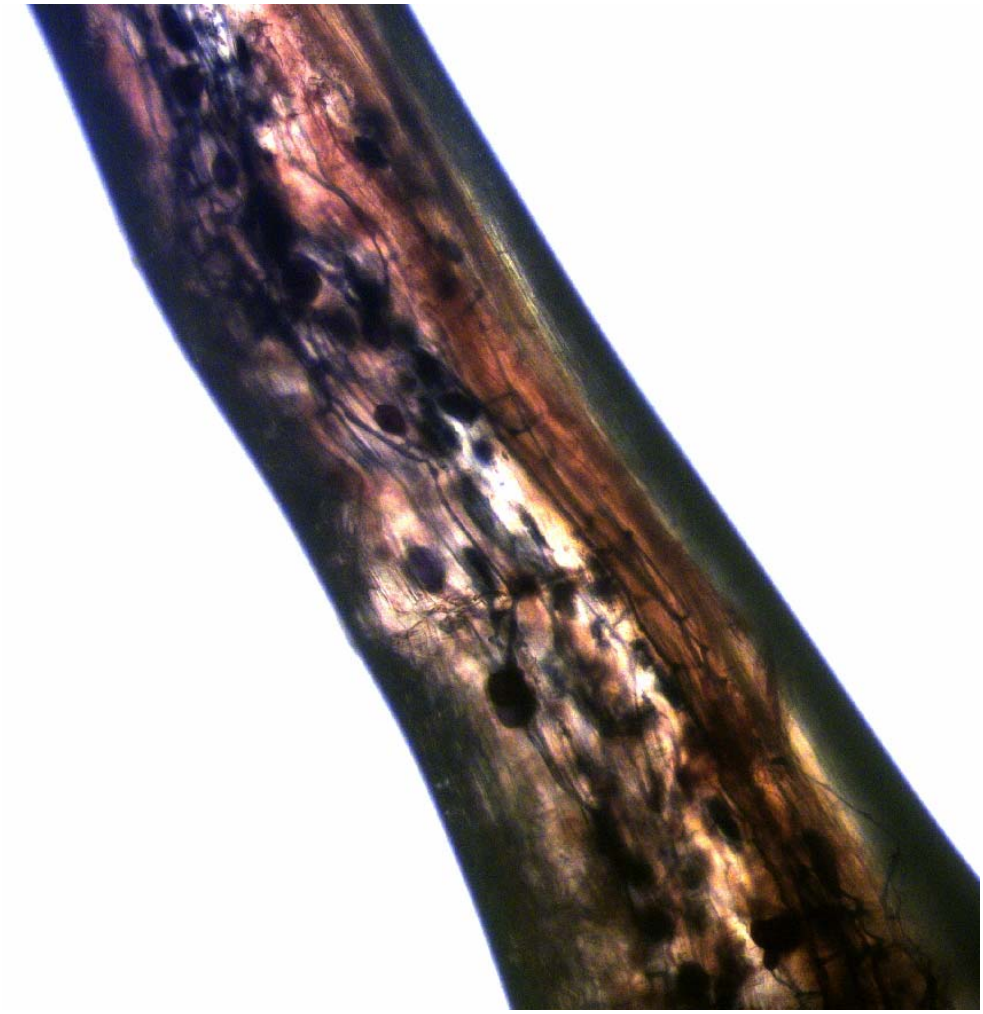
Soil samples were taken during harvesting by mixing the soil of each pot after the plants had been removed and putting a representative sample into a plastic bag. The bags were immediately frozen at -20 °C.

3.2.2 Plant analyses

3.2.2.1 Mycorrhizal colonization

Before dry matter determination of roots, root segments (1 cm in length) below the upper 2 cm of the roots had been sampled to estimate root colonization by AMF. The roots were cleared with a 10% KOH solution and stained with a 5% ink solution (Shaeffer jet black + acetic acid). Evaluation of AMF colonization (Fig. 3.1) was done under a microscope by a grid line method according to Vierheilig et al. (1998).

Figure 3.1: Colonized root of chickpea by arbuscular mycorrhiza



3.2.2.2 Rhizobial infection

On two harvests, three plants were taken from each plot, shaken free of superfluous soil, and any nodules carefully removed (Fig.3.2). The number of nodules per pot was counted, dried at 105 °C for 24 h, and weighed.

Figure 3.2: Nodules on chickpea root

3.2.2.3 Dry matter production

Plants were divided into shoots and roots, at maturity shoots were further divided into straw and pods or ears, respectively. Root samples were washed out carefully with water and a sieve (250 µm mesh size). Shoot and root samples were dried at 105 °C for 24 h and amounts of dry matter were measured gravimetrically. Thereafter samples to determine nutrient content were ground.

3.2.2.4 Nutrient content

The concentration of nitrogen in the samples was determined using an element analyzer based on the Dumas principle (LECO CN). For the plant tissue concentration of other elements, dried samples (ca. 500 mg) were digested in a tri-acid (HNO_3 + $HClO_4$ + H_2SO_4) mixture (Nabrzyski and Gajewska, 1998). Contents of Ca, Mg, K, Fe, Mn, Cu and Zn in the digest were measured using atomic absorption spectrometry (Varian SpektrAA 300) (Beaty and Kerber, 1993). The P content in the plant tissues was analyzed by the vanadomolybdate method after the wet digestion followed by photometry (Varian DMS 200) (Cavell, 1955).

3.2.3 Soil mineral N

From the frozen soil samples, subsequently the content of soil mineral N (nitrate and ammonium N) was extracted by a $CaCl_2$ solution and measured by a photometer method on a FIASTAR 5000 apparatus (FOSS GmbH, D). In all soil samples NH_4 concentrations were very low, i.e. below 0.1 kg NH_4-N ha^{-1}, thus we report only NO_3-N results.

3.3 Statistical analysis

The statistical analysis of all observations was done by the procedure MIXED of the SAS software. With significant factorial effects, the t-test was used to compare means and least significant differences (LSD) were calculated. The significance threshold was assumed at p=0.05.

4 Results

4.1 Arbuscular mycrorrhizal and nitrogen nutrition effects on chickpea and barley growth

The results section starts with a table showing characteristics of soil biological and chemical conditions of the experiments as obtained by inoculation, fertilization and soil sterilization treatments (Tab. 4.1). The subsequent presentation of dry matter results is based on the analyses of variance that have been performed for four experimental units, i.e. orthogonal subsets of data. Due to our research objectives, significant interactions of AMF inoculation and nitrogen nutrition with soil sterilization or crop species were of primary interest. These have been frequently modified by environmental conditions or plant development, i.e. by year or harvest date. Due to the heterogeneity of significant interaction levels, and because interactions between AMF inoculation and nitrogen nutrition were only rare, the general results are presented in two comprehensive figures (Fig. 4.1, 4.2), giving an overview about AMF or nitrogen nutrition effects, respectively. These are supplemented by individual specific effects of relevance for our objectives.

4.1.1 Soil biological and chemical conditions of the experiments

The inoculation with AMF has been successful, because all inoculated plant samples were substantially colonized (Tab. 4.1). The colonization level was higher in 2006 than in 2007, without than with soil sterilization and with chickpea compared to barley. Without sterilization, the soil obviously contained indigenous populations which were able to colonize chickpea and barley. Yet, additional inoculation increased colonization also in non-sterilized soil. After soil sterilization without AMF inoculation no colonized roots were found at all. Nitrogen fertilization had no effect on mycorrhizal root colonization (data not shown).

There was a significant interaction between harvest date and year on percentage of AMF colonization for chickpea in sterilized soil. The colonization levels varied in different years from flowering to maturity. In 2006 chickpea roots showed the highest colonization rate at the flowering stage (58%) that decreased to 51% at maturity, while in 2007 the higher mycorrhizal colonization rate of 35% was observed at maturity time, compared to only 14% at flowering. But also with barley in 2006 or 2007, colonization increased from 14% or 6% at flowering to 21% or 18% at maturity, respectively.

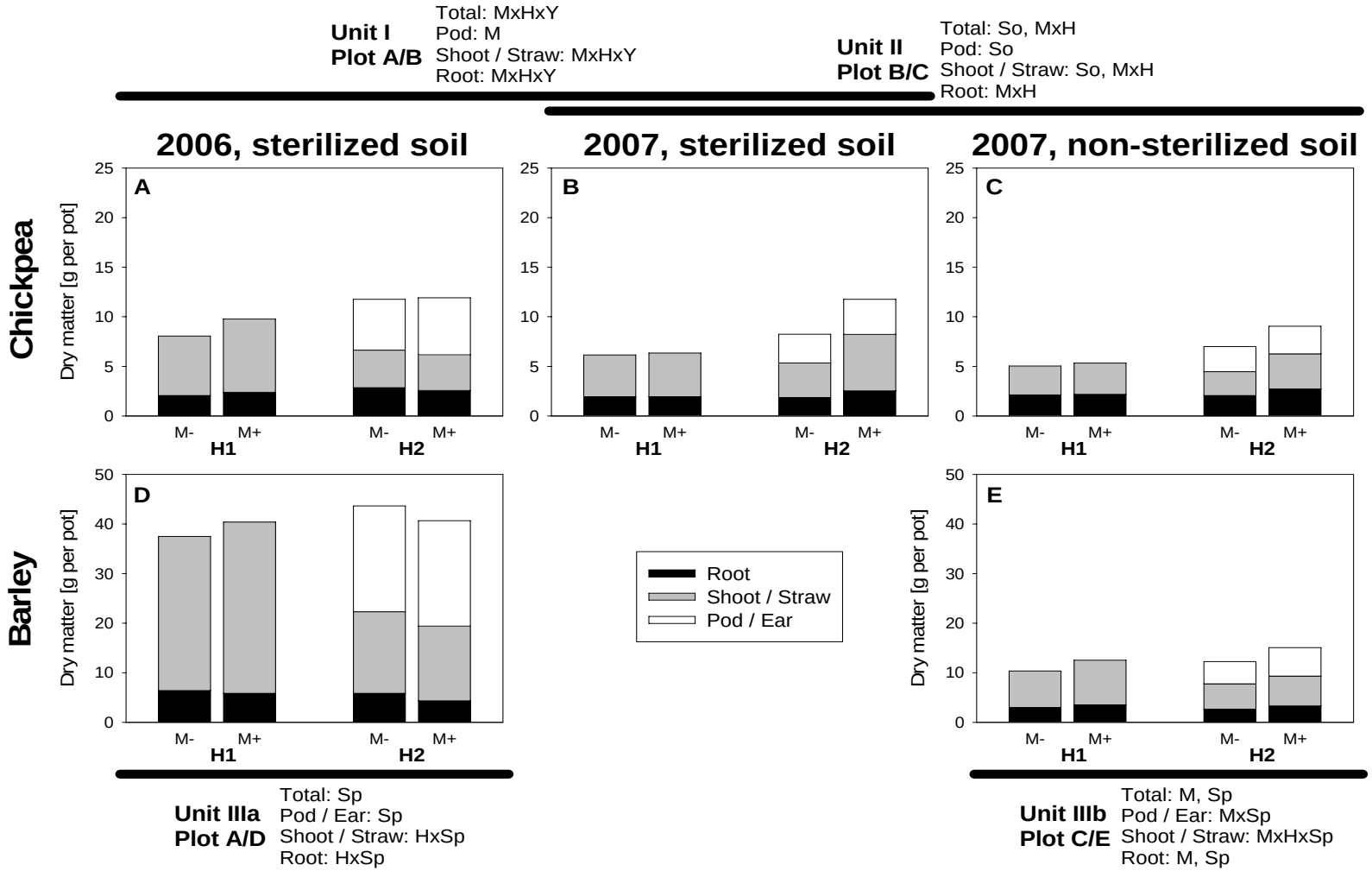
Similar to AMF, rhizobia inoculation resulted in nodule production while without rhizobia addition no nodules were observed. Missing nodulation also in non-sterilized soil indicates that the soil from our experimental station was not colonized by rhizobia strains capable of chickpea infection. The inoculation success was much more pronounced in 2006 and also in non-sterilized soil.

Soil NO_3-N concentrations at final harvest were slightly higher in 2007 than in 2006, but the difference is about the same as the difference in initial concentrations of the substrates (cf. Tab. 3.1). Nitrate concentrations were substantially increased after addition of fertilizer N. They were generally lower under barley or in non-sterilized soil compared to chickpea or sterilized soil, respectively. Under chickpea we observed a tendency of lower soil NO_3-N concentrations after rhizobia inoculation compared to the untreated control, i.e. N- R- (data not shown).

Table 4.1: Characterization of soil biological and chemical conditions, i.e. percentage of AMF colonized roots, rhizobial nodule dry weight and number (all on average across two harvest dates) and soil NO_3-N at maturity. Means and standard deviations (in brackets) indicated according to the subsets of data submitted to statistical analysis (experimental units I, II, IIIa, IIIb). Treatments without (M-) or with (M+) AMF inoculation, without (N-) or with (N+) mineral fertilizer, without (R-) or with (R+) rhizobia inoculation

Unit	Year	Species	Soil sterilization	AMF Colonization (%)		Nodule weight (mg per pot)		N. number (per pot)		Soil NO_3-N (mg kg^{-1})		
				M+	M-	N- R-	N+ R-	N- R+		N- R-	N+ R-	N- R+
I	2006	Chickpea	with sterilization	54.5 (11.8)	0 (0)	0 (0)	0 (0)	46.1 (30.1)	41 (22)	12.0 (14.0)	25.3 (19.2)	6.1 (3.6)
	2007			24.9 (19.8)	0 (0)	0 (0)	0 (0)	3.2 (8.2)	5 (4)	15.1 (6.5)	31.6 (5.9)	11.5 (6.0)
II	2007	Chickpea	with sterilization	24.9 (19.8)	0 (0)	0 (0)	0 (0)	3.2 (8.2)	5 (4)	15.1 (6.5)	31.6 (5.9)	11.5 (6.0)
			without sterilization	44.1 (22.4)	17.8 (12.3)	0 (0)	0 (0)	52.5 (96.8)	18 (18)	5.1 (4.7)	14.6 (4.8)	3.5 (3.5)
IIIa	2006	Chickpea	with sterilization	55.1 (12.4)	0 (0)	for chickpea: see above for barley: not applicable				12.0 (14.0)	25.3 (19.2)	not tested
		Barley		17.7 (10.0)	0 (0)					2.9 (2.1)	3.0 (1.7)	
IIIb	2007	Chickpea	without sterilization	43.5 (20.8)	19.7 (13.3)					5.1 (4.7)	14.6 (4.8)	
		Barley		17.6 (9.0)	6.6 (6.9)					6.7 (1.7)	14.1 (7.3)	

Figure 4.1: Dry matter of crops, divided into fractions, as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated. Note that axis scales for chickpea and barley are different.



4.1.2 Mycorrhizal effects

Analyzing chickpea in sterilized soil in two years (exp. unit I), the interaction of mycorrhizal inoculation x harvest date x year showed that mycorrhiza increased total dry matter in both years, but this enhancing effect was significant only on the first harvest in 2006 and on the second harvest in 2007 (Fig. 4.1). This resulted from significant differences in all plant fractions (i.e. roots, shoots and pods) between inoculated and non-inoculated plants.

Comparing chickpea 2007 in sterilized vs. non sterilized soil (unit II), the positive mycorrhizal effect at maturity can be confirmed also for the non-sterilized soil. Only pod yield was not improved.

With regard to the comparison of barley and chickpea, in 2006 in sterilized soil (unit IIIa) AMF inoculation did not affect growth. Contrastingly, in 2007 in non-sterilized soil (unit IIIb) total dry matter and all plant fractions were enhanced by AMF with slightly stronger effects on barley than on chickpea. For pod/ear dry matter, no significant differences in the pod dry matter were observed between AMF inoculated and non-inoculated chickpea, while inoculated barley showed higher ear dry matter than non-inoculated plants. There was a favorable effect of AMF inoculation on barley shoot/straw dry matter at both sampling dates, while chickpea straw production was only enhanced at maturity.

AMF also affected soil NO_3-N concentrations under chickpea, but only in 2007 in sterilized soil (Tab. 4.2). Only under these conditions we found substantially more NO_3-N after AMF inoculation than in non-inoculated pots.

Table 4.2: Soil NO_3-N concentrations under chickpea as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year or soil sterilization. Experimental units I and II, means across harvest dates

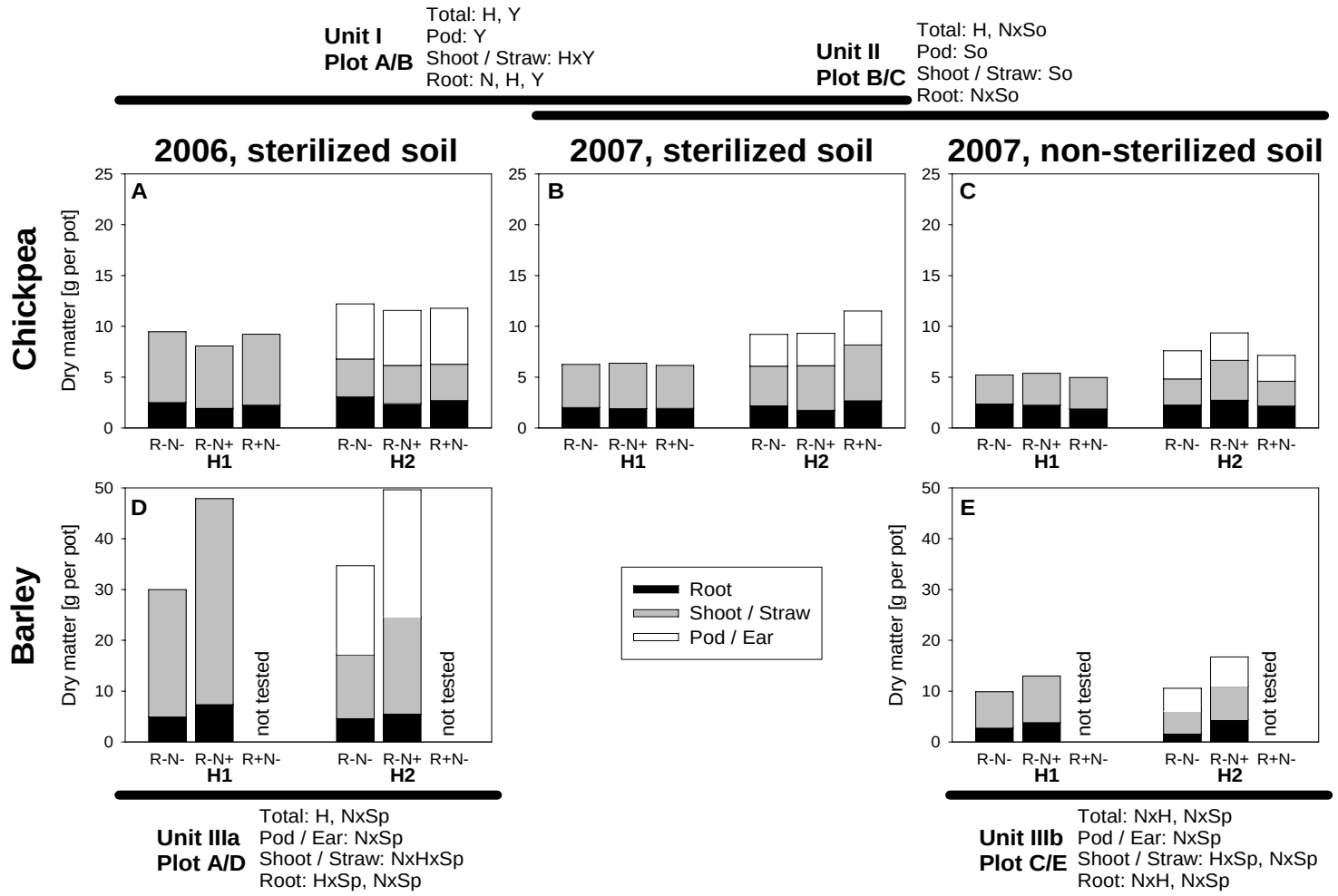
Exp. unit		Year	Soil sterilization	Soil NO_3-N (mg kg^{-1})		LSD	
				M+	M-		
I		2006	with sterilization	10.8	14.8	4.0	
	II	2007		22.2	14.2		2.6
		2007	without sterilization	9.1	9.3		

4.1.3 N nutrition effects

With chickpea in sterilized soil (unit I), we found hardly any consistent effect of nitrogen nutrition on chickpea growth, neither due to fertilizer N nor to rhizobial infection (Fig. 4.2). Only root dry matter was significantly affected by nitrogen nutrition. On average across years and harvest dates, mineral N fertilizer application (R-N+) reduced root growth compared to the unfertilized crops with or without rhizobia inoculation.

Comparing the soils in 2007 (unit II), shoot/straw and pod yields were generally higher in sterilized soil. Chickpea showed different reactions to N nutrition treatments in total and root dry matter. The root depressing effect of mineral fertilization did not appear in the

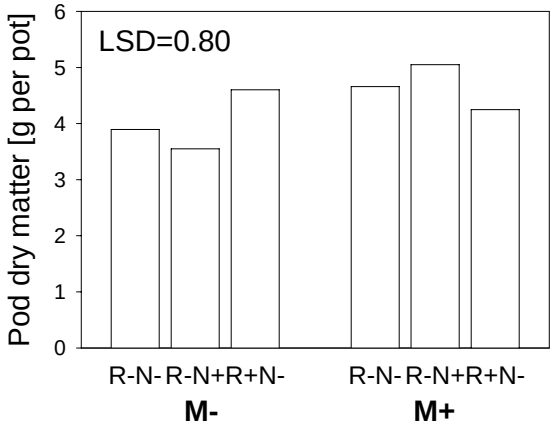
Figure 4.2: Dry matter of crops, divided into fractions, as affected by nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+), in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated. Note that axis scales for chickpea and barley are different.



non-sterilized soil and total dry matter was even enhanced by the fertilizer. On the other hand in sterilized soil rhizobia inoculation caused the highest root and total dry matter.

With view to the two species (units IIIa, b), barley growth was strongly enhanced by mineral fertilizer in contrast to chickpea in both soil treatments and years, respectively.

Figure 4.3: Dry matter of chickpea pods as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+). Experimental unit I, sterilized soil, harvest date H2, means across years



4.1.4 Interaction effects between AMF and N nutrition

Chickpea pod yield across two years (unit I) in the presence of AMF colonization was improved by fertilizer N while pod yield of rhizobia treated plants was lower (Fig. 4.3). Contrastingly, without AMF inoculation rhizobia infected plants produced more pod dry matter than those with fertilizer N.

On average across both species, chickpea and barley, in 2006 in sterilized soil (unit IIIa), root growth of AMF colonized plants was hardly affected by N fertilization, while without AMF it was significantly enhanced by fertilizer N (Tab. 4.3).

Table 4.3: Dry matter (D.M.) of roots as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with nitrogen nutrition without (R-N-) or with (R-N+) mineral fertilizer. Experimental unit IIIa, means across species and harvest dates

Unit	Year	Soil sterilization	Nitrogen nutrition	Root D.M. (g per pot)		LSD
				M+	M-	
IIIa	2006	with sterilization	R-N-	3.97	3.58	0.90
			R-N+	3.67	4.90	

4.2 Arbuscular mycrorrhizal and nitrogen nutrition effects on N concentration and total N uptake in chickpea and barley

This presentation of results is based on the analyses of variance that have been performed for four experimental units; i.e. orthogonal subsets of data (cf. part 4.1). The soil biological and chemical conditions of the experiments have been mentioned in Table 4.1.

Due to the heterogeneity of significant interaction levels, and because AMF effects and interactions between AMF inoculation and nitrogen nutrition were only rare, the general results are presented in two comprehensive figures in the appendix (Appendix Fig. A1, A2), giving an overview about concentrations and uptake of N, respectively. These are subsequently supplemented by individual specific effects of significance and relevance for our objectives, focusing on nitrogen nutrition effects (Tab. 4.4).

4.2.1 Mycorrhizal effects

Effects of mycorrhiza on N concentrations were scarce. Only in sterilized soil across both years, 2006 and 2007, N concentration was significantly lower in AMF chickpea than in control plants (means not shown).

Analyzing chickpea in sterilized soil in two years (exp. unit I), the interaction of mycorrhizal inoculation x harvest date x year showed that mycorrhiza increased total N uptake only in 2007, but this enhancing effect was significant only on the second harvest (Fig. A2) reflecting significant differences in total dry matter between AMF colonized and non-colonized plants (cf. part 4.1.2).

Comparing chickpea 2007 in sterilized vs. non sterilized soil (unit II), the positive mycorrhizal effect at maturity on N uptake can be confirmed also for the non-sterilized soil (Fig. A2).

With regard to the comparison of barley and chickpea, in 2006 in sterilized soil (unit IIIa) AMF inoculation did not affect N uptake. Contrastingly, in 2007 in non-sterilized soil (unit IIIb) total N uptake was enhanced by AMF with slightly stronger effects on barley than on chickpea (Fig. A2).

4.2.2 N nutrition effects

With view to units I and II, chickpea N concentration was strongly enhanced by mineral fertilizer in contrast to rhizobial inoculation in both years and soil treatments, respectively (Tab. 4.4). With regard to the comparison of barley and chickpea, in 2006 in sterilized soil (unit IIIa) the positive effect of nitrogen fertilizer on N concentration is not confirmed for barley. Contrastingly, in 2007 in non-sterilized soil (unit IIIb) N concentrations were enhanced by nitrogen application in both plant species (Tab. 4.4).

Comparing the soils in 2007 (unit II), N concentration (Fig. A1) and N uptake were generally higher in sterilized soil (Tab. 4.4 and Fig. A2).

With chickpea in both units I and II, we found a positive effect due to nitrogen fertilizer but no effect of rhizobia inoculation on N uptake (Tab. 4.4). Only in sterilized soil in 2007 chickpea showed a different reaction to N nutrition treatments in N uptake. On average across mycorrhizal and non-mycorrhizal chickpea, rhizobia inoculation (R+N-) elevated N uptake to level of, mineral fertilizer application (R-N+). At maturity the N uptake was even highest after rhizobia application (means not shown).

Table 4.4: Nitrogen concentrations (g kg^{-1}), uptake (mg pot^{-1}) and soil NO_3-N (mg kg^{-1}) in total plant biomass affected by nitrogen nutrition levels (all on average across mycorrhiza treatments and harvest dates). Means indicated according to the subsets of data submitted to statistical analysis (experimental units I, II, IIIa, IIIb). Treatments are without (N-) or with (N+) mineral fertilizer, without (R-) or with (R+) rhizobia inoculation.

Unit	Year	Species	Soil sterilization	Plant N concentration (g kg^{-1})			Plant N uptake (mg pot^{-1})		
				N- R-	N+ R-	N- R+	N- R-	N+ R-	N- R+
I	2006 2007	Chickpea	with sterilization	24.3	29.1	23.5	208.7	241.3	213.4
$LSD_{0.05}$					1.2			18.8	
II	2007	Chickpea	with sterilization	26.4	31.1	26.3	238.4	265.0	256.7
			without sterilization				126.7	189.1	130.0
$LSD_{0.05}$					2.0			20.5	
IIIa	2006	Chickpea	with sterilization	16.6	23.1	not tested	179.0	217.7	not tested
		Barley		9.6	11.2		311.1	534.7	
$LSD_{0.05}$					2.1			74.6	
IIIb	2007	Chickpea	without sterilization	17.0	23.6		126.7	189.1	
		Barley					136.2	293. 0	
$LSD_{0.05}$					1.0			15.3	

With view to the two species (units IIIa, b), N uptake in barley was strongly enhanced by mineral N fertilizer in both sterilized soil (unit IIIa) and non-sterilized soil (unit IIIb), whereas this enhancement was not significant with chickpea in sterilized soil (unit IIIa) but positive effect of mineral N fertilizer on N uptake appeared with chickpea in non-sterilized soil (unit IIIb) (Tab. 4.4).

4.3 Arbuscular mycrorrhizal and nitrogen fertilizer effects on micro and macro element concentrations and uptake in chickpea and barley

4.3.1 Mycorrhizal effects

The results are presented in tables based on the significance of effects detected by ANOVA. We found with high consistence either main effects of AMF inoculation or interactions of AMF with another specific factor, i.e. year (unit I, Tab. 4.5, 4.6) or soil sterilization (unit II, Tab. 4.7, 4.8) or crop species (unit III, Tab. 4.9, 4.10), respectively. The rarely observed interactions of AMF with nitrogen nutrition are indicated in Table 4.11.

4.3.1.1 Chickpea in sterilized soil

a) Concentration

Across both years, 2006 and 2007, the P concentration was higher and N concentration was lower in AMF chickpea than in control plants (Tab. 4.5). Only Mg and Zn were unaffected by mycorrhizal inoculation.

The effect of AMF inoculation on the concentrations of K, Ca, Fe, Mn and Cu partly depended on the year. AMF induced higher Mn concentration in both years but this effect was pronounced in 2007. Mycorrhizal inoculation decreased the Ca concentration in 2006 significantly while in 2007 it was slightly increased. Only in 2006 mycorrhizal inoculation resulted in higher K, Cu and Fe concentrations. The high absolute level of Fe concentration was due to high Fe concentration in roots (data not shown).

While the average concentrations of P, K, Ca, Mg and Fe were similar in both 2006 and 2007, the concentrations of Mn, Cu and N in 2007 exceeded those in 2006, while the Zn concentration was higher in 2006 than in 2007 (main year effects not shown).

Table 4.5: Nutrient concentrations in total chickpea biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year (exp. unit I, sterilized soil, means across nitrogen nutrition levels)

Nutrient element		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		($g\ kg^{-1}$)					($mg\ kg^{-1}$)			
M+	2006	24.26	1.55	14.71	16.42	3.11	2101	107.81	39.59	10.74
	2007			13.02	20.12	3.42	2051	166.62	35.41	11.53
M-	2006	27.38	0.96	11.59	22.39	3.09	891	85.52	38.61	9.06
	2007			12.77	18.60	3.19	1944	120.31	34.94	11.81
$LSD_{0.05}$		2.14	0.12	1.17	2.20	n.s.	628	14.32	n.s.	0.88

b) Uptake

As a reflection of differences in dry matter, the uptake of P, K, Mg, Fe, Mn, Zn and Cu per pot in both years and Ca in 2007 increased with AMF inoculation (Tab. 4.6). However, the magnitude of Mn increment was substantially higher in 2007.

Table 4.6: Nutrient uptake in total chickpea biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year (exp. unit I, sterilized soil, means across nitrogen nutrition levels)

Nutrient element		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		(mg pot^{-1})					(µg pot^{-1})			
M+	2006	218.1	18.51	163.1	202.7	38.38	24296	1335.9	440.0	130.4
	2007	334.4			228.0			1879.8		
M-	2006	221.8	9.16	112.0	257.4	29.63	12597	987.8	350.0	96.4
	2007	263.2			145.4			917.1		
$LSD_{0.05}$		n.s.	2.59	15.7	57.8	5.45	5419	345. 8	50.6	17.6

4.3.1.2 Comparing sterilized with non-sterilized soil

a) Concentration

On average across AMF treatments, in sterilized soil the concentrations of N, K and Mn were higher than in the non-sterilized soil while soil sterilization resulted in lower P and Fe concentrations than in non-sterilized soil (main sterilization effects not shown).

The Mg concentration was positively affected by AMF treatments across sterilized or non-sterilized soil (Tab. 4.7). AMF colonization resulted also in significantly higher Mn concentration in both soils, but with a stronger effect in sterilized soil. In the non-sterilized soil, there was no clear difference in P concentration due to AMF inoculation. In contrast, in the sterilized soil, the P concentration of plants with AM inoculation was significantly higher than of those without AMF. We did not find any effect of AMF colonization on N, K, Ca, Fe, Zn and Cu concentrations.

Table 4.7: Nutrient concentrations in total chickpea biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with soil sterilization (So+, sterilized soil or So-, non-sterilized soil) (exp. unit II, 2007, means across nitrogen nutrition levels)

Nutrient element		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		(g kg^{-1})					(mg kg^{-1})			
M+	So+	30.83	1.45	13.02	20.12	3.45	2051	166.62	35.41	11.53
	So-	21.73	1.46	10.83	20.27		2979	115.00	35.88	12.26
M-	So+	35.23	0.98	12.77	18.60	3.18	1944	120.31	34.94	11.81
	So-	22.37	1.35	9.44	20.29		2746	97.44	35.07	12.71
$LSD_{0.05}$		n.s.	0.17	n.s.	n.s.	0.18	n.s.	17.46	n.s.	n.s.

b) Uptake

The average uptake of P, Ca, Mg, Zn and Cu was similar in both sterilized and non-sterilized soils, but the N, K and Mn uptake was enhanced while the Fe uptake was reduced after soil sterilization compared to non-sterilized treatments (main sterilization effects not shown).

AMF inoculation in both sterilized and non-sterilized soil led to a higher uptake of N, P, K, Ca, Mg, Fe, Mn, Zn and Cu (Tab. 4.8). There were no significant interactions in nutrient uptake between AMF inoculation and sterilization of soil for any element except for Mn: The effect of AMF inoculation on the uptake of Mn was more pronounced in sterilized soil.

Table 4.8: Nutrient uptake in total chickpea biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with soil sterilization (So+, sterilized soil or So-, non-sterilized soil) (exp. unit II, 2007, means across nitrogen nutrition levels)

Nutrient element		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		(mg pot^{-1})					(µg pot^{-1})			
M+	So+	270.3	15.59	125.4	209.4	35.97	25527	1879.8	364.9	121.8
	So-							1076.6		
M-	So+	211.1	8.72	81.7	145.9	23.35	17224	917.1	258.7	90.9
	So-							694.6		
$LSD_{0.05}$		38.2	2.46	17.3	31.7	4.67	4394	324.3	50.0	20.3

4.3.1.3 Comparing chickpea with barley in non-sterilized soil

a) Concentration

Compared to barley, all chickpea plants, either mycorrhizal or non-mycorrhizal, contained higher concentrations of all nutrients except K. The K concentration in barley was about twice as high as in chickpea plants (main species effects not shown).

We found hardly any interactions between AMF inoculation and species. Only the elevated Mn and Mg concentrations after AMF inoculation in chickpea were not confirmed in barley (Tab. 4.9).

Table 4.9: Nutrient concentrations in total chickpea and barley biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with plant species (exp. unit III, non-sterilized soil, 2007, means across nitrogen nutrition levels)

Nutrient element		N*	P*	K*	Ca*	Mg	Fe*	Mn	Zn*	Cu*
		(g kg^{-1})					(mg kg^{-1})			
M+	Chickpea	21.73	1.46	10.82	20.27	3.48	2979	115.00	35.88	12.26
	Barley	15.35	0.94	21.24	6.84	1.97	1302	52.91	21.28	9.01
M-	Chickpea	22.37	1.35	9.44	20.29	3.17	2746	97.44	35.07	12.71
	Barley	14.29	0.99	22.01	7.64	2.10	1493	61.66	24.26	8.48
$LSD_{0.05}$		n.s.	n.s.	n.s.	n.s.	0.24	n.s.	11.11	n.s.	n.s.

*nutrient elements with significant species effect

b) Uptake

The N, K and Cu uptakes were higher but the Ca uptake was lower with barley compared to chickpea (main species effects not shown).

AMF inoculation increased the uptake of all elements by both chickpea and barley plants but differences of Mn uptake were not significant in barley plants (Tab. 4.10).

Table 4.10: Nutrient uptake in total chickpea and barley biomass as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with plant species (exp. unit III, non-sterilized soil, 2007, means across nitrogen nutrition levels)

Nutrient element		N*	P	K*	Ca*	Mg	Fe	Mn	Zn	Cu*
		(mg pot^{-1})					(µg pot^{-1})			
M+	Chickpea	222.0	14.28	213.9	148.0	31.75	24743	1076.6	332.5	126.0
	Barley							814.4		
M-	Chickpea	170.7	10.89	173.2	121.1	24.32	19183	694.6	274.3	99.6
	Barley							773.9		
$LSD_{0.05}$		16.2	1.62	19.8	18.0	3.47	4435	150.9	41.2	14.6

*nutrient elements with significant species effect

4.3.2 Interaction effects between AMF inoculation and nitrogen fertilizer

Only with Ca and in one case with K concentrations we found significant interactions between AMF and nitrogen fertilization (Tab. 4.11). On average across both years (unit I), sterilization treatments (unit II) and species (unit III), the Ca concentration of the AMF inoculated plants was hardly affected by the N fertilization, while N application significantly enhanced the Ca concentration in non-inocullated plants. Without N application, the K concentration of chickpea plants across years significantly increased after AMF inoculation. In contrast, with N there were no clear effects on the K concentration due to the AMF factor.

Table 4.11: Concentrations of potassium and calcium in total plant biomass (g kg^{-1}) as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with nitrogen nutrition (N-, without or N+, with) (exp. Units I-III, means across years or soil sterilization treatments or species)

Experimental unit		I		II	III
Nutrient element		K	Ca	Ca	Ca
M+	N-	14.74	18.53	20.85	13.66
	N+	13.00	18.02	19.55	13.45
M-	N-	11.76	18.78	18.02	12.56
	N+	12.60	22.21	20.87	15.37
$LSD_{0.05}$		1.17	2.20	2.29	1.78

5 Discussion

5.1 The effects of arbuscular mycorrhiza and nitrogen nutrition on growth and N acquisition of chickpea and barley

5.1.1 Success of inoculations

In all experiments, we were able to establish a suitable level of AMF colonization, although the percentage of AMF colonized roots was markedly affected by environment and crop species. Seasonal patterns in the formation of mycorrhiza have also been found to vary considerably from year to year (Sanders and Fitter, 1992; Muthukumar and Udaiyan, 2002). On the other hand, non-inoculated pots with sterilized soil were obviously kept completely free of AMF and also in non-sterilized soil, a distinct increase in AMF colonization was observed after inoculation.

As chickpea had never been grown before on the experimental fields we had used as soil source for our pot experiments, it was not surprising that the non-sterilized soil contained no suitable rhizobia strains for chickpea infection. The inoculation with rhizobia ("Radicin®"), however, induced nodulation with substantially better results in 2006 and in 2007.

5.1.2 Mycorrhiza and N nutrition effects on chickpea

AMF colonization caused substantial growth improvement by up to +43% total dry matter at maturity as compared to the non-inoculated control. Positive effects of AMF inoculation on chickpea and other legume species have been explained by an enhancement of root hair length and mycorrhizal mycelium length at early stages of plant development (Weber et al., 1993; Schweiger et al., 1995). The present results confirm a positive effect on root growth, although the growth promotion on shoots in general was more pronounced.

AMF are known to be effective in increasing nutrient uptake, particularly phosphorus, and biomass accumulation of many crops in soils low in phosphorus (Turk et al., 2006) or soils that fix phosphorus due to a high concentration of calcium and high pH values. In those situations, organic acids produced by AMF may partially explain enhanced nutrient uptake by the roots of mycorrhizal plants (Sharif and Moawad, 2006). The soil material in the present study was not low in plant available phosphorus, however of an elevated pH (cf. Tab. 3.1).

AMF inoculation increased the available soil NO_3-N content at maturity in sterilized soil in 2007 under chickpea. This might be explained because AMF plants have access to N pools in the soil which are not equally available to non-mycorrhizal plants (Ames et al., 1984), thus saving plant available soil N.

In contrast with the AMF treatment, we found no significant growth or N acquisition enhancement due to rhizobial infection. As also mineral N fertilization of chickpea did not improve dry matter production, N was obviously sufficiently available in control pots and not growth limiting.

After inoculation, nodulation of chickpea varied with soil sterilization and between years. Nodules were not always observed. When they were present their number often exceeded 10 per plant, but most of them were of small size and apparently ineffective. This suggests that compatible, effective rhizobia were not present in the inoculum product or their environmental demands were not fulfilled. *Bradyrhizobium* strains that infect chickpea are specific and rarely exist especially in soil where chickpea were not grown before (El Hadi and Elsheikh, 1999; Date, 2000). Aouani et al. (2001) showed that some rhizobial species were able to nodulate chickpea, but the symbiosis they formed took a longer time to establish than when formed with specific mesorhizobia, and was ineffective, which indicated that they were not really compatible. Ineffective or hardly effective cross nodulation is sometimes reported for other legumes (Mutch and Young, 2004;

Nandasena et al., 2004). It is evident that there was hardly any cross-inoculation between nodulating rhizobia of *C. arietinum* and those infecting other legumes. Only strains of *Sesbania-Rhizobium* were found to be able to form nodules on *C. arietinum* (Gaur and Sen, 1979).

Although some minor interaction effects between AMF and N nutrition were significant, they were not consistent across experimental units and thus cannot be generalized.

5.1.3 Effects of soil sterilization

The additional colonization potential of the natural AMF population present in non-sterilized soil resulted in higher colonization rates, but did not additionally improve dry matter production. This result indicates that native AMF was less effective to promote plant growth than the commercial product “Symbivit®”. On the contrary, other authors had observed substantial competition of the indigenous microflora in non-sterilized soil, which greatly reduced the efficacy of applied mycorrhizal inoculum (Abbott and Robson 1981; Ibijbijen et al., 1996; Biró et al., 2000). This is not confirmed by our results.

5.1.4 Comparing chickpea with barley

Barley produced substantially more biomass than chickpea, which was presumably biased by the different densities of ten barley plants as compared to three chickpea plants per pot. The density chosen for chickpea had been based on a much stronger growth of individual plants in previous field experiments. Improved plant growth of chickpea and barley was observed following inoculation with AMF. These positive effects were accompanied by variable percentages of root colonization in years or soil and crop types.

Our findings of relatively high levels of AMF root colonization in chickpea compared with barley differ from a study on the same plant species by Sharif and Moawad (2006). Although colonization levels of barley were generally lower, the fostering of growth was even slightly more pronounced than with chickpea. Chaurasia and Khare (2005) also found more growth promotion with barley as compared to legume species but in their study also barley root colonization was higher, while Zhu et al. (2000) showed less colonization of grass compared with clover roots.

It seems that barley was more efficiently supported by mycorrhiza than chickpea because after inoculation with AMF it produced finally additional ear dry matter, while no additional enhancement of chickpea pod yield was observed. This finding is in agreement with Weber et al. (1993) who reported that 'high mycorrhizal' chickpea plants at maturity had a lower harvest index compared to 'low mycorrhizal' plants, and tended to give less seed yield despite greater shoot biomass. They hypothesized that the colonization with AMF improved P uptake and growth of chickpea early in the season thereby increasing water stress during seed development. But water shortage must not be suspected in our pot trials.

5.2 Arbuscular mycrorrhizal and nitrogen fertilizer effects on concentrations and uptakes of macro and micro nutrients in chickpea and barley

5.2.1 Mycorrhiza effects on chickpea

The fungal mycelium in soil can absorb nutrients beyond the zone depleted through root uptake (Toro et al., 1997; Ruiz-Lozano et al., 2001; Bi et al., 2003; Rohyadi et al., 2004; Smith and Read, 2008). Positive effects were explained by increased root lengths, coupled with the extra soil volume that can be exploited by hyphae (Daft, 1991; Turk et al., 2006). As reported above, our study confirms a positive effect on root growth after successful inoculation of chickpea with AMF, but the growth promotion on shoots in general was more pronounced (cf. Appendix ANOVA Tab.1).

In the present study in chickpea, AMF colonization significantly reduced the N concentrations compared to the control irrespective of the N supply level (Tab. 4.5). This might be attributed to a dilution effect due to higher dry matter production in the AM treatment (cf. Fig. 4.1). This result is in accordance with those of other studies (Hirata et al., 1988; Gavito et al., 2000). However, it must be emphasized that plant N in our experiments originated only from the soil medium and not from rhizobial N fixation.

On contrary, under sterilized soil conditions we found in chickpea a positive impact of AM colonization on the P, K, Fe, Mn and Cu concentrations, partly depending on the year (Tab. 4.5). This is even more remarkable due to the simultaneous increase in plant biomass (cf. part 4.1.2). Our results are in accordance with those of previous work on chickpea by Akhtar and Siddiqui (2007 cf. Tab. 1.2) showing enhancement levels of the P and K concentrations in mycorrhizal plants. Other experiments on nutrient acquisition of mycorrhizal chickpea (Hirata et al., 1988) and pea (Gavito et al., 2000) under sterile soil conditions and high soil P levels have shown that the P and K concentrations were not increased. The soil material in the present study showed moderate (in 2006) to high (in 2007) P, K and Mg concentrations (cf. Tab. 3.1). Ibijbijen et al. (1996) showed the significant differences of the responses of P and K concentration to mycorrhizal inoculation between AMF species and bean varieties.

In chickpea plants grown on the sterilized soil our analyses also revealed a negative effect of AMF colonization on the Ca concentration and no effect on the Mg concentration compared to non colonized plants. Several previous studies have reported inconsistent results regarding Ca and Mg benefits of different species due to mycorrhizal colonization in combination with various soil pH or initial P values (Clark, 1997; Alloush et al., 2000; Bagayoko et al., 2000). However, it has been suggested that in mycorrhizal plants it is favorable to maintain low Ca concentrations as the presence of Ca-loaded polyphosphates possibly could harm the functioning of the arbuscules (Marschner and Dell, 1994).

The elevated concentrations of Fe and Mn that we found in the mycorrhizal plants resulted mainly from very high concentrations in the root fraction (data not shown). Extremely high Fe concentrations in root material may be explained by soil particles that were not removed completely. But this is unlikely because roots were washed thoroughly. The high Fe and Mn concentration in our study are in line with Nogueira et al. (2007). Improved plant root development and acquisition of P may be involved in enhanced Fe acquisition by mycorrhizal plants (Clark and Zeto, 1996). Enhanced concentration of Mn in AMF plants has commonly been reported (Al-Karaki and Clark, 1999; Bi et al., 2003). The benefits of AMF on Fe and Mn acquirement varied among AMF genotypes and inoculation rate, different levels of soil nutrient supply and plant species (Clark and Zeto, 1996; El-Ghandour et al., 1996; Al-Karaki and Al-Raddad, 1997; Caris et al., 1998; Liu et al., 2000).

No consistent variation of the Mg and Zn concentrations was observed after mycorrhizal inoculation. Eventual reductions could be related to increases in dry matter and thus reflect dilution effects (Bagayoko et al., 2000; Liu et al., 2000).

A mycorrhizal effect on chickpea was also observed when looking at the nutrient uptake (Tab. 4.6). We found an increase of the P, K, Mg, Fe, Mn, Zn and Cu uptake after AM inoculation in sterilized soil partly as a reflection of differences in dry matter. Only changes in Ca and N uptakes in AM chickpea were relatively small. Positive effects of AMF on nutrient uptake in legumes had been reported earlier (El-Ghandour et al., 1996; Zaidi et al., 2003; Ilbas and Sahin, 2005; Lin et al., 2007).

The higher P, K, Mg, Fe, Mn, Zn and Cu uptake of the mycorrhizal chickpea plants in parallel with the lack of difference in N uptake of mycorrhizal and non-mycorrhizal plants in a growth substrate with adequate N levels might indicate an improving effect of AMF on dry matter by increasing the uptake of nutrients other than N (Jia et al., 2004). They showed no effect on N uptake but increasing level of the P uptake in mycorrhizal broad bean plants.

5.2.2 Effects of soil sterilization

Mycorrhizal effects can differ largely between sterile or non-sterile soils. Competition between inoculated AMF, indigenous mycorrhiza and other soil microorganism in non-sterilized soil may affect mycorrhizal effects (Abbott and Robson, 1981; Baas, 1990). We found with chickpea across both soils, sterilized and non-sterilized, that inoculated AMF significantly enhanced the Mg and Mn concentration. AMF inoculation resulted in an increased P concentration in the sterilized soil as compared to the non-sterilized soil. The enhancement of the P concentration in non-inoculated chickpea plants grown in non-sterilized soil vs. sterilized soil, 1.35 vs. 0.98 g kg^{-1} (cf. Tab. 4.7, p=0.02), indicates that the indigenous mycorrhiza was as effective as inoculated mycorrhiza when looking at the P concentration or that other microorganisms present in the non-sterilized soil influenced either the nutrient solubility or the indigenous mycorrhiza function (Toro et al., 1997; Mukherjee and Rai, 2000; Khan and Zaidi, 2006; Zaidi and Khan, 2006 and 2007). In other studies mycorrhizal chickpea accumulated considerably greater P and N concentrations in combined inoculations (*Rhizobium* sp., *Pseudomonas striata* and *Glomus fasciculatum*) in comparison to single inoculation with *Glomus fasciculatum*, Zaidi et al., 2003).

While the K, N, Ca, Zn, Cu and Fe concentrations remained unaffected by AMF inoculation, the subsequent increases in uptake of these nutrients reflect the enhancing effect of AMF on dry matter production.

The colonization potential of the indigenous AMF population present in non-sterilized soil resulted in successful root colonization (18%) and substantially increased the P concentration in the non-inoculated plants. However, the presence of the indigenous AM did not preclude a positive response to AM inoculation in P uptake, and dry matter production was not improved beyond the levels achieved by artificial inoculation (Fig 4.1). Similarly, Singh and Tilak (1989) found in chickpea under non-sterilized soil that indigenous mycorrhizal fungi enhanced root colonization and nutrient concentrations more than dry matter in chickpea. Root colonization by AM can significantly improve the P uptake per unit root length (Bagayoko et al., 2000) due to the enhancement of the root absorption surface by hyphal growth (Li et al., 1991). Biró et al. (2000) found that in alfalfa, when an AM strain (*G. fasciculatum* M 107) was inoculated, it was more effective in the N, P and K uptake if indigenous AMF were excluded. However, it was frequently reported that the presence of indigenous AM does not preclude a positive response to AM inoculation (Smith and Smith, 1981; Barea et al., 1987; Ortas, 2003). With regard to differences in plant growth response to indigenous AMF vs. commercial inoculant treatments, it has been suggested that for traditional cultivars adequate management of the indigenous AMF to increase the inoculum potential of the soil, rather than artificial inoculation, is the best strategy to improve yield. Contrastingly, modern, high-input agriculture cultivars are not adapted to the indigenous

AMF, thus inoculation may be beneficial, especially when used in low-input agriculture (Quilambo et al., 2005).

5.2.3 Comparing chickpea with barley

One of the major factors that determine potential benefits from mycorrhizal inoculation is crop species (Owusu-Bennoah and Mosse, 1979; Caris et al., 1998; Nwoko and Sanginga, 1999; Bagayoko et al., 2000). The response of plant species to AMF when looking at nutrient concentration and growth differs also depending on the mycorrhizal isolate (Jensen, 1982; Rohyadi et al., 2004) and crop cultivar (Zhu et al., 2003). Differences in responsiveness to AMF between crop species or even cultivars could also be related to plant factors such as root structure. E.g. plant species with coarser, less hairy roots like legumes are more responsive to AM fungi than crops like cereals with an extensive fine root system (Al-Raddad, 1991; Nwoko and Sanginga, 1999; Bagayoko et al., 2000). Al-Raddad (1991) showed that broad bean plants have few fine roots and depend to some degree on the network of AM hyphae which acts as a bridge for conveying nutrients from the soil solution to plant cells. This is in agreement with the data presented by Bagayoko et al. (2000) who showed that cowpea was more dependent on AMF inoculation to acquire nutrients in comparison to millet, because of its coarser rooting system. Millet due to its high root length density appeared to have even a better spatial access to nutrients in soil than mycorrhizal cowpea.

In contrast with our results, in a field study with barley and a low availability of P it was found that the infection by indigenous AMF increased concentration of P but did not result in an increased growth (Jensen, 1983). Mendoza and Borie (1998), however, reported both an increased P concentration and an increased yield in mycorrhizal barley.

The higher nutrient uptake but lower or unaffected nutrient concentrations due to AMF inoculation in barley are certainly due to dilution effects given the much larger dry matter after AMF inoculation. Other studies confirm our findings that inoculation with AMF is effective in increasing P, Mn, Zn and Cu uptake of barley (Jensen, 1982; Al-Karaki and Clark, 1999; Mohammad et al., 2003; cf. Tab. 1.2).

Although colonization levels of barley (on average 18%) were generally lower than those of chickpea (43%), the fostering of growth was even slightly more pronounced than with chickpea (Tab. 4.1 and Fig. 4.1). Al-Karaki and Al-Raddad (1997) also found that strongly colonized roots did not result in higher nutrient uptake. Crops sometimes benefit from symbionts with little correlation to the degree of root colonization (Al-Raddad, 1991).

Differences in the nutrient acquisition by AMF between legumes and cereal species may be not only due to various ability of their root system to take up nutrient from the soil (see above) but also because of a different response to nutrient deficiency (Caris et al., 1998; Bagayoko et al., 2000; cf. Tab. 1.2; Gunes and Inal, 2008). Gamper et al. (2004) showed in well-fertilized agricultural ecosystems, that grasses benefit from improved N nutrition and legumes benefit from increased protection against pathogens and/or herbivores. This is different from what is expected in nutritionally limited plant communities (Nwoko and Sanginga, 1999). It has been commonly observed that nutritional benefits from AMF under P-limited soil conditions are particularly important in the production of leguminous crops since N_2-fixing legumes have high requirements for phosphorus and iron (Robson et al., 1981; Jakobsen, 1985; Israel, 1987 and 1993; Cadisch et al., 1989; Thomson et al., 1992; Yahiya and Samiullah Fatma 1995; Yang, 1995; El-Ghandour et al., 1996; Wall et al., 2000; Miao et al., 2007).

In our study we observed that AMF colonization influenced the growth of mycorrhizal chickpea and barley differently. Chickpea plants benefit from AMF via increased nutrient concentrations while mycorrhizal inoculation of barley improved nutrient uptake in parallel with enhanced growth.

5.2.4 Interaction effects between AMF inoculation and nitrogen fertilizer

Due to the application of N fertilizer, either ammonium or nitrate, variations in the nutrient movement between AMF and associated plants have been identified (Liu et al., 2000; Ortas and Rowell, 2004; Cornejo et al., 2008), but so far underlying mechanisms were not revealed in detail.

In our study interactions between AMF inoculation and nitrogen nutrition were only rare. We found that N application significantly reduced the positive mycorrhizal effect on the K concentration of chickpea only in sterilized soil (unit I), but the mycorrhizal effect on K concentration was small and inconsistent in years. In our study N fertilizer added to sterilized soil suppressed root dry matter (Fig 4.2) while N application did not influence AMF colonization and total dry matter of plant. Azcón et al. (2003) observed that the roots of plants grown at high N supply had less extraradical hyphae and less absorbtion potential for nutrients than plants grown in a soil with strongly developped extraradical hyphae at less available N.

On the other hand; AMF colonization inhibited the positive effect of N application on the Ca concentration across all experimental units (cf. Tab. 4.11). This might also be explained by a dilution effect due to more dry matter after mycorrhizal inoculation. Azcón et al. (2003) also found an increased Ca concentration in non-mycorrhizal plants in contrast to the Ca reduction in mycorrhizal plants when increasing N rates were applied.

6 Conclusions

The percentage of AMF colonized roots was markedly affected by environment, crop species and sterilization of soil. However, AMF inoculation of chickpea and barley consistently led to AM root colonization and subsequently enhanced dry matter production. This growth stimulation was hardly related to nitrogen nutrition, since nitrogen was presumably not a growth limiting factor even in control pots (N-R-) of our experiments. Native AMF in non-sterilized soil were less effective to promote plant growth than the commercial product “Symbivit®” and did not reduce the efficacy of applied mycorrhizal inoculum.

Inoculated rhizobia were able to nodulate chickpea, but most nodules were of small size and apparently ineffective. Thus, no increase of growth or N acquisition after rhizobial inoculation was observed. This suggests that compatible, effective rhizobia were not present in the inoculum product or their environmental demands were not fulfilled.

In all our experiments a moderate level of AMF colonization had a supporting effect on the macro and micro nutrient uptake of chickpea and barley plants. This effect may be due to both enhanced nutrient concentrations and improved growth of mycorrhizal plants. In many cases the nutrient concentrations were also higher, despite the simultaneous increase in plant biomass. A supplement N supply was of minor importance for these effects. From the results obtained in the present study, it can be concluded that both indigenous and inoculated AMF have positive effects on nutrient status of chickpea and barley plants grown under moderate soil nutrient supply. AMF inoculation caused a better response in chickpea than in barley when looking at the nutrient concentration, but in barley the dry matter production was more efficiently supported by mycorrhiza than in chickpea.

7 Outlook

Adequate selection of rhizobia strains is an important agronomic task to enhance legume grain yield. Chickpea as a legume has shown increased yield under symbiosis with effective rhizobia. However, if chickpea shall become a more widely used crop in Austria, introducing efficient rhizobia strains is a main challenge. It should be clarified, which rhizobia strains are compatible with chickpea under prevalent environmental conditions and able to enhance grain yield. Additionally it would be useful to explore the effect of dual inoculation with mycorrhiza and rhizobia on chickpea in the field.

We think that our study has provided some elements and ideas for further efforts to study the soil indigenous mycorrhiza. Furthermore, phenological effects of AMF have not been studied in detail. The beneficial potential of indigenous mycorrhiza could be cleared via amplification of local populations with subsequent re-inoculation in different density levels.

In certain cases, previous inoculation with one of the endophytes (such as rhizobia and mycorrhiza) can depress the development of the other. The competitive advantage of mycorrhiza vs. rhizobia could be investigated via co-inoculation with different inoculation dates or densities.

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11 Appendix

11.1 ANOVA tables

ANOVA tables of four experimental units (I-IIIb), as affected by AMF inoculation (M, with or without), nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+, only for chickpea) in interaction with year (Y, 2006 or 2007), soil sterilization (So, with or without sterilization), crop species (Sp, chickpea and barley) and harvest date (H, flowering or physiological maturity)

Table 1: Analysis of variance of the data on dry matter of crops, divided into fractions (root, straw/shoot and pod), percentage of AMF colonized roots, rhizobial nodule dry weight and number and soil NO_3-N (experimental unit I, chickpea in sterilized soil)

Source of variation	Root	Straw/Shoot	Pod	Total	AMF colonization	Nodule dry weight	Nodule number	soil NO_3-N
	Dry matter (g pot^{-1})				(%)	(mg pot^{-1})		(mg kg^{-1})
M	ns	**	*	**	***	ns	ns	ns
N	***	ns	ns	ns	ns	***	***	***
H	***	***	–	***	ns	ns	ns	ns
Y	***	*	***	***	***	***	***	**
M*N	ns	ns	*	ns	ns	ns	ns	ns
M*H	ns	ns	–	ns	*	ns	ns	ns
M*Y	ns	ns	ns	ns	***	ns	ns	**
N*H	ns	ns	–	ns	ns	ns	ns	ns
N*Y	ns	ns	ns	ns	ns	***	***	ns
H*Y	ns	***	–	ns	**	ns	ns	ns
M*N*H	ns	ns	–	ns	ns	ns	ns	ns
M*N*Y	ns	ns	ns	ns	ns	ns	ns	ns
M*H*Y	*	**	–	**	**	ns	ns	ns
N*H*Y	ns	ns	–	ns	ns	ns	ns	ns
M*N*H*Y	ns	ns	–	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant, – means there is not effect.

Table 2: Analysis of variance of the data on dry matter of crops, divided into fractions (root, straw/shoot and pod), percentage of AMF colonized roots, rhizobial nodule dry weight and number and soil NO_3-N (experimental unit II, chickpea in 2007)

Source of variation	Root	Straw/Shoot	Pod	Total	AMF colonization	Nodule dry weight	Nodule number	soil NO_3-N
	Dry matter (g pot^{-1})				(%)	(mg pot^{-1})		(mg kg^{-1})
M	**	***	ns	***	***	ns	ns	***
N	ns	ns	ns	ns	ns	**	***	***
H	ns	ns	–	***	***	ns	*	ns
So	ns	***	*	***	***	*	**	***
M*N	ns	ns	ns	ns	ns	ns	ns	ns
M*H	*	**	–	**	**	ns	ns	ns
M*So	ns	ns	ns	ns	ns	ns	ns	***
N*H	ns	ns	–	ns	ns	ns	**	ns
N*So	*	ns	ns	*	ns	**	***	ns
H*So	ns	ns	–	ns	ns	ns	ns	*
M*N*H	ns	ns	–	ns	ns	ns	ns	ns
M*N*So	ns	ns	ns	ns	ns	ns	ns	ns
M*H*So	ns	ns	–	ns	ns	ns	ns	ns
N*H*So	ns	ns	–	ns	ns	ns	*	ns
M*N*H*So	ns	ns	–	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant, – means there is not effect.

Table 3: Analysis of variance of the data on dry matter of crops, divided into fractions (root, straw/shoot and pod/ear), percentage of AMF colonized roots and soil NO_3-N (experimental unit IIIa, sterilized soil in 2006)

Source of variation	Root	Straw/Shoot	Pod/Ear	Total	AMF colonization	soil NO_3-N
	Dry matter (g pot^{-1})				(%)	(mg kg^{-1})
M	ns	ns	ns	ns	***	ns
N	ns	***	**	***	ns	***
H	ns	***	–	*	ns	ns
Sp	***	***	***	***	***	***
M*N	*	ns	ns	ns	ns	ns
M*H	ns	ns	–	ns	ns	ns
M*Sp	ns	ns	ns	ns	***	ns
N*H	ns	*	–	ns	*	ns
N*Sp	**	***	**	***	ns	***
H*Sp	*	***	–	ns	**	ns
M*N*H	ns	ns	–	ns	*	ns
M*N*Sp	ns	ns	ns	ns	ns	ns
M*H*Sp	ns	ns	–	ns	*	ns
N*H*Sp	ns	**	–	ns	ns	ns
M*N*H*Sp	ns	ns	–	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant, – means there is not effect.

Table 4: Analysis of variance of the data on dry matter of crops, divided into fractions (root, straw/shoot and pod/ear), percentage of AMF colonized roots and soil NO_3-N (experimental unit IIIb, non-sterilized soil in 2007)

Source of variation	Root	Straw/Shoot	Pod/Ear	Total	AMF colonization	soil NO_3-N
	Dry matter (g pot^{-1})				(%)	(mg kg^{-1})
M	*	***	**	***	***	ns
N	***	***	*	***	ns	***
H	ns	***	–	***	***	ns
Sp	***	***	***	***	***	ns
M*N	ns	ns	ns	ns	ns	ns
M*H	ns	ns	–	ns	ns	ns
M*Sp	ns	ns	*	ns	**	ns
N*H	**	ns	–	**	ns	*
N*Sp	***	**	**	***	ns	ns
H*Sp	ns	***	–	ns	**	*
M*N*H	ns	ns	–	ns	ns	ns
M*N*Sp	ns	ns	ns	ns	ns	ns
M*H*Sp	ns	*	–	ns	ns	ns
N*H*Sp	ns	ns	–	ns	ns	ns
M*N*H*Sp	ns	ns	–	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant, – means there is not effect.

Table 5: Analysis of variance of the data on N concentration and N uptake in total chickpea biomass (experimental unit I, chickpea in sterilized soil)

Source of variation	N concentration (g kg^{-1})	N uptake (mg pot^{-1})
M	**	*
N	***	**
H	**	***
Y	***	***
M*N	ns	ns
M*H	ns	ns
M*Y	ns	**
N*H	ns	ns
N*Y	ns	ns
H*Y	ns	**
M*N*H	ns	ns
M*N*Y	ns	ns
M*H*Y	ns	***
N*H*Y	ns	ns
M*N*H*Y	ns	*

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 6: Analysis of variance of the data on N concentration and N uptake in total chickpea biomass (experimental unit II, chickpea in 2007)

Source of variation	N concentration (g kg^{-1})	N uptake (mg pot^{-1})
M	ns	***
N	***	***
H	***	***
So	***	***
M*N	ns	ns
M*H	ns	***
M*So	ns	ns
N*H	ns	ns
N*So	ns	*
H*So	ns	***
M*N*H	ns	ns
M*N*So	ns	ns
M*H*So	ns	*
N*H*So	ns	*
M*N*H*So	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 7: Analysis of variance of the data on N concentration and N uptake in total chickpea and barley biomass (experimental unit IIIa, sterilized soil in 2006)

Source of variation	N concentration (g kg^{-1})	N uptake (mg pot^{-1})
M	ns	ns
N	***	***
H	ns	**
Sp	***	***
M*N	ns	ns
M*H	ns	ns
M*Sp	ns	ns
N*H	*	ns
N*Sp	**	**
H*Sp	**	*
M*N*H	ns	ns
M*N*Sp	ns	ns
M*H*Sp	ns	ns
N*H*Sp	ns	ns
M*N*H*Sp	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 8: Analysis of variance of the data on N concentration and N uptake in total chickpea and barley biomass (experimental unit IIIb, non-sterilized soil in 2007)

Source of variation	N concentration (g kg^{-1})	N uptake (mg pot^{-1})
M	ns	***
N	***	***
H	***	**
Sp	***	***
M*N	ns	ns
M*H	ns	ns
M*Sp	ns	*
N*H	ns	*
N*Sp	ns	***
H*Sp	ns	***
M*N*H	ns	ns
M*N*Sp	ns	ns
M*H*Sp	ns	ns
N*H*Sp	ns	ns
M*N*H*Sp	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

ANOVA tables of three experimental units (I-III), as affected by AMF inoculation (M, with or without) and nitrogen fertilization (N, with or without) in interaction with year (Y, 2006 or 2007), soil sterilization (So, with or without sterilization), crop species (Sp, chickpea and barley) at the physiological maturity.

Table 9: Analysis of variance of the data on nutrient concentration in total chickpea biomass (exp. unit I, sterilized soil)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	($g\ kg^{-1}$)					($mg\ kg^{-1}$)			
M	*	***	**	*	ns	*	***	ns	ns
N	**	ns	ns	ns	ns	**	ns	***	ns
Y	***	ns	ns	ns	ns	ns	***	**	***
M*N	ns	ns	*	*	ns	ns	ns	ns	ns
M*Y	ns	ns	**	***	ns	*	*	ns	*
N*Y	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*N*Y	ns	ns	ns	ns	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 10: Analysis of variance of the data on nutrient uptake in total chickpea biomass (exp. unit I, sterilized soil)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	($mg\ pot^{-1}$)					($\mu g\ pot^{-1}$)			
M	ns	***	***	ns	*	***	***	**	**
N	ns	ns	ns	ns	ns	**	ns	*	ns
Y	**	*	***	ns	ns	ns	ns	***	ns
M*N	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*Y	ns	ns	ns	**	ns	ns	*	ns	ns
N*Y	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*N*Y	ns	ns	ns	ns	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 11: Analysis of variance of the data on nutrient concentration in total chickpea biomass (exp. unit II, 2007)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	(g kg^{-1})					(mg kg^{-1})			
M	ns	***	ns	ns	*	ns	***	ns	ns
N	**	ns	*	ns	ns	*	ns	**	**
So	***	*	***	ns	ns	***	***	ns	ns
M*N	ns	ns	ns	*	ns	ns	ns	ns	ns
M*So	ns	*	ns	ns	ns	ns	*	ns	ns
N*So	ns	ns	*	ns	ns	ns	ns	ns	ns
M*N*So	ns	ns	ns	ns	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 12: Analysis of variance of the data on nutrient uptake in total chickpea biomass (exp. unit II, 2007)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	(mg pot^{-1})					(µg pot^{-1})			
M	*	***	***	**	***	**	***	**	*
N	*	ns	ns	ns	ns	ns	ns	ns	ns
So	***	ns	**	ns	ns	*	***	ns	ns
M*N	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*So	ns	ns	ns	ns	ns	ns	*	ns	ns
N*So	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*N*So	ns	ns	ns	ns	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 13: Analysis of variance of the data on nutrient concentration in total chickpea and barley biomass (exp. unit III, non-sterilized soil, 2007)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	(g kg^{-1})					(mg kg^{-1})			
M	ns	ns	ns	ns	ns	ns	ns	ns	ns
N	***	ns	ns	ns	ns	ns	ns	ns	ns
Sp	***	***	***	***	***	***	***	***	***
M*N	ns	ns	ns	*	ns	ns	ns	ns	ns
M*Sp	ns	ns	ns	ns	*	ns	**	ns	ns
N*Sp	ns	ns	***	ns	ns	**	**	ns	**
M*N*Sp	ns	ns	ns	*	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

Table 14: Analysis of variance of the data on nutrient uptake in total chickpea and barley biomass (exp. unit III, non-sterilized soil, 2007)

Source of variation	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	(mg pot^{-1})					(µg pot^{-1})			
M	***	**	**	*	**	*	**	*	**
N	***	**	***	***	***	**	***	**	***
Sp	**	ns	***	***	ns	ns	ns	ns	*
M*N	ns	ns	ns	ns	ns	ns	ns	ns	ns
M*Sp	ns	ns	ns	ns	ns	ns	*	ns	ns
N*Sp	***	ns	***	ns	ns	*	**	*	***
M*N*Sp	ns	ns	ns	ns	ns	ns	ns	ns	ns

* ≤ 0.05, ** ≤ 0:01, *** ≤ 0.001, ns = not significant.

11.2 Additional results

11.2.1 Tables

Table A1: Recognized genera and species of legume root- and stem-nodule bacteria (Graham, 2008, modified)

Genera/species	Principal and other reported hosts	References
Allorhizobium		
A.. undicola	*Neptunia natans, Acacia, Faidherbia, Lotus*	de Lajudie et al., 1998a; considered within *Rhizobium* by Sawada et al., 2003
Azorhizobium		
A.. caulinodans	*Sesbania rostrata*	Dreyfus et al., 1988
A.. doebereinerae	*Sesbania virgata*	Moreira et al., 2006
Blastobacter		
B. denitrificans	*Aeschynomene indica*	van Berkum and Eardly, 2002; considered within *Bradyrhizobium* by van Berkum et al., 2006
Bradyrhizobium		
B. canariense	*Chamaecytisus, Lupinus*	Vinuesa et al., 2005a,b; Stepkowski et al., 2005
B. elkanii	*Glycine max*	Kuykendall et al., 1993
B. japonicum	*Glycine max*	Jordan, 1984
B. liaoningense	*Glycine max*	Xu et al., 1995
B. yuanmingense	*Lespedeza, Medicago, Melilotus*	Yao et al., 2002
Burkholderia		
B. caribensis	*Mimosa diplotricha, M. pudica*	Achouak et al., 1999; Vandamme et al., 2002
B. cepacia	*Alysicarpus glumaceus*	Vandamme et al., 2002
B. phymatum	*Machaerium lunatum, Mimosa*	Vandamme et al., 2002; Elliott et al., 2006
B. tuberum	*Aspalathus* spp.	Vandamme et al., 2002
Devosia		
D. neptuniae	*Neptunia natans*	Rivas et al., 2003
Ensifer		
E. adhaerens		Willems et al., 2003; considered within *Sinorhizobium* by Sawada et al., 2003

Mesorhizobium		
M. amorphae	*Amorpha fruticosa*	Wang et al., 1999, 2002a
M. chacoense	*Prosopis alba*	Velasquez et al., 2001
M. ciceri	*Cicer arietinum*	Nour et al., 1994
M. huakuii	*Astragalus sinicus, Acacia*	Chen et al., 1991; Jarvis et al., 1997
M. loti	*Lotus corniculatus*	Jarvis et al., 1982, 1997
M. mediterraneum	*Cicer arietinum*	Nour et al., 1995; Jarvis et al., 1997
M. plurifarium	*Acacia senegal, Prosopis juriflora, Leucaena*	de Lajudie et al., 1998b
M. septentrionale	*Astragalus adsurgens*	Gao et al., 2004
M. temperatum	*Astragalus adsurgens*	Gao et al., 2004
M. tianshanense	*Glycyrrhiza pallidiflora, Glycine, Caragana, Sophora*	Chen et al., 1995; Tan et al., 1997
Ralstonia (Cupriavidus)		
R. taiwanensis	*Mimosa*	Chen et al., 2001
Rhizobium		
R. etli	*Phaseolus vulgaris, Mimosa, affinis*	Segovia et al., 1993; Wang et al., 1999
R. galegae	*Galega orientalis, G. officinalis*	Lindstrom, 1989
R. gallicum	*P. vulgaris, Leucaena, Macroptilium, Onobrychis*	Amarger et al., 1997
R. giardinii	*P. vulgaris, Leucaena, Macroptilium, Desmanthus*	Amarger et al., 1997; Beyhaut et al., 2006
R. hainanense	*Desmodium sinuatum, Stylosanthes*, *Vigna, Arachis, Centrosema*	Chen et al., 1997
R. huautlense	*Sesbania herbacea*	Wang et al., 1998
R. indigoferae	*Indigofera*	Wei et al., 2002
R. leguminosarum		
biovar (bv) *ciceri*	*Cicer arietinum*	El Hadi and Elsheikh, 1999; El-Ghandour and Galal, 2002; Kantar et al., 2003
bv *trifolii*	*Trifolium*	Dangeard, 1926; Jordan, 1984
bv *viciae*	*Lathyrus, Lens, Pisum, Vicia*	Dangeard, 1926; Jordan, 1984
bv *phaseoli*	*P. vulgaris*	Dangeard, 1926; Jordan, 1984
R. loessense	*Astragalus, Lespedeza*	Wei et al., 2003
R. mongolense	*Medicago ruthenica, Phaseolus vulgaris*	van Berkum et al., 1998

R. sullae	*Hedysarum coronarium*	Squartini et al., 2002
R. tropici	*P. vulgaris, Dalea, Leucaena, Macroptilium, Onobrychis*	Martinez-Romero et al., 1991
R. yanglingense	*Amphicarpaea, Coronilla, Gueldenstaedtia*	Tan et al., 2001
Sinorhizobium		
S. abri	*Abrus precatorius*	Ogasawara et al., 2003
S. americanus	*Acacia spp.*	Toledo et al., 2003
S. arboris	*Acacia senegal, Prosopis chilensis*	Nick et al., 1999
S. fredii	*Glycine max*	Scholla and Elkan, 1984; Chen et al., 1988
S. indiaense	*Sesbania rostrata*	Ogasawara et al., 2003
S. kostiense	*Acacia senegal, Prosopis chilensis*	Nick et al., 1999
S. kummerowiae	*Kummerowia stipulacea*	Wei et al., 2002
S. medicae	*Medicago truncatula, M. polymorpha, M. orbicularis*	Rome et al., 1996
S. meliloti	*Medicago, Melilotus, Trigonella*	Dangeard, 1926; de Lajudie et al., 1994
S. morelense	*Leucaena leucocephala*	Wang et al., 2002b
S. saheli	*Acacia, Sesbania*	de Lajudie et al., 1994; Boivin et al., 1997
S. terangae	*Acacia, Sesbania*	de Lajudie et al., 1994; Lortet et al., 1996

11.2.2 Figures

Figure A1: N concentration of crops, as affected by treatments without (M-) or with (M+) AMF inoculation, nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+) in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated.

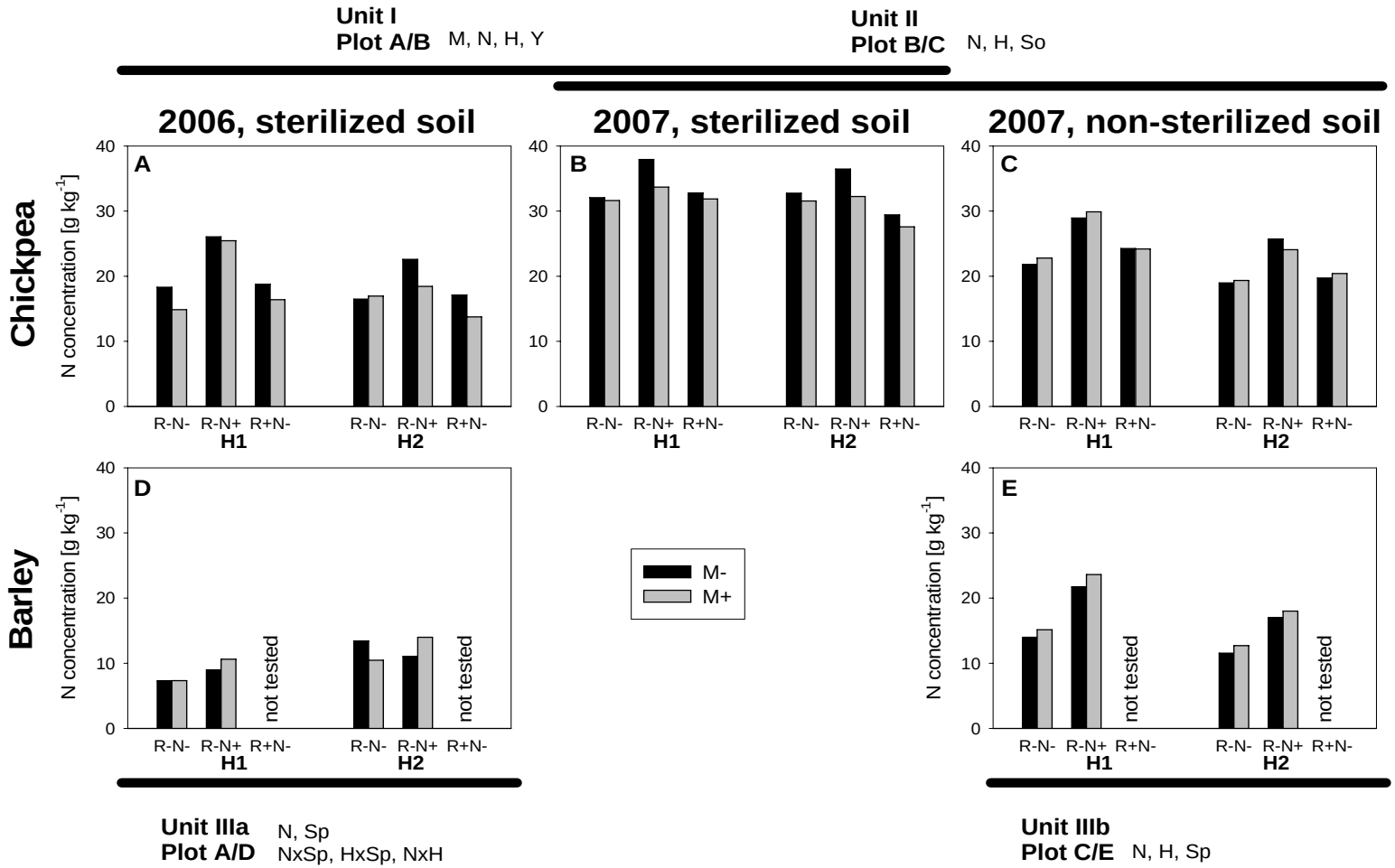
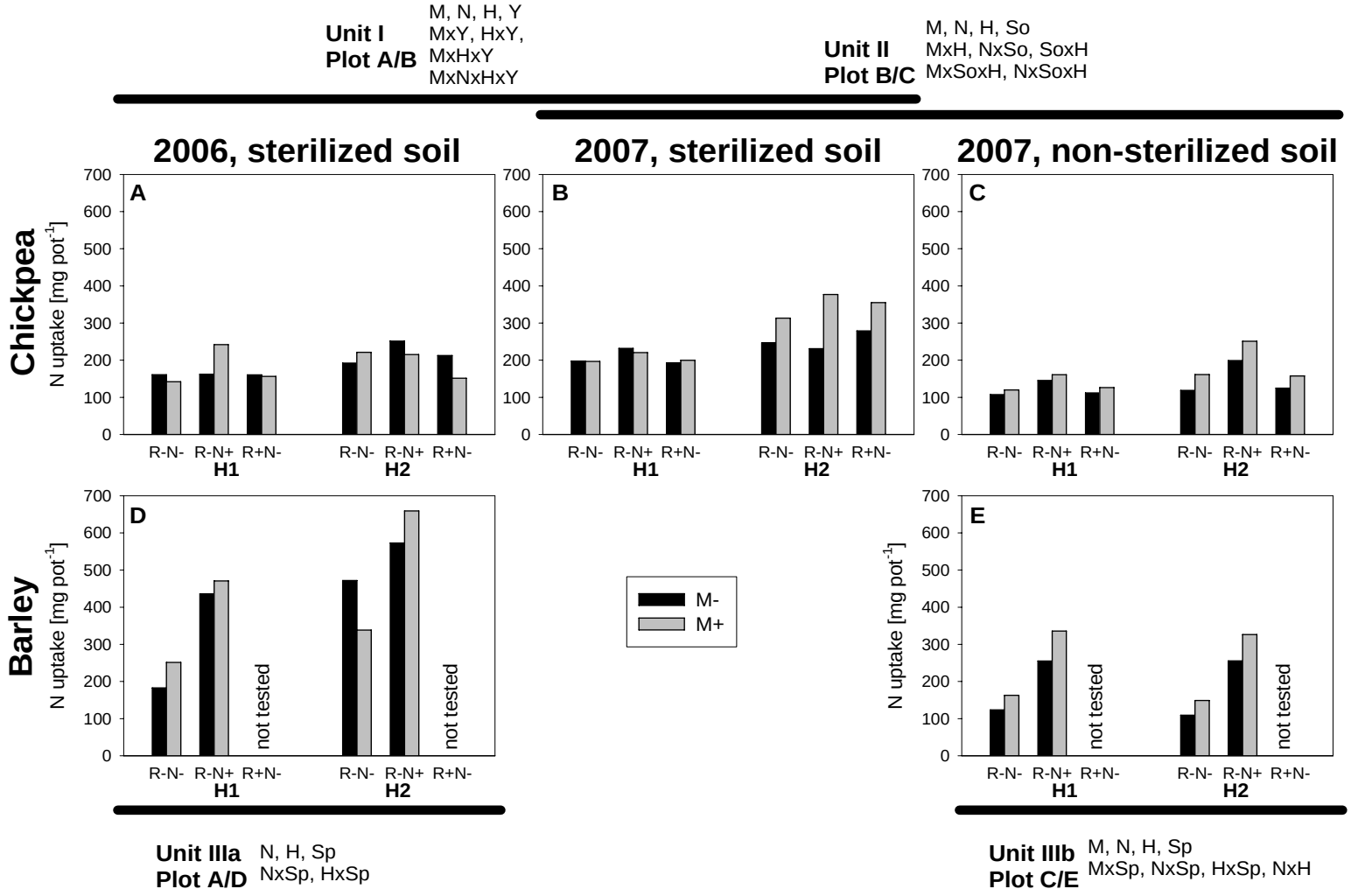


Figure A2: N concentration of crops, as affected by treatments without (M-) or with (M+) AMF inoculation, nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+) in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated.



12 Curriculum Vitae

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Education and Professional Experience

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Bachelor of Science: Study at the University of Ferdowsi Mashhad: Agronomy and Plant Production

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List of publications

2009

Farzaneh, M., S. Wichmann, H. Vierheilig, H.-P. Kaul (2009): The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley. Pflanzenbauwissenschaften, 13 (1), 15-22; ISSN 1431-8857

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Wichmann, S., M. Farzaneh, H.-P. Kaul (2007): Yield performance and N accumulation of chickpea (*Cicer arientinum* L.) compared to peas (*Pisum sativum* L.) in eastern Austria. In: Mendel University of Agriculture and Forestry Brno (Eds.), Faculty of Agronomy, Plant nutriton and its prospects, 5. - 6. September 2007, Brno, 119-121; ISBN: 978-80-7375-068-8

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