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The Influence of Singular and Combined Applications of Arbuscular Mycorrhizal Fungi and *Enterobacter radicincitans* on Growth and Nutrition of Plants

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Symbols and Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
AMF	Arbuscular mycorrhizal fungi
C_{mic}	Soil microbial biomass carbon
DL	Doppel-Lactat
FAO	Food and Agriculture Organization
G	*Glomus*
IFA	International Fertilizer Industry Association
K	Potassium
Mg	Magnesium
N	Nitrogen
P	Phosphorus
PGPR	Plant-growth-promoting rhizobacteria
PGRs	Plant growth regulators
rpm	Revolutions per minute
SIR	Substrate-induced respiration method
SOM	Soil organic matter
YIB	Yield-increasing bacteria

Abstract

Arbuscular mycorrhizal fungi (AMF) and plant-growth-promoting rhizobacteria (PGPR) can be beneficial to crop plants due to their nutrient acquisition properties and stimulation of plant growth. The present work focuses on the prospects of AMF (1) to solve plant nutritional problems and (2) to reduce the negative effects of chemical fertilizers on the environment due to reducing chemical inputs in agriculture.

The contributions of AMF and PGPR to plant nutritional problems were investigated with barley and faba bean plants in field and greenhouse conditions. Additionally, maize was investigated in greenhouse conditions. The effects of the singular and combined applications of the microbial inoculants were investigated in field and greenhouse conditions. To investigate the effects of the different fertilizers on the functions of the microbial inoculants, mineral and organic fertilizers were combined with AMF and/or with *E. radicincitans* in greenhouse conditions, and organic fertilizer was combined with AMF or with *E. radicincitans* in the barley experiment in field conditions. Grain yield, shoot dry weight, and N, P, K and Mg concentrations in the plants were measured. Also, soil basal respiration, soil biomass and the most probable number of P-solubilizing bacteria in the soil were measured. This was done since soil microbial parameters can be significant indicators of soil quality and nutritional status.

Plant inoculation with the microbial inoculants improved the plant yield nutrient status under the described experimental conditions; however, plant responses to the microbial inoculants were different between the field and greenhouse conditions and depending on the plant species. The effects of the addition of organic fertilizer on the functions of AMF and *E. radicincitans* were mostly related to the soil conditions (soil pH and nutrient content). Soil microbial analyses were generally affected by the singular inoculation or in combination with fertilizers, but the effect was also related to the plant species and to the type of fertilizer.

Kurzfassung

Arbuskuläre Mykorrhizapilze (AMF) und Rhizobakterien können das Wachstum und die Nährstoffaufnahme von Kulturpflanzen positiv beeinflussen. Dadurch können Düngemittel eingespart und wertvolle ressourcen gespart werden.

In mehreren Feld- und Gefäßversuchen wurden Einflüsse von AMF und dem Bakterium *Enterobacter radicicitans* auf das Wachstum und die Nährstoffaufnahme von Gerste, Mais und Ackerbohne untersucht. Hierbei wurden sowohl die Einzel- als auch die Kombinationswirkungen mit erfasst. Zudem wurden mineralische und organische Düngemittel mit den Mikroorganismen kombiniert.

Es wurde der Kornertrag, das Sprossgewicht und die Nährstoffaufnahme (N, P, K und Mg) der Pflanzen ermittelt. Im Boden wurden die Basalatmung, die mikrobielle Biomasse und die Anzahl P-lösender Bakterien gemessen. Diese mikrobiellen Parameter sind wesentliche Indikatoren für die Fruchtbarkeit des Bodens .

Die Inokulation der Pflanze mit den Mikroorganismen führte gewöhnlich zur einer Erhöhung derErtrages und des Nährstoff-Status der Pflanzen unter teikontrollierten Bedingungen und Feldbedingungen. Allerdings hing die Effektivität der Mikroorganismen von den kultivierten Pflanzen und den Versuchsbedingungen ab. So führten geringe pH-Werte des Bodens im Feldversuch zu einer Verringerung der Wirkung der Mikroorganismen. Deren Wirksamkeit erhöhte sich unter diesen Bedingungen, wenn sie zusammen mit einer organischen Düngung appliziert wurden.Ebenso wie die Pflanzenparamter wurden auch die bodenbiologischen Parameter durch die Applikation der Mikroorganismen und der Düngung beeinflusst.

Chapter 1

1 General Introduction and Literature Review

1.1 Introduction

The work in this thesis aimed to investigate the contributions of arbuscular mycorrhizal fungi (AMF) and the plant-growth-promoting rhizobacterial (PGPR) strain *Enterobacter radicincitans* DSM 16656 (*E. radicincitans*) to the yield and nutrient uptake of crop plants and to increase the potential of crop production through more efficient fertilization by inoculating crop plant seeds and young plants with beneficial AMF and *E. radicincitans* bacteria. These microbial inoculants have the potential to improve the sustainability of crop plant production by increasing yield and plant health and by consequently reducing input levels to achieve the same yield. Reducing external inputs lowers the harmful effects of agricultural chemicals, which are the main cause of many environmental problems such as eutrophication of water bodies by excessive applications of chemical fertilizers and the depletion of non-renewable resources. Increasing the quality of crops by increasing the nutritional situation also improves the sustainability of economical agricultural crops, thus achieving a secure and healthy food supply for the human population.

Intensive agricultural production requiring an excessive addition of chemical fertilizers may increase crop productivity but at the same time can cause extensive damage to ecosystems (Pimental et al. 1973; Montgomery 2007; Evans et al. 2011). The intensive application of fertilizers leads to the accumulation of nutrients in the upper layer of the soil (McDowell and Sharpley 2001), which increases the possibility of leaching and run-off of different nutrients such as N and P, leading to environmental pollution (Turtola and Kemppainen 1998; Kimmell et al. 2001; Kröger et al. 2009; Bertol et al. 2010).

The low use efficiency of fertilizers and their continuous long-term usage are the main reasons for the environmental problems mentioned above (Adesemoye et al. 2008). The rapid growth of the human population worldwide is creating a high demand on agricultural production to fulfil the food and nutritional gap. According to a United Nations report (2004), the world population was 6.1 billion in 2000, and it is expected to grow to 8.9 million in 2050. It is expected that the current food production will not be sufficient in the coming years, and therefore a quantum leap in agricultural production is required (Glick 2012). Because of the need to produce more food for the increasing world population, higher levels of agricultural production will lead to increased use of chemical fertilizers, in spite of the harmful effects on the environment (Donald et al. 2001; Townsend et al. 2003; Green et al. 2005; Kleijn et al. 2009).

According to the International Fertilizer Industry Association (IFA), the levels of N, P and K fertilizer use have increased enormously in the last five decades. It is reported that the three countries with the highest fertilizer use in 2007 were China, India and the USA, consuming 51.17, 22.58 and 19.54 million tons of NPK fertilizer, respectively, compared with consumption in 1961 of 1.01, 0.42 and 7.88 million tons, respectively (International Fertilizer Industry Association 2010). Many harmful effects of the use of mineral fertilizers on the environment were reported by the IFA (IFA 2000), including:

- Soil: nutrient depletion, soil degradation, soil acidity and soil erosion
- Water: ground water pollution and eutrophication
- Air: air pollution, which can be caused by the loss of nitrogen from agricultural systems, the depletion of ozone in the upper atmosphere and greenhouse gases

The challenge therefore is to continue agricultural productivity in a way that minimizes harmful environmental effects from chemical fertilizers (Adesemoye et al. 2009). To avoid more disadvantages to the environment, there is now a way to undertake safer agricultural production by using biofertilizers and organic fertilizers as complements to mineral fertilizers to improve production yield and quality (Abdelhamid et al. 2011).

Various definitions for sustainable agriculture have been proposed. Wolf and Snyder (2003) considered that agriculture is sustainable when there is an adequate production of agricultural products that could be enough for the present generation's requirements, does not damage the ecosystem by pollution, degradation, etc. and is able to provide adequate production for future generations. Lichtfouse et al. (2009) stated that "agricultural systems are considered to be sustainable if they sustain themselves over a long period of time, that is, if they are economically viable, environmentally safe and socially fair". This means that agricultural systems can be considered sustainable when the agricultural activities increase production yield and quality while decreasing chemical inputs (Welch and Graham 1999). It has been suggested that it could be possible to reduce chemical inputs worldwide without reducing food production by using organic farming systems (Hewlett and Melchett 2008).

Sustainable agricultural production could be especially interesting for developing countries, which have many problems (including agricultural production problems) (Regmi and Weber 2000).

Furthermore, nutrient poverty in soil produces poor nutrient density in grain crops, which have increasingly become an essential food since the Green Revolution (Cakmak et al. 1999). Consequently, many human diseases may be caused by nutrient deficiencies in soil (Rengel et al. 1999).

1.2 Role and Mechanisms of the Beneficial Microorganisms in Plant Growth and Nutrition

The application of beneficial microbes in agricultural production systems could have the potential of providing an integrated solution to the environmental problems resulting from chemical inputs into agricultural production systems, since these beneficial microorganisms are able to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Kirk et al. 2004; Araujo et al. 2012). According to Adesemoye and Kloepper (2009), these bio-inoculants can be categorized into three main groups: (1) AMF, (2) PGPR and (3) nitrogen-fixing rhizobia.

In the context of PGPR and AMF, several benefits can be achieved from applications of bio-inoculants in agricultural systems, such as increasing plant nutrition and growth when they are used as biofertilizers or as bio-control tools (Pandya and Saraf 2010; Hridya et al. 2012).

The advantages to plants of AMF application include many aspects, such as increased plant growth, nutrient uptake and water uptake (Khalvati et al. 2005; Neumann and George 2009; Ardakani and Mafakheri 2011) and facilitating water flow in roots under well-watered and drought conditions (Bárzana et al. 2012). An important benefit of AMF is increasing the available P in soil, since AMF have the ability to collect P in soil and then provide it to plants due to the mycorrhizal hypha net in the soil (Li et al. 1991; Kothari et al. 1991). On the other hand, high available P content in soil can negatively affect AMF due to the inhibited growth of the hypha net and spore production (Nagahashi et al. 1996; Tawaraya et al. 1996), which decreases the plant benefits from AMF (Grant et al. 2005; Stewart et al. 2005).

The second group is PGPR, which are considered beneficial since they have many positive effects on plants, such as increasing yield (Bashan et al. 2004, Turan 2010), root growth, root surface area and volume (Mia et al. 2012); nodulation and nitrogen fixation (Figueiredo et al. 2008); increasing iron availability (Vansuyt et al. 2007); the production of plant hormones, such as indole-3-acetic acid (IAA) (Bianco and Defez 2009), gibberellins (Kang et al. 2012), cytokinins and auxins (Ryu et el. 2003; Glick et al. 2007); tolerance to biotic and abiotic stresses (Timmusk and Wagner 1999; Bhattacharyya and Jha 2012); and providing bio-control tools against pathogens (Murphy et al. 2000; Hynes et al. 2008). Furthermore, some strains of PGPR are able to enhance phosphorus availability in the soil (Martínez-Viveros et al. 2010; Bhattacharyya and Jha 2012). These beneficial effects of PGPR result in several complex mechanisms (Dobbelaere et al. 2003). Some of these affect root growth and increase the growth of root hairs and hence increase the ability of the roots to access more nutrients (Mia et al. 2010; Mia et al. 2012).
The possible use of PGPR and AMF together as a mixture could be an important issue for sustainable agricultural systems. Jaizme-Vega et al. (2006) and Couillerot et al. (2012) found that the interaction between AMF and PGPR increased plant development. The dual inoculation can result in

higher yields (Dhillion 1992) and better nutrient acquisition compared to the singular application (Singh and Kapoor 1998). Yousefi et al. (2011) found that dry matter yield, the number of seed grain spikes and grain yield were increased after the combined application of AMF and P-solubilizing bacteria. The hypha net of the AMF can be used by the PGPR to access a wide area in the rhizosphere (Kim et al. 1998; Morrissey et al. 2004). Kim et al. (1998) suggested a synergistic interaction between AMF and PGPR after the combined application of *Glomus etunicatum* (AMF) and *E. radicincitans*. It was confirmed by Yasmeen et al. (2012) that the combined application of a mix of AMF and PGPR inoculants was more effective in crop production than the singular application of AMF or the bacteria.

1.3 Use of Beneficial Microorganisms as Biofertilizers

Sustainable agriculture is “ecologically sound, economically viable, socially just and humane” (Gips 1987). Sustainable agricultural systems management should involve appropriate methods to release soil minerals instead of adding them as synthetic compounds to reduce the external inputs, to maintain the soil biodiversity and to optimize the use of plant microbe interactions for the benefits of plant nutrition and countering pathogens (Edwards et al. 1990). Vessey (2003) defined a biofertilizer as a substance that contains living microorganisms that (when applied to seeds, plant surfaces or soil) colonize the rhizosphere or the internal tissue of a plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. The main sources of biofertilizers are PGPR, beneficial fungi such as AMF and *Penicillium bilaii*, and cyanobacteria (blue-green algae), which have long been known to have plant-growth-promoting effects by increasing the nutrient status of host plants. Beneficial microorganisms can be an important factor when used as biofertilizers to achieve sustainable agricultural systems. Since biofertilizers are considered to be environmentally friendly and because of their role in plant nutrition, the use of biofertilizers for sustainable agriculture has increased considerably in various parts of the world during the last few decades (International Plant Nutrition Institute (IPNI) 2011).

Various studies have demonstrated the positive influence of biofertilization on plant growth, development and yield (Singh and Prasad 2011). Significant increases in the growth and yield of agronomically important crops in response to inoculation with biofertilizers have been reported (Mia and Shamsuddin 2010). Moreover, AMF products are now commercially available as biofertilizers around the world (IPNI 2011).

Benefits from biofertilizers include:

- Increasing crop yield by 20–30%
- Replacing chemicals N and P by 25%
- Activating the soil biologically
- Restoring natural soil fertility
- Providing protection against drought and some soil-borne diseases (ICRISAT 2012)

A simplified methodology of using biofertilizers is presented in Fig. 1.

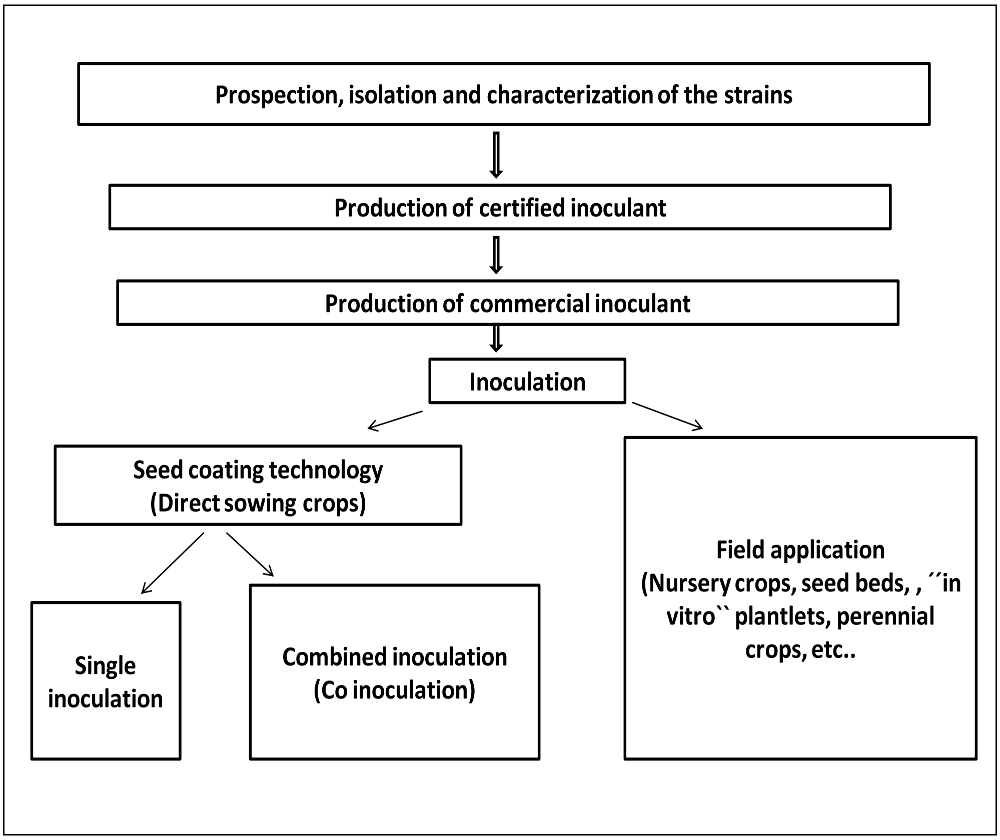

Fig. 1 General methodology for obtaining and using biofertilizers (Basso and Díaz 2004)

1.3.1 AMF: Characterization and Plant Response

AMF belong taxonomically to the phylum Glomeromycota (Schüssler et al. 2001) and can establish a symbiotic relationship with more than 80% of plant species (Wang and Qiu 2006).

Mycorrhiza forms a beneficial relationship with plant roots, which can provide plants with many benefits. Hence, AMF have become a tool for sustainable systems, which have important roles in natural ecosystems and could be considered as beneficial to humanity in terms of filling the shortage of food and achieving sustainability (Gianinazzi et al. 2010). The majority of crop plants form relationships with AMF, and their responsiveness to AMF depends on many factors, including genotype (Eason et al. 2001; An et al. 2010), plant population (Pánková et al. 2008), P supply rates (Stevens et al. 2002), soil properties (Douds et al. 1993) and chemical inputs into the agricultural system (Vosatka and Albrechtova 2009).

Some soils do not have sufficient nutrient levels; therefore, mineral and organic fertilizers are added as nutrient resources to fill the gap and to optimize crop productivity. However, the use of chemical inputs into agricultural systems cannot be sustainable for a long period of time (Khan et al. 2007).

AMF are considered to be an essential component of sustainable agroecosystems (Schreiner et al. 2003). As a sustainability tool, beside the aforementioned benefits, AMF can provide the following solutions to different environmental and agricultural production problems:

1- Increasing carbon sequestration in land ecosystems to stabilize the amount of atmospheric CO_2.
2- Acting as a soil reclamation tool, leading to sustainable agroecosystems.
3- Improving soil properties to resist increasing erosion and reducing the risks of water pollution and eutrophication.

The possible morphological effects of AMF on the rhizosphere are shown in Fig. 2.

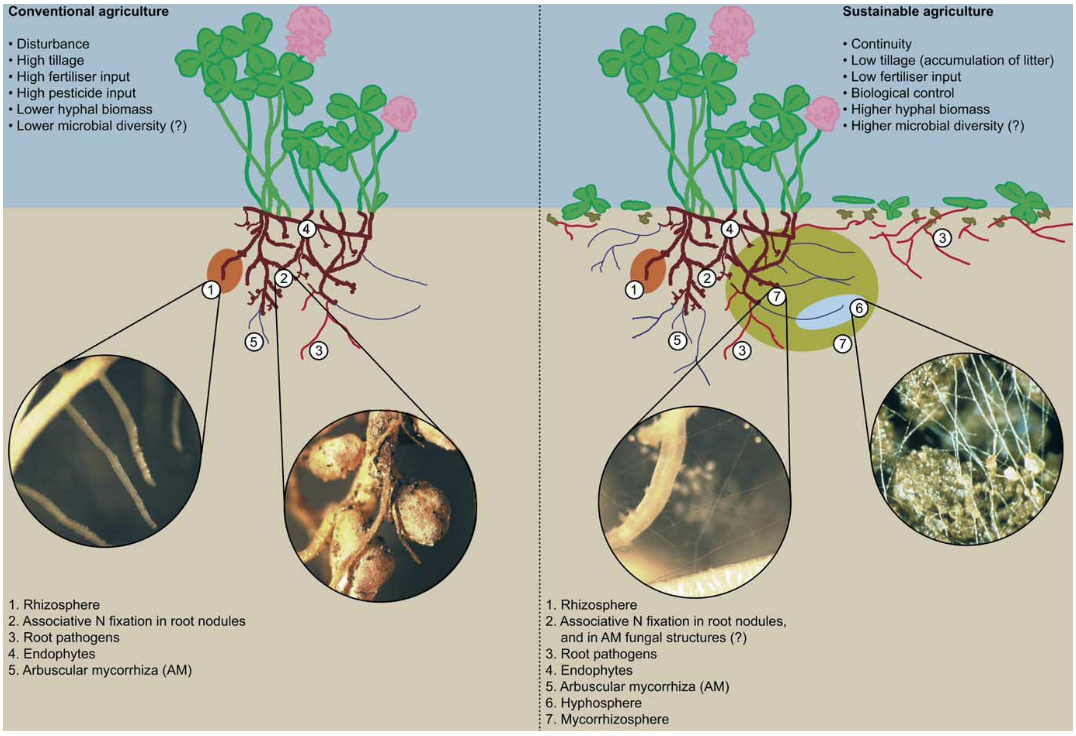

Fig. 2 View of the mycorrhizosphere in contrast to the rhizosphere: features of conventional agricultural soils and sustainably managed agricultural soils are indicated, with emphasis on mycorrhizosphere components and possible effects on them (Johansson et al. 2004)

Furthermore, in addition to AMF's ability to colonize plant roots, increase plant health and increase nutrient availability in soil (Dalpe and Monreal 2004; Lehnert et al. 2012), AMF can reabsorb the nutrients lost due to root exudation (Hamel 2004). AMF play an important role in reducing nutrient loss by leaching (Van der Heijden 2010; Asghari and Cavagnaro 2012). Several further benefits can be achieved by the application of AMF, such as affecting the soil fertility by the production of glomalin, thus accumulating organic matter and forming stable soil aggregates. Other benefits include providing protection against erosion (Bearden and Petersen 2000); enhancing seedling growth; improving the rooting of cuttings (Vosatka 1995); reducing chemical fertilizers and pesticides (Douds et al. 2007); increasing tolerance to biotic and abiotic stresses (Lehnert et al. 2012); increasing leaf area, flowering and fruiting (Shamshiri et al. 2012); and affecting the biochemical and molecular responses in host plants, with the benefit of improving plant resistance to pathogens (Khan et al. 2010).

Plants use two P uptake pathways: one involves obtaining P directly from the soil via P membrane transporters located in the root hairs, and the second involves obtaining P through the extraradical hyphal network and by delivery to the arbuscules. In the arbuscules, it is absorbed by plant phosphate transporters in the periarbuscular membrane (Smith et al. 2011).

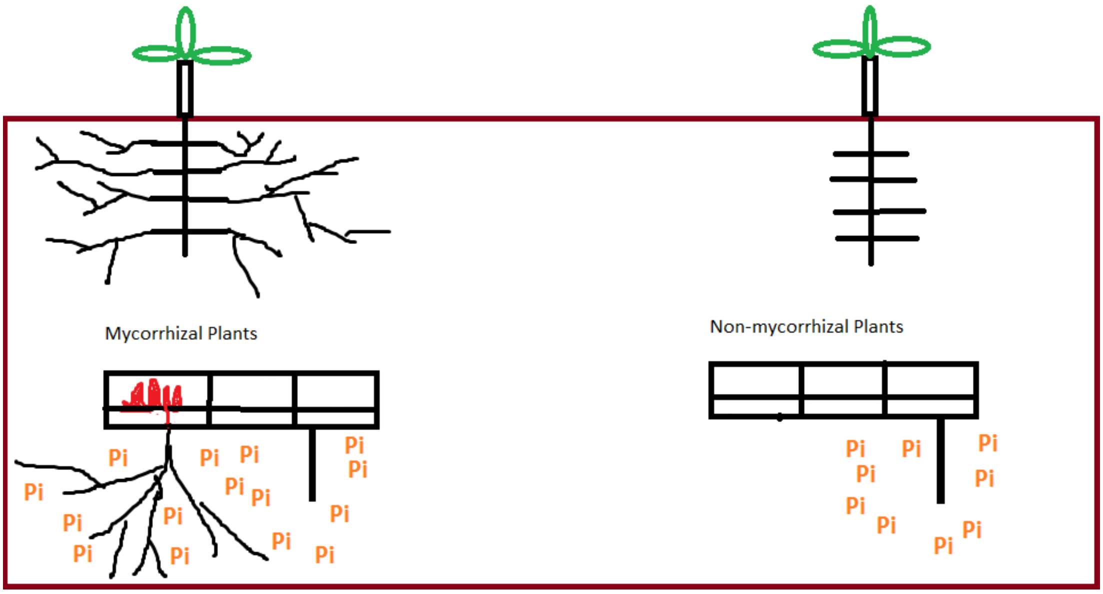

Fig. 3 Phosphate uptake pathways: phosphate (Pi) uptake by non-mycorrhizal and mycorrhizal plant roots. In mycorrhizal plants, P uptake is performed directly by the root hairs or by the mycorrhizal hyphae. In the case of non-mycorrhizal plants, P is obtained from the soil through the extraradical hyphal network and by delivery to the arbuscules (Sawers et al. 2008)

In dry soils, AMF mycelium development is important for nutrient uptake (Smith et al. 2010).

One of the main aspects affecting the mycorrhizal effectiveness is the dependence of plants upon mycorrhiza, which can be explained by the level of plant dependence upon mycorrhiza during the growth stages (Saha and Mandal 2009). Plant responses to AMF can vary from a high positive effect to a negative effect (Smith et al. 2011; Herrera-Peraza et al. 2011). Different plant responses to AMF can be found among the cultivars of the same plant and can differ among the plant species (Tawaraya 2003). The same plant may respond differently to different species of AMF inoculants (Othira et al. 2012). According to Jonas (2007), plant responsiveness to AMF can be an indicator of the effectiveness of AMF, but this can also be “represented by the difference in growth between plants with and without mycorrhizas at any designated level of phosphorus availability”.

Smith and Smith (2011) defined plant responsiveness to AMF as “a change in plant biomass that results from the symbiosis”. The mycorrhizal growth response can be described by the following equation:

Mycorrhizal growth responsiveness = 100 (AM – NM) / NM

Where AM is the biomass of mycorrhizal plants and NM refers to the biomass of non-mycorrhizal plants.

1.3.1.1 Inoculation with AMF

Agricultural systems that depend on tillage and intensive chemical fertilization probably have a poor AMF community (Daniell et al. 2001), and it will thus take a long time to establish an efficient AMF community after turning to an organic farming system (Scullion et al. 1998). Therefore, the application of AMF as a commercial product to farming systems that have turned from conventional to organic systems could be the best solution to improve the diversity of AMF communities (Eason et al. 1999) and could result in many benefits, such as an increase in biomass after the application of a mixed inocula containing AMF (Van der Heijden et al. 2006).

The application of AMF to plants, either directly or to the soil, has been shown to have the ability to increase P uptake and yield and to improve plant resistance to disease (Gosling et al. 2006). Many studies have proved the positive effects of AMF applications. Khan et al. (2008) reported that AMF inoculation has increased nutrient uptake and yield in greenhouse experiments. Furthermore, the application of AMF to plants in field conditions has shown a positive effect on plants (Bever et al. 2001; Guissou 2009; Sidibe et al. 2012; Ortas 2012). However, it is still difficult to predict the effects of AMF on growth and nutrition, since plant responses to AMF application are difficult to determine, even with the same plant species in the same soil (Charron et al. 2001; Ortas et al. 2002). Plants show a wide range of response differences to AMF inoculation, which are related to the available P concentration in the soil (Gavito and Varela 1995; Al-Karaki 2002).

AMF inoculation can be beneficial in some situations when the native AMF community is slight (Grant et al. 2005). The application of AMF to the soils of conventional agricultural systems with high available P content can be more effective in such circumstances, since a native AMF community can be inhibited in soils with high available P rates (Hamel et al. 1997). Many problems can arise before or after the application of the AMF inoculant. Choosing the most efficient AMF species can be difficult because of (1) differences in plant responses to AMF species and (2) the aim of the inoculation, which could be increasing nutrient availability or improving plant resistance to pathogens. Therefore, some AMF applications fail to achieve the aim of the inoculation when the chosen AMF inoculant is not suitable, even when colonization was high (Gosling et al. 2006). A single AMF species can affect many plant species (Smith and Read 1997), but AMF's ability to colonize and affect the plant can be different from one plant to another (Khalil et al. 1999). Single AMF species may reduce the yield of crop plants because of the difficulties in choosing a suitable inoculum (Khaliq and Sanders 2000).

After application, the AMF inoculant may face many problems, such as competition with the native AMF, which could be more adapted to the soil conditions than the added AMF (Harinikumar and Bagyaraj 1996). The native AMF could have the same effectiveness as the applied AMF and hence the AMF inoculant could have no effects on plant growth (Klironomos 2002) or could even negatively affect plant performance due to competition with other microorganisms (Wilson et al. 2001). Furthermore, some AMF species could cause negative growth responses due to the differences in plant responses to the different AMF species (Gogoi et al. 2011). Different AMF species require different amounts of time until they are established with the plant roots (Wang et al. 2008). AMF inoculants could disturb the nutrient exchange balance of host plants (Mack and Rudgers 2008). In view of this, the inoculum should be carefully chosen to guarantee achieving the aim of the application (Azcón-Aguilar and Barea 1997; Klironomos and Hart 2002).

Production of the AMF Inoculant

The interest in AMF formulation technique and application is increasing due to the promosing beneficial effect of the mycorrhiyal fungi (Vassilev et al. 2005). Many companies worldwide produce mycorrhiyal preperations as bio inoculants for commercial porpuses. These products are used in agriculture, horticulture and forestery (Schwartz et al. 2006). According to Siddiqui and Kataoka (2011), the commercial products of mycorrhiyal will be produced in pots, nursery plots, containers with different substrates and plants, aeroponic systems, nutrient film technique, or in vitro. The mycorrhizal products are presented in many different types, some companies present a mix of single mycorrhizal strain and the carrier material, or as powder, liquid, or tablets (Siddiqui and Kataoka 2011).

1.3.2 PGPR – Potential Resources to Increase Crop Productivity and Mechanisms of Action

PGPR are free-living bacteria (Kloepper et al. 1989) and could be a trend for the future of agriculture worldwide (Siddiqui 2006). PGPR are bio-resources that may be considered as a potential tool for providing important advantages to agriculture (Richardson 2001; Saghir Khan et al. 2007). Plant root exudates can offer a suitable active environment in the rhizosphere for PGPR to colonize onto seeds and roots and hence affect plant growth (Khalid et al. 2009). Many microorganisms are highly dependent on compounds of plant root exudates for their survival (Khalid et al. 2006).

Many species of bacteria, including species of *Rhizobium, Bradyrhizobium, Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsiella, Enterobacter, Xanthomonas, Serratia* and many others, have been shown to promote plant growth by different mechanisms. These microorganisms are potential tools for sustainable agriculture because they enhance the availability of essential nutrients to plants and also enhance nutrient use efficiency (Khalid et al. 2009). Several studies have proved a significant increase in growth and yield of agricultural crops in response to PGPR inoculants in field conditions (Shaharoona et al. 2006; Ahmad et al. 2008; Adjanohoun et al. 2011) and greenhouse applications (Srinivasan and Mathivanan 2011; Jarak et al. 2012), increasing the efficiency of applied fertilizers (Jilani et al. 2007; Ahmad et al. 2008; Ramanjaneyulu et al. 2010), increasing plant resistance to pathogens (Mafia et al. 2009) and enhancing abiotic stress tolerance (Gururani et al. 2012).

Furthermore, an improvement in the success rate of the application of biofertilizers in agricultural production systems could be achieved due to better understanding of the plant–bacterial interaction (Ruppel et al. 2006). Many promising microorganisms have been isolated and marketed as biofertilizers; however, their effects on crop yields fluctuate from crop to crop, from place to place and from season to season, depending on the survival of the introduced microorganisms on seeds, on roots and in the soil (Nowak 1998; Khalid et al. 2004; Hafeez et al. 2006).

Beneficial PGPR that increase yield (YIB) (Kilian et al., 2000) can affect plant growth and yield in a number of ways, including improvements in the vegetative and reproductive growth of crops like cereals, legumes, ornamentals, vegetables, plantation crops and some trees (Medeot et al. 2010). The mechanisms of the effects of PGPR are not yet fully understood (Figueiredo et al. 2010; Glick 2012).

Glick (2012) suggested some mechanisms that describe how PGPR affects plant growth. Some of the mechanisms are direct, such as (1) providing plants with nutrients such as P solubilization, N fixation and Fe sequestering and (2) providing growth hormones such as cytokinins and gibberellins, indoleacetic acid (IAA) and ethylene. Alternatively, other mechanisms are indirect, such as (1) the production of antibiotics; (2) the production of siderophores (some PGPR strains are able to produce siderophores more efficiently than pathogens, hence the pathogens will not be able to multiply due to the lack of iron); (3) causing competition due to the beneficial PGPR colonization of plant roots, resulting in growth that leads to high competition with the pathogens that exist in the rhizosphere; (4) the reduction of plant ethylene production as a response to the pathogens; and (5) the induction of systemic resistance due to producing compounds that work as signals to stimulate plants' systemic resistance to pathogens.

1.3.2.1 *Enterobacter radicincitans* as PGPR – Characteristics and Function

The bacterial strain *Enterobacter radicincitans* DSM 16656 (formerly *Pantoea agglomerans*) is a rhizobacterium that belongs to the family of *Enterobacteriaceae* (Kämpfer et al. 2005). *Enterobacter radicincitans* spp. are one type of PGPR showing an ability to increase the growth and yield of different agricultural plants, such as wheat, corn and beans, and also evidence of being a plant-growth-promoting factor (Höflich et al. 1992; Ruppel 2000). These bacteria have also shown the ability to colonize different parts of plants and to survive on the surface and in the internal tissues of plants (Figs. 4a and 4b) (Remus et al. 2000).

E. radicincitans bacterial cells can fix atmospheric nitrogen (Ruppel and Merbach 1995), solubilize calcium phosphate (Schilling et al. 1998), inhibit the growth of phytopathogenic fungi (Ruppel et al. 2006) and produce phytohormones (auxin-like compounds: indole-3-lactic acid and indole-3-acetic acid, and cytokinine-like compounds: N6-isopentyladenosine and N6-isopentyladenine) (Scholz-Seidel and Ruppel 1992).

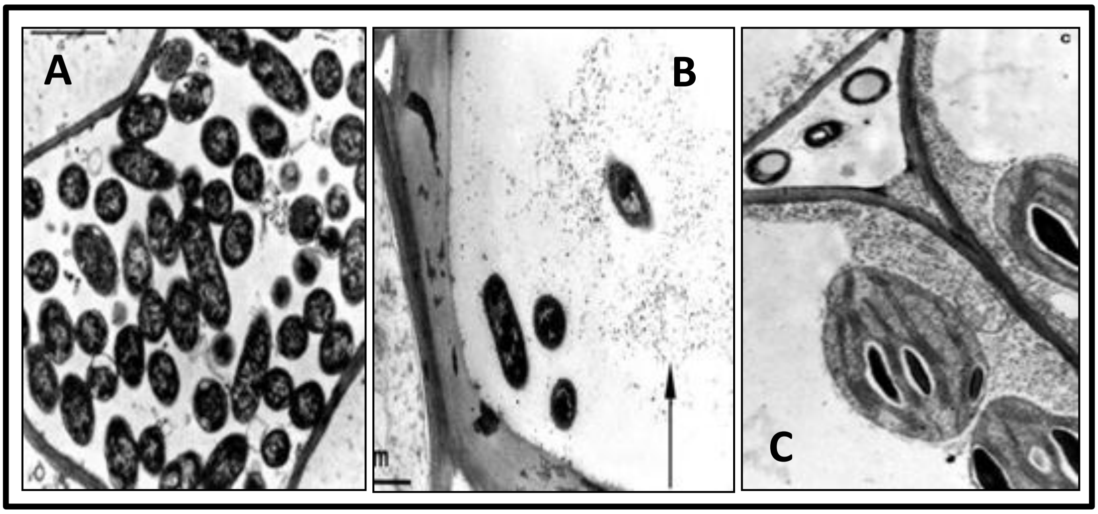

Fig. 4a Transmission electron micrographs of *E. radicincitans* in association with winter wheat (cv. Miras): (A) Bacterial cells in the intercellular space of the root cortex, (B) Bacterial cells in a xylem vessel of the stalk (the bacteria are ensheathed in the granular electron-dense material; *marked with an arrow*) and (C) Bacteria in intercellular spaces of the mesophyll (Remus et al. 2000)

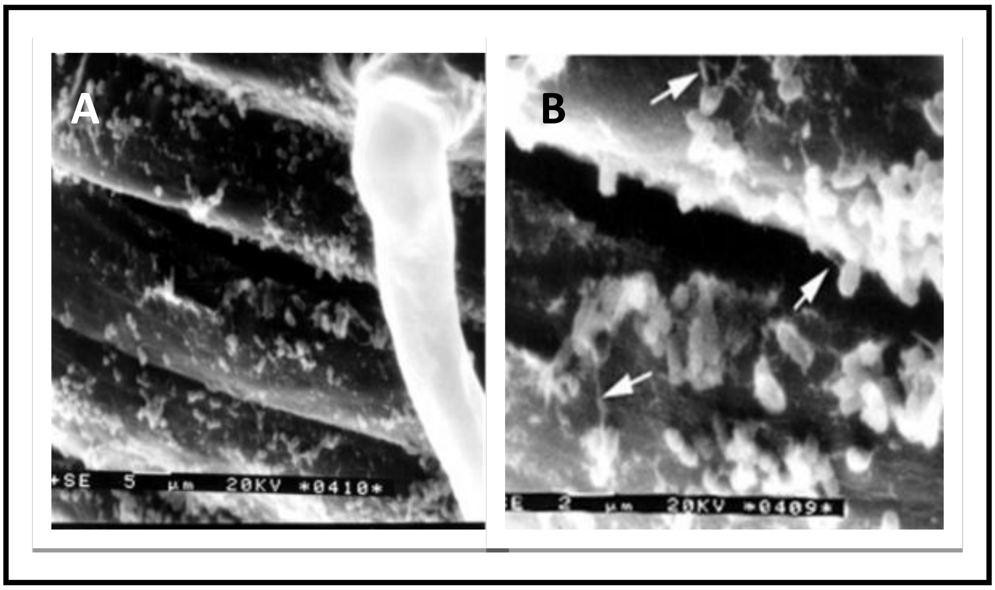

Fig. 4b Scanning electron micrograph of the colonization of *E. radicincitans* cells on the root surface (root hair zone) of winter wheat (cv. Miras) cultivated in a hydroponic system: (A) A magnified section showing the filamentous structure (probably extracellular polysaccharides; *marked with arrows*) between bacteria and the root surface and (B) The bacteria were inoculated into the plant growth medium (Remus et al. 2000)

1.4 Research Objectives

This work is focusing on alternatives for intensive use of mineral fertilizers in crop production to reduce the environmental impact by the combination of fertilizers with plant growth promoting microorganisms.

The prospects of AMF and PGPR (*E. radicincitans*) in single applications, in co-inoculation or in combination with various fertilizers to increase the yield, promote the nutrient supply and soil microbial activities were tested in field and greenhouse applications.

The optimisation of microbial plant growth promoting applications was intended for a further use mainly in organic farming. The main objectives of the present work were:

1. To measure the responses of crops (*Hordeum vulgare*, *Zea mays* and *Vicia faba*) to inoculation and co-inoculation with AMF or PGPR spp. (*E. radicincitans*), focusing on crop yield and foliar concentrations of P, N, K and Mg.

2. To study the crop- species effect on the efficiency of the microbial applications either singular or in combination with the fertilizers.

3. To study the effects of the combined application of AMF and/or *E. radicincitans* with mineral or organic fertilizers.

4. To study the effects of the application of AMF and *E. radicincitans* on soil microbial parameters (soil biomass, soil basal respiration and bacterial communities).

5. To investigate the influence of the microbial inoculation singularly or in combination with organic fertilizers on plant growth under sub-optimal soil conditions such low soil pH.

The following hypotheses were assumed:

- The application of AMF and *E. radicincitans* are able to promote plant growth after root or rhizosphere colonisation.
- The inoculation and co-inoculation of AMF and *E. radicincitans* can increase the grain or shoot yield and the nutrient supply.
- The combined application of AMF and/or *E. radicincitans* with fertilizers will promote the impact on the yield and nutrient supply.
- Soil microbial parameters will be affected by the application of the microbial inoculants and the changes in these parameters will be indicator to the changes in soil fertility.

Chapter 2

2 Materials and Methods

2.1 Description of the Experiments' Location, Soils and Climatic Conditions and the Experimental Design

This study was conducted in 2007 and 2008 in Rostock in north-eastern Germany. The experiments were established in the greenhouse and the field of the experimental station of the University of Rostock, about 15 km from the Baltic Sea. The study area is strongly affected by marine conditions. The annual average temperature is 8.1 °C. In Rostock, the annual rainfall is 593 mm.

The soil texture in all experiments was loamy sand. The soil pH, organic matter and nutrient content for each experiment are presented in Tables 1 and 2.

2.1.1 Field Experiment

Seven treatments were conducted for the barley experiment in 2007: (1) control (without any additions), (2) mineral fertilizer, (3) organic fertilizer, (4) *E. radicincitans*, (5) AMF, (6) organic fertilizer + *E. radicincitans,* and (7) organic fertilizer + AMF.

Four treatments for faba bean were established: (1) control (without any additions), (2) mineral fertilizer, (3) *E. radicincitans* and (4) AMF.

In 2008, an extra treatment combining organic fertilizer + *E. radicincitans* + AMF in the barley experiment was conducted, and a treatment of *E. radicincitans* + AMF in the fava bean experiment was conducted.

Table 1 Soil properties of the field experiments

Year of experiment	pH	OM	P	K	Mg
2007	5.8	2.27	6.27	7.40	14.10
2008	4.9	2.23	2.87	4.51	23.26

P, K and Mg in mg 100 g^{-1} soil; OM: organic matter (%)

Both field experiments were established in the same field, but in different locations; therefore, there were differences in the soil properties. Plots were prepared and distributed randomly. The plot size was 12 m^2 in 2007 (1.5 × 8 m) and 7.5 m^2 (1.5 × 5 m) in 2008.

2.1.2 Greenhouse Experiments

Soil for the greenhouse experiments was taken from different plots of the experimental station of the University of Rostock. The soil was mixed and sieved but not sterilized to allow for competition from the indigenous microorganisms. A total of 6 kg of soil per pot was used, with four replications of each treatment.

Table 2 Soil properties used in the greenhouse experiments

Year of experiment	pH	OM	P	K	Mg
2007	5.8	2.44	5.30	8.20	28.10
2008	6.6	3.03	4.00	5.70	30.50

P, K and Mg in mg 100 g^{-1} soil; OM: organic matter (%)

In the 2007 experiment, seven treatments were established with barley and maize and four treatments with faba bean.

The treatments with barley and maize were as follows: (1) organic fertilizer, (2) *E. radicincitans*, (3) AMF, (4) organic fertilizer + *E. radicincitans*, (5) organic fertilizer + AMF, (6) AMF + *E. radicincitans* and (7) organic fertilizer + AMF + *E. radicincitans*.

The treatments with faba bean were as follows: (1) control (without any additions), (2) *E. radicincitans*, (3) AMF and (4) AMF + *E. radicincitans*.

In the greenhouse experiment of 2007, the effects of the combination of AMF and *E. radicincitans* with organic fertilizer on barley and maize were investigated. In the 2008 experiment, additional treatments were established: the microbial inoculants were combined with organic fertilizer and with mineral fertilizer since the functions of these microbes could have been affected due to the application of nutrients.

Therefore, eleven treatments with barley and maize and six treatments with faba bean were established in the greenhouse.

The treatments with barley and maize can be summarized as follows: (1) control treatment (without any additions), (2) mineral fertilizer (the amounts of the fertilizers used are listed in Table 4), (3) organic fertilizer (cattle manure), (4) *E. radicincitans*, (5) AMF, (6) AMF + *E. radicincitans*, (7, 8) combined treatments of mineral fertilizer with AMF and with *E. radicincitans* and (9, 10, 11) combined treatments of organic fertilizer with (a) AMF, (b) *E. radicincitans* and (c) AMF + *E. radicincitans.*

The treatments with faba bean can be summarized as follows: (1) control treatment (without any additions), (2) Mineral fertilizer (the amounts of the fertilizers used are listed in Table 4), (3) *E. radicincitans*, (4) AMF and (5, 6) combined treatments of Mineral fertilizer with (a) AMF and (b) *E. radicincitans.*

2.1.3 Tested Plants, Microbial Inoculants, Mineral and Organic Fertilizers

The plants used and their varieties in the experiments were:

1- Barley (*Hordeum vulgare*) Barke cultivar

2- Faba bean (*Vicia faba*) Scirocco cultivar

3- Maize (*Zea mays*) Arabica cultivar

Experiments with barley, maize and faba bean were established in the greenhouse. However, only experiments with barley and faba bean were established in the field.

2.1.3.1 Microbial Inoculants

AMF

The AMF preparation used was a commercial product, it was a mix of three *Glomus* species (*Glomus etunicatum, G. intraradices* and *G. claroideum*). The AMF preparation was obtained from INOQ Company in Germany. 100 ml m^{-2} of the used AMF preparation was added in all experiments (according to the manufacturer's instructions).

The plant inoculation process with AMF was different between the experiments in 2007 and 2008.

In 2007, barley and maize seeds used were treated with fungicides: the barley seeds were treated with Aagrano (chemical compound = Imazalil) and the maize seeds were treated with Fludioxonil + Metalaxyl-M + Thirame. Therefore, it was necessary to wait after sowing the seeds before inoculating the plants with AMF spores (the producer recommended inoculating the plants with the AMF preparation at least 12 days after sowing). In the greenhouse, plants were inoculated with AMF spores 12 days after seeding. The spores were added into the root zones of the young plants. This was more difficult in the field, because the plant roots were still weak and not stable yet in the soil; therefore, it was necessary to wait until the roots became stronger and more stable in the soil. The fungi were applied to the plants four weeks after seeding: cracks in the soil among the plant rows were made using a mattock and then the AMF spores were added into the cracks along the rows. Following this, the soil was put back over the spores. The delay in AMF inoculation was necessary to reduce the inhibition effect of the fungicides on the AMF.

In the 2008 experiment, to avoid the delay of AMF application because of the fungicides, the seeds were not treated with fungicide. Instead, the seeds were treated by x-ray in the Fraunhofer Institute for Electron Beam and Plasma Technology in Dresden, Germany. Using this technology, seeds are treated with low energy electrons for seed dressing to inactivate the pathogenic organisms on their surfaces and in the seed coats (Eschrig et al. 2007). Therefore, the application of AMF was possible by sowing the AMF directly. AMF were added to the AMF treatment plots in the field experiment using the sowing machine before the seeds were added at the required seeding depth. Also, the AMF preparation was applied to the pots after filling the pots with soil and one day before seeding in the greenhouse experiment.

Enterobacter radicincitans

The bacterial inoculants for the experiments were prepared at the microbiology laboratory of the Leibniz-Institute of Vegetable and Ornamental Crops Groβbeeren/Erfurt e.V. (IGZ), Germany.

E. radicincitans cells were grown in a standard nutrient solution (MERCK) at 29 °C in a rotary incubator at 100 rpm for 48 hrs (Ruppel et al. 2006).

Seed and Plant Inoculation with *E. radicincitans*

A suitable dilution of 10^8 bacterial cells mL^{-1} suspension of *E. radicincitans* was used to inoculate the seeds. The seeds were inoculated with *E. radicincitans* by coating them with the bacterial suspension for 5–10 mins. Afterwards, the seeds were dried in the dark at room temperature. During the two-leaf growth stage of the plants, the bacterial suspension (10^8 cells mL^{-1}) was sprayed with a hand pump onto the young plants (1 mL per plant) in all experiments. The aim of the second inoculation was to improve the opportunity for the bacterial cells to colonize and establish on the plants, as well as successfully compete with the native bacterial communities.

2.1.3.2 Mineral Fertilizers

Table 3 shows the fertilizers and the amounts used in the greenhouse experiments. All the plants received the same amounts of Mineral fertilizer except that NH_4NO_3 was not added to the faba bean pots.

Table 3 Mineral fertilizer used in the greenhouse experiments

Fertilizer	Amount of element (g pot^{-1})	Amount of fertilizer used (g pot^{-1})
KH_2PO_4	0.23 P 0.29 K	1.00
NH_4NO_3	0.49 N	1.40
$MgSO_4$	0.29 Mg	1.46
KH_2SO_4	0.85 K	3.00

In the field experiments, 120 kg ha^{-1} of Mineral fertilizer (calcium ammonium nitrate – 27% N) was added in two batches to barley plots in the mineral fertilization treatment. The first application was 80 kg ha^{-1} added directly after sowing, and the second application (40 kg ha^{-1}) was added five weeks after the first application.

In the faba bean field experiment, the amounts of the fertilizers applied per hectare were: 20 kg P (as Triple Super Phosphate 46% P_2O_5), 20 kg Mg (as Kieserite 25% Mg) and 100 kg K (as potassium salt (KCl) 60% K_2O). The mineral fertilizers were applied manually four weeks after seeding.

2.1.3.3 Organic Fertilization

Cattle manure as an organic fertilizer was used singularly and in combined treatments with AMF and *E. radicincitans* in the field and greenhouse experiments. Table 4 shows the properties and nutrient contents of the organic fertilizer used in 2007 and 2008. The manure was analysed at LUFA laboratory (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Rostock der LMS Landwirtschaftsberatung Mecklenburg – Vorpommern).

In the field experiments, 3 l m^{-2} of cattle manure was added to the barley organic fertilizer treatment plots. The same amount of manure was applied to the pots in the barley and maize greenhouse experiments; the manure was mixed with the soil of each pot.

Table 4 Properties and nutrient contents of the organic fertilizer used

Parameter	Content g l^{-1}	
	2007	2008
Dry substance	54.11	86.00
pH (value)	7.90	7.90
N	2.20	3.40
P (as P_2O_5)	1.50	2.02
K (as K_2O)	2.90	4.16
Mg (as MgO)	1.03	1.03

2.2 Plant and Soil Analyses

2.2.1 Soil Analyses before Seeding

Soil samples from the field and the greenhouse were collected before sowing and fertilization to determine the pH, organic matter content, and P, K and Mg content (the data are shown in Tables 1 and 2). Soil samples were dried at room temperature and then sieved using a 2 mm sieve.

pH Determination

A total of 10 g of the sieved soil was put in a flask, then 25 ml 0.01 N $CaCl_2$ was added to the soil. After the addition of $CaCl_2$, the suspension was stirred with a glass rod. After 30 mins, the suspension was stirred again. After 1 hr, the pH value of the suspension was measured with an electrode (pH Electrode SenTix 81: name of the electrode; TM-38- pH evaluator- Sensor technique Meisberg GmbH).

Soil Organic Matter Determination

Soil organic matter was determined by drying fine soil in a crucible at 105°C for 4 hrs and by weighing the crucible with the soil (w1). Afterwards, the samples were put into a muffle furnace at 550°C for 4 hrs and weighed again (w2).

Soil organic matter (SOM) was calculated as:

SOM % = (w1 – w2)/w2 ×100

P, K and Mg Determination

A total of 10 g of air-dried soil was dissolved in 125 ml DL solution (Doppel-Lactat); the solution was shaken for 1.5 hrs and then filtered.

P was estimated from the filtrated soil solution: 25 ml from the filtrated soil solution was mixed with 15 ml vanadate-molybdate mixture and 50 ml DL solution in a volumetric flask. After 2 hrs, P was measured by spectrophotometer at a wavelength of 430 nm (Spekol 11, Carl Zeiss, Jena).

Soil-filtrated suspension was also used for K and Mg determination: K was measured by flame photometer (Elex 6361, Eppendorf) and Mg was measured by spectrometer (Epos Analyzer 5060, Com Eppendorf).

2.2.2 Harvest and Plant Analyses

The plants were harvested when mature. The period of growth differed between 2007 and 2008. In the field, the barley and faba bean plants were harvested 17 weeks after sowing in 2007 and after 16 weeks in 2008.

In the greenhouse experiments, the maize, barley and faba bean plants were harvested 53 days after seeding in 2007 and after 51 days in 2008.

After harvesting, the plant material (shoots from the greenhouse experiment and seeds from the field experiment) was dried for 48–96 hrs at 60 °C to provide a constant weight for dry matter determination. The dry weight of the seeds and shoots was measured. Following this, the plant material was subsequently milled for chemical analyses. Grain yield was also measured per plot.

P, K, Mg and N Determination

The vanadate-molybdate method (Page et al. 1982) was used to determine the P in the plant material. Dried subsamples of 2 g were put in a muffle furnace at 550 °C for 4 hrs. Afterwards, the plant material ash was digested in 22 ml HCl (25%) in 50 ml volumetric flasks and put on an electric heater for 15–20 mins. After cooling, the digestion solution was supplemented with distilled water. Later, the solution was transferred and filtered into 50 ml flasks. After filtration, 10 ml from the solution was put into 100 ml volumetric flasks and then the flasks were filled up with distilled water to the mark. Afterwards, 15 ml from the solution was transferred into 50 ml volumetric flasks and the volume was supplemented with vanadate-molybdate mixture. After 2 hrs, P was measured by spectrophotometer at a wavelength of 430 nm (Spekol 11, Carl Zeiss, Jena).

K and Mg were estimated from the filtered suspension using flame photometer (Elex 6361, Eppendorf) for K and spectrophotometer (Epos Analyzer 5060, Com Eppendorf) for Mg. Nitrogen was analysed as total N from the subsamples. The Kjeldahl method (Bremner and Mulvaney 1982) was used to determine the nitrogen content in the plant material.

2.2.3 Soil Sampling and Microbial Analyses

Three random soil samples were taken from each plot in the field (the 0–30 cm soil layer) after harvesting, and about 1 kg of soil was taken from each pot in the greenhouse. Soil samples were stored at -20°C until the microbial parameters were investigated.

Soil Microbial Biomass

Several methods are used nowadays to study soil microbial biomass (Solaiman 2007). Among them is the substrate-induced respiration (SIR) method. This method uses an infrared gas analyser to analyse microbial biomass (Heinemeyer et al. 1989). The operating principle of the infrared gas analyzer offers an automated system for continuous soil respiration and microbial biomass measurements based on infrared gas analysis. The switching device is computer controlled and allows hourly measurements of up to 24 samples when switching intervals of 2.5 mins are selected. This allows the use of the SIR method for biomass determination. A software package to run the system is available (Heinemeyer et al. 1989).

The microbial biomass carbon (C_{mic}) content of the soil was determined using the SIR method with an automatic infrared gas analyser. The C_{mic} content was calculated according to the correlation of SIR with the fumigation incubation method (Anderson and Domsch 1978). The soil was mixed with glucose (2 mg g^{-1} soil) and analysed under continuous gas flow at 20 °C ± 1 K. C_{mic}, which includes all respiratory active soil organisms that are able to metabolize glucose, is expressed as µg C_{mic} g^{-1} dry soil (Ruppel et al. 2007).

Soil basal respiration activity was measured by an infrared gas analyser without the addition of substrates (20 °C ± 1 K); the values for basal respiration (CO_2 production) are given as µg CO_2-C (g^{-1} soil h^{-1}).

P-Solubilizing Bacteria

The number of P-solubilizing bacteria (PSB) was determined in the soil samples that were collected from the greenhouse and the field. The soil samples were sieved using a 2 mm sieve. A total of 5 g of sieved soil was added to 45 ml of sterilized 0.05 M NaCl with 10 sterilized glass beads in 500 ml glass flasks; the flasks were shaken at 290 rpm at 4 °C for 1 hr. The soil suspension was then centrifuged at 664 g for 3 mins. Following this, the separated soil microorganisms (in soil suspension) were transferred to new centrifuge tubes. The soil suspension was twice made up to 50 ml and centrifuged at 2,872 g for 30 mins at 5 °C. After centrifuging, the liquid was discarded and the deposited bacteria were collected (to reduce the nutrient content in the suspension).

Three tenfold dilutions of soil suspension (10^{-3}, 10^{-4} and 10^{-5}) were cultured on Muromcev solid media with three replications of each dilution. Therefore, 100 ml of each dilution was plated on identical agar plates and incubated at 29° C for two weeks. The Muromcev medium consists of [(g L^{-1}) glucose 10, L-asparagine 1, K_2SO_4 0.2, $MgSO_4.7H_2O$ 0.4, agar-agar 20, $CaCl_2$ 2.2, $Na_3PO_4.12H_2O$ 3.8].

The number of PSB on the media was determined after two weeks of growth. P-solubilizing bacteria appeared on the media with a clear spot around the bacterial colony.

2.3 Statistical Analysis

All the analyses were carried out with four replications and the mean values of the four replicates were reported. The data in all the experiments were subjected to a one-way analysis of variance. One way ANOVA was performed to test the differences among the treatments. The mean values were compared with a post-hoc test followed by a Tukey's HSD test at $P < 0.05$. The data were analysed using Statistica 6.0 (StatSoft 2001) software.

Chapter 3

3 The Effects of Singular and Combined Treatments on Yield, Nutrient Uptake and Soil Microbial Parameters in the Field Experiment

This chapter deals with the effect of the application of AMF and *E. radicincitans* inoculation separately and in combination with organic manure on the grain yields of barley (*Hordeum vulgare*) and faba bean (*Vicia faba*), the nutrient uptake, soil microbial activity and soil bacterial communities under field experimental conditions in 2007 and 2008.

3.1 Results

3.1.1 Barley Grain Yield and Nutrient Content in the Field Experiment

The effect of the singular and combined treatments on the barely grain yield of the 2007 field experiment is given in Fig. 5. The observed result shows that the grain yield was generally significantly increased in all the treatments in comparison to the non-fertilized control treatment. Among all the treatments, the singular application of organic fertilizer and mineral fertilizer demonstrated the highest barely grain yield. It is notice worthy that the combined application of microorganisms and organic fertilizer did not give an additional yield effect over the singular application of the organic fertilizer or the microbial inoculants.

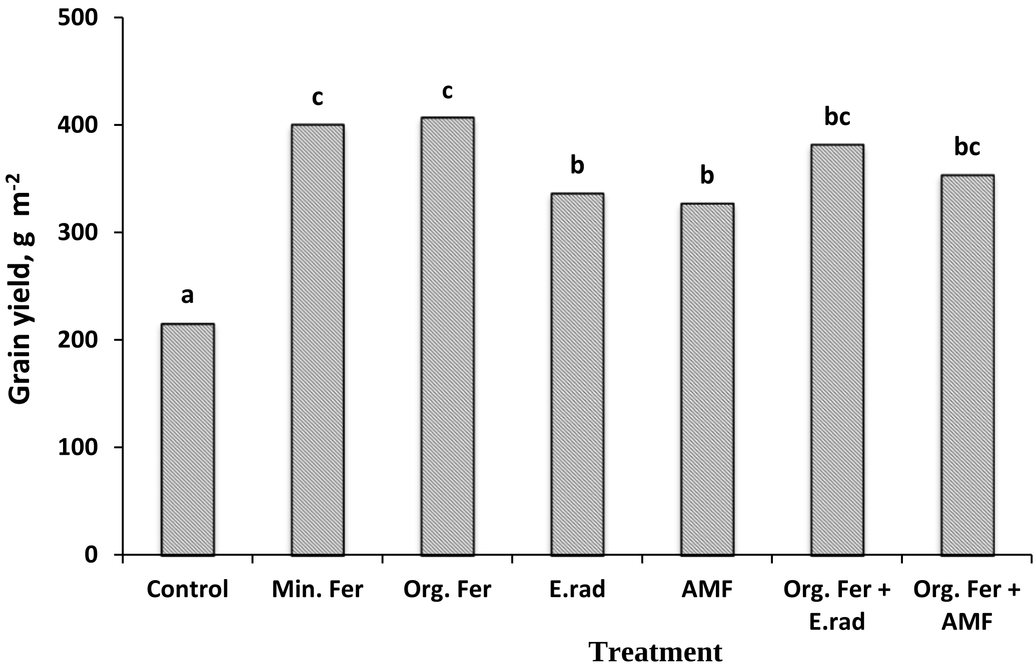

Fig. 5 The effect of the singular and combined treatment on the barely grain yield (g m^{-2}) in the field experiment of 2007; Mineral fertilizer (Min. Fer), Organic fertilizer (Org. Fer) and *E. radicincitans* (E. rad); **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The effect of the singular and combined treatments on the barely grain yield in 2008 field experiment is graphically presented in Fig. 6. The result showed that the singular application of the mineral fertilizer, organic fertilizer, AMF or *E. radicincitans* as well as the combined application of AMF and *E. radicincitans* had no significant effect on the barley grain yield. On the other hand, it was observed that the combined application of organic fertilizer + AMF or organic fertilizer + *E. radicincitans* considerably increased the grain yield in compassion to the control treatment. However, the barely grain yield in 2007 was greater than grain yield in the 2008.For instance, the grain yield in the control treatment in 2007 was 216.3 g m^{-2} but it was 96.7 g m^{-2} in the control treatment in 2008, that clearly indicates the difference in the grain yield of the 2007 and 2008 field experiments under different soil conditions.

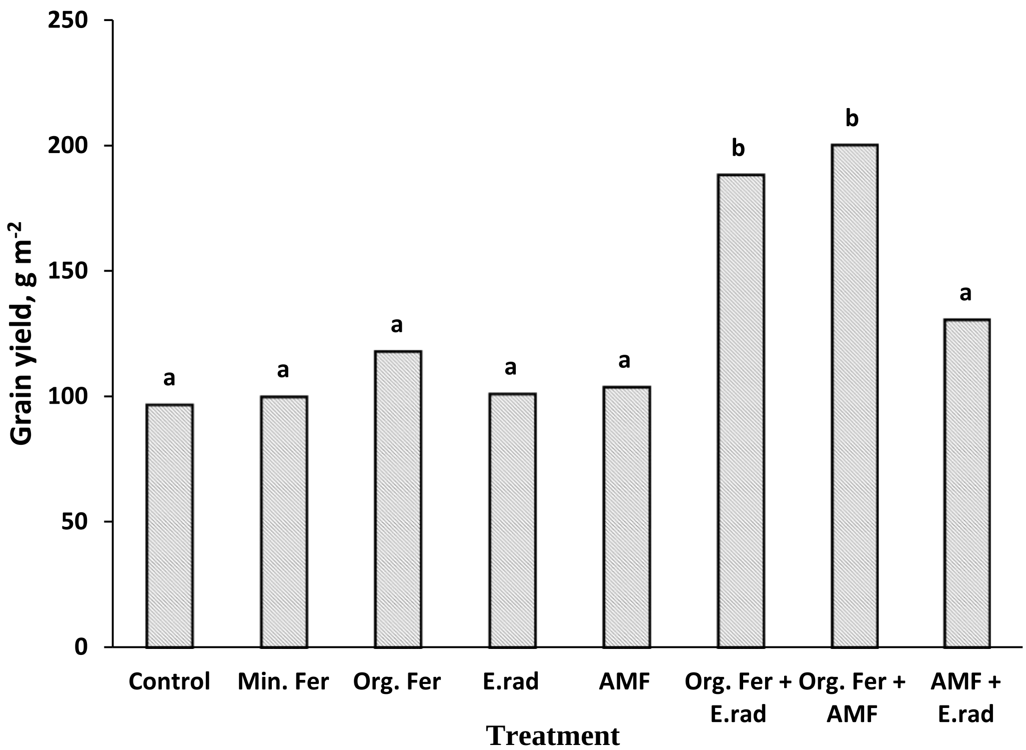

Fig. 6 The effect of the singular and combined treatments on the barely grain yield (g m^{-2}) obtained in the field experiment of 2008; Mineral fertilizer (Min. Fer), Organic fertilizer (Org. Fer) and *E. radicincitans* (E. rad); **Note:** bar graphs with different letters are significantly different according to Tukey's test at p ≤ 0.05

The contents of P, N, K and Mg were analysed in the barely grain after the singular and combined treatments in the field experiment of 2007 are summarized in Table 5. The results showed that the application of the microbial inoculants significantly increased P and N uptake, but there was no significant effect on K and Mg uptake compared to the control treatment in the experiment. The combined application of the organic manure with AMF or with *E. radicincitans* increased the N, P, K and Mg content of the barley grain, but it was lower than the effect of the singular application of the manure. When considering the effect of the application of mineral fertilizer only on the uptake of P and N, the uptake of N and P was higher than the uptake of N and P under the application of AMF and *E. radicincitans* separately or in combination with organic fertilizer However, the uptake of K and Mg under the application of mineral fertilizer was not significantly different. The effect of the application of Mineral fertilizer was statistically similar to the effect of the application of organic fertilizer.

Table 5 Grain content of N, P, K and Mg (g m^{-2}) in the different treatments in barley field experiment in 2007

Treatment	P	N	K	Mg
Control	0.89 a	3.27 a	0.87 a	0.28 a
Mineral fertilizer	1.83 d	8.17 d	1.78 b	0.56 bc
Organic fertilizer	1.76 cd	7.20 cd	2.02 b	0.61 c
E. radicincitans	1.37 b	5.50 b	1.33 ab	0.42 ab
AMF	1.40 bc	5.49 b	1.37 ab	0.43 abc
Organic fertilizer + *E. radicincitans*	1.60 bcd	6.12 bc	1.62 b	0.52 bc
Organic fertilizer + AMF	1.50 bcd	5.88 bc	1.51 ab	0.46 bc

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at p ≤ 0.05

The effect of the singular and combined treatments of soil on the N, P, K and Mg content in the barely grain of the 2008 field experiment was examined and presented in Table 6. According to the results, the N, P, K and Mg content in the barley grain harvested after the combined application of *E. radicincitans* with organic fertilizer was significantly increased when compared to the control treatment and to the singular application treatments of the manure or the bacteria. It was also observed that there was no significant effect of the singular application of the microbial inoculants into soil on the nutrient uptake in comparison to the control treatment.

Table 6 Grain content of N, P, K and Mg (g m^{-2}) in the different treatments in barley field experiment in 2008

Treatment	P	N	K	Mg
Control	0.41 a	1.80 a	0.35 a	0.14 a
Mineral fertilizer	0.42 a	2.25 ab	0.37 a	0.14 a
Organic fertilizer	0.48 ab	2.30 ab	0.44 ab	0.17 ab
E. radicincitans	0.42 a	1.93 a	0.36 a	0.13 a
AMF	0.38 a	1.99 a	0.35 a	0.13 a
Organic fertilizer + *E. radicincitans*	0.75 b	3.57 c	0.65 b	0.26 c
Organic fertilizer + AMF	0.68 ab	3.32 bc	0.59 ab	0.24 bc
E. radicincitans + AMF	0.56 ab	2.52 ab	0.47 ab	0.20 abc

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at p ≤ 0.05

3.1.2 Grain Yield of Faba Bean and Nutrient Content in the Field Experiment of 2007

The yield of faba bean significantly increased after treating soil with AMF as depicted in Fig. 7. It was also observed that the application of mineral fertilizer also increased the yield of faba bean as compared to the control treatment yield result. The singular application of *E. radicincitans* did show little increase in the grain yield which was not statistically significant.

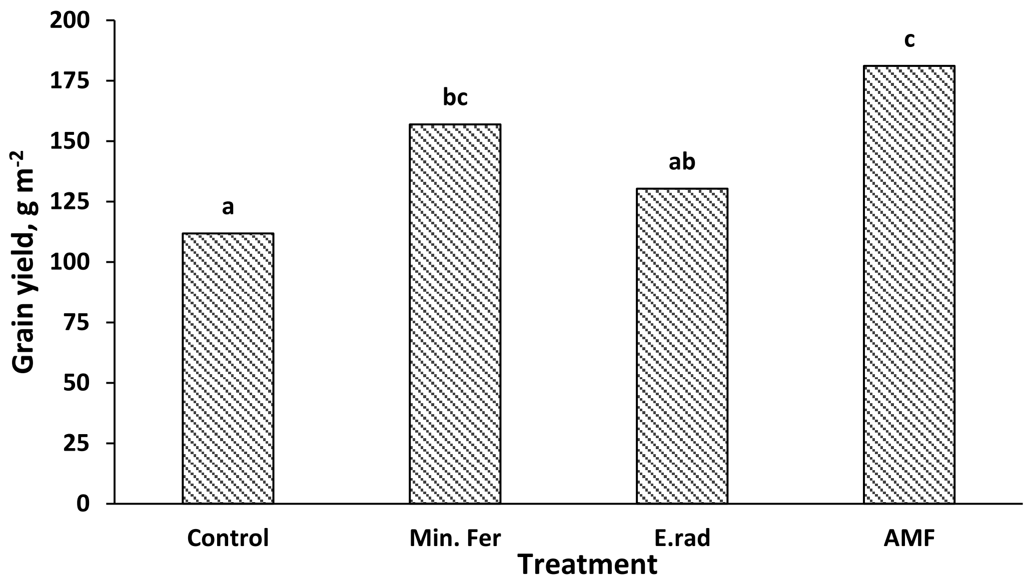

Fig. 7 The effect of singular treatment on the grain yield of faba bean (g m^{-2}) obtained in the field experiment of 2007; Mineral fertilizer (Min. Fer), Organic fertilizer (Org. Fer) and *E. radicincitans* (E. rad); **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The N, P, K and Mg contents in the faba bean after singular application of mineral fertilizer, *E. radicincitans* and AMF are presented in Table 7. According to the results, it was observed that there was an increase in the content of the nutrient after the singular application in comparison to the control treatment. The result showed that the contents of all the nutrients were significantly increased after inoculation with AMF. The application of *E. radicincitans* and mineral fertilizer separately also increased nutrient uptake, but the increase was not statistically significant.

Table 7 Grain content of N, P, K and Mg (g m^{-2}) in the different treatments in faba bean field experiment in 2007

Treatment	P	N	K	Mg
Control	0.70 a	5.37 a	1.10 a	0.1 8a
Mineral fertilizer	1.00 bc	7.63 bc	1.63 bc	0.26 bc
E. radicincitans	0.87 ab	6.26 ab	1.3 ab	0.21 ab
AMF	1.15 c	8.57 c	1.82 c	0.30 c

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$

3.1.3 Soil Microbial Analyses

3.1.3.1 Barley Experiment

To analyse the soil microbial parameters, the soil sample was taken after harvesting barely from the field experiment during dry time. The soil microbial parameters measured after the singular and combined treatments of the barely field experiment in 2007 are given in Table 8. The results showed that there was no significant effect of AMF and *E. radicincitans* with or without the application of organic fertilizer on the soil basal respiration or soil biomass. However, it was observed that the number of P-solubilizing bacteria was increased significantly in the soils that were taken from the combined treatment of *E. radicincitans* with organic fertilizer.

Table 8 Soil microbial parameters in the different treatments in barley field experiment in 2007

Treatment	BR	SMB	PSB
Control	6.36 a	130.8 a	1.39E+07 a
Organic fertilizer	7.23 a	135.1 a	1.47E+07 a
E. radicincitans	8.30 a	122.1 a	1.63E+07 ab
AMF	8.22 a	136.0 a	1.42E+07 a
Organic fertilizer + *E. radicincitans*	7.40 a	160.1 a	2.85E+07 b
Organic fertilizer + AMF	7.21 a	156.8 a	1.48E+07 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})]; SMB: soil microbial biomass µg C g^{-1} soil; PSB (Bacterial Cells g^{-1} soil

Table 9 shows the soil microbial parameters measured in the barely field experiment of 2008 after the singular and the combined treatments of soil. The results showed that there was no significant effect of the singular or combined application of AMF and *E. radicincitans* on the measured microbial parameters of soil in the barley experiment in 2008. The number of P-solubilizing bacteria in the treatment of the combined application of the organic fertilizer with AMF could not be counted because of the enormous growth of fungi on the nutritional media in the Petri dishes.

Table 9 Soil microbial parameters in the different treatments in barley field experiment in 2008

Treatment	BR	SMB	PSB
Control	4.22 a	70.5 a	3.08E+05 a
Organic fertilizer	5.11 a	93.9 a	4.92E+05 a
E. radicincitans	4.64 a	77.2 a	2.33E+06 a
AMF	4.56 a	70.1 a	1.18E+06 a
Organic fertilizer + *E. radicincitans*	5.24 a	101.5 a	1.68E+06 a
Organic fertilizer + AMF	5.98 a	91.7 a	-
AMF + *E. radicincitans*	5.24 a	86.8 a	1.79E+06 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})]; SMB: soil microbial biomass µg C g^{-1} soil; PSB (Bacterial Cells g^{-1} soil)

3.1.3.2 Faba Bean Experiment

The measured values of the soil microbial parameters in soil of the faba bean experiment in 2007 are summarized in Table 10. According to the results, the singular and combined treatments did not cause significant increase in all the measured soil microbial parameters in comparison to the non-fertilized control treatment.

Table 10 Soil microbial parameters in the different treatments in faba bean field experiment in 2007

Treatment	BR	SMB	PSB
Control	5.89 a	129.9 a	2.06E+07 a
Mineral fertilizer	5.35 a	129.0 a	3.88E+07 a
E. radicincitans	6.38 a	141.1 a	4.42E+07 a
AMF	6.31 a	133.4 a	4.33E+07 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})]; SMB: soil microbial biomass µg C g^{-1} soil; PSB (Bacterial Cells g^{-1} soil)

3.2 Faba Bean Experiment in 2008

The faba bean plants in the 2008 experiment grew until about flowering time but they were very weak. Afterwards, the plants began to dry until there were only some dry stems left in the plots. Therefore, the faba bean plants in this experiment were not harvested, and hence data were not collected for the faba bean experiment in 2008.

3.3 Discussion

Effects of the Singular Application of AMF and *E. radicincitans* on Grain Yield and Nutrient Content in the Field Experiment

The singular application of AMF or *E. radicincitans* significantly increased the yield of the inoculated barely plants, which support the hypothesis that treatment of soil with AMF or *E. radicincitans* can increase the yield. As well the singular application of mineral fertilizer or organic fertilizer increased the barely yield in the 2007 experiment. The increase in the barely yield in the singular treatments may be due to the suitable soil conditions for the availability of optimum amount nutrients for the inoculated barely as compared to the non-inoculated control experiment. On the contrary, the barely yield of the 2008 experiment was not insignificantly different in the singular application of AMF or *E. radicincitans* from the control treatment, which could be due to the sub-optimal soil conditions associated with the low soil pH that could have toxic effect on plants and cause low nutrient availability. The result of the 2008 experiment is similar to several previous studies conducted for different plants (Kucey and Diab 1984; von Uexküll 1986; Marschner 1991; Ryan et al. 1994; Marschner 1995; von Uexküll and Mutert 1995; Varga and Kytöviita 2010).

In addition, the grain yield and nutrient content of faba bean plants were increased after the singular application of AMF and *E. radicincitans*, but the increases were significant only in the application of AMF in comparison to the control in the 2007 field experiment. The significant increase in the grain yield of faba bean and nutrient content after the singular application of AMF as compared to the other singular treatment and control treatment can be due to the application of AMF can produce several growth promoting substances that can influence plant nutrient uptake and yield in the alkaline soil pH. The result is identical to the previous studied conducted for different plants applying singularly AMF (Clarke and Mosse 1981; Powell 1981; Jensen 1984; Achatz et al. 2010).

The effect of *E. radicincitans* on the grain yield of wheat and maize was reported by Ruppel et al. (1989). They reported that inoculation experiments with bacterial strains of *E. radicincitans* on wheat and barley in temperate regions have demonstrated the possibility of increasing the yield up to 500 kg ha^{-1}. Also, Remus et al. (2000) found that the grain yield of winter wheat was increased by 23.5% after the application of *E. radicincitans*. However, the concentration of P, N and K in the grain was not affected by the inoculation.

Effect of the Combined Application of the Organic Fertilizer and AMF or *E. radicincitans*

Grain yield (Figure 5) and nutrients uptake (Table 5) were decreased by the combined application of the manure with the microbial inoculants in comparison to the singular application of the manure, but the decrease was not significant. The combined application of the organic fertilizer either with AMF or with *E. radicincitans* did not affect the grain yield and nutrient uptake of barely experiment in 2007 in comparison to the singular application of the manure, which could be due to the high competition in the rhizosphere between the native microorganisms and the inoculants for nutrients after the introduction of the organic fertilizer. The combined application of organic manure with AMF could enhance AMF development in the soil thereby increasing the competition between the inoculants and native microorganisms leading to the nutrient uptake and grain yield (Harinikumar and Bagyaraj 1989; Sattelmacher et al. 1991; Ryan et al. 1994; Joner et al. 2000; Muthukumar and Udaiyan 2002; Picone 2002; Gryndler et al. 2006). In addition, the decrease in the yield can be in some cases associated to carbon draining by AMF due to the prolific AMF colonization in organic systems (Dann et al. 1996 Kitchen et al. 2003, Ryan et al. 2004). Another stipulated reason in the decrease of the yield and nutrient uptake in the combined application of AMF and *E. radicincitans* was the increase of the soil pH from pH = 5.8 to more higher pH level which might be not optimal pH for microorganisms due to the addition of organic manure thus causing the decrease in the nutrient uptake and yield of the plants.

On the other hand, the results of the 2008 experiment (Fig. 6 and Table 6) show that the addition of the organic manure in combination with both AMF and *E. radicincitans* significantly increased grain yield and nutrient uptake. The increase in yield and nutrient uptake could be due to the improvement of the soil conditions such as the soil pH (4.9) following the addition of organic manure of pH = 7.9 to optimum pH for effectual activities of the microorganisms from which the plant benefits. In the same way, Whalen et al. (2000) observed the increase in the pH of an acidic soil following manure addition.

Effects of the Singular and Combined Applications of the Bio-Inoculants and the Organic Fertilizer on Soil Microbial Parameters

The effect of the microbial inoculants on the measured soil microbial parameters when they were applied singularly or with the organic manure was little, except in the combined application of *E. radicincitans* with the manure. From the presented results it can be understood that there were few differences among, the soil basal respiration, soil biomass and the P-solubilizing bacteria on the Muromcev solid media. The time of soil sampling, which was after harvesting the plants when their roots were dry and had died, could be the reason for the observed results. In addition, the soil was dry too during the sampling time, so the microbial rhizosphere activity could have been reduced because there was no activity in the rhizosphere as the soil samples had been taken. The present observation is in agreement with the results reported by Lang et al. (2007), who found that the microbial community and the population of the soil bacteria *E. coli* increased in cool and moist soil during autumn and winter, and reduced in warm, dry soil in spring and summer.

The faba bean field experiment in 2008 was failed and data were not gathered. The failure of the faba bean field experiment in 2008 could be because of the field conditions such as low soil pH ~ 4.9, low available nutrient content and bad drainage. Soil acidity is associated with chemical changes in the soil which could restrict the availability of the essential plant nutrients, such as P, Ca, Mg, K and micronutrients such as molybdenum and boron. Soil acidity also creates a condition in which elements such as Fe, Al and Mn become toxic to plants (Schroth et al. 2003) and as well, Fe and Al may combine with P to form insoluble compounds in the low soil pH (Hollier and Reid 2005). Furthermore, low soil pH could cause the leaching of essential plant nutrients to below the rooting zone thereby the nutrients are not available for the plants to be absorbed by the roots for normal growth. Bacteria populations require optimum pH condition, which is a slightly acidic soil environment, to survive and be effectually beneficial to plant growth. However, the low pH soil condition, which is below the optimum pH for bacteria population to survive, can inhibit the survival of useful bacteria such as rhizobia bacteria that fix nitrogen for legumes (Hollier and Reid 2005). Due to these all factors the low soil pH environment could create unfavourable conditions for normal plant growth, which was also the case in the faba bean field experiments in 2008. The failure of the faba bean field experiment in 2008 is similar to the observation made by El-kherbawy et al. (1989) where alfalfa plants failed to survive in soil with pH values of 4.3 and 5.3.

Chapter 4

4 The Effects of Singular and Combined Applications of AMF and *E. radicincitans* on Shoot Growth, Shoot Nutrient Content and Soil Microbial Parameters in the Greenhouse Experiment

The experiment was conducted in 2007 and 2008 in the greenhouse of the University of Rostock. The aim of the experiment was to investigate the effects of the applications of AMF (*Glomus etunicatum, G. intraradices* and *G. claroideum*) and *E. radicincitans* singularly or in combination with organic or mineral fertilizers on the plant growth, nutrient content and soil microbial parameters (soil basal respiration, soil microbial biomass and the most probable number of PSB) of barley (*Hordeum vulgare*), maize (*Zea mays*) and faba bean (*Vicia faba*) under greenhouse conditions.

The greenhouse experiment in 2007 aimed mainly to investigate the effect of the combined application of the organic fertilizer (cattle manure) with AMF and/or *E. radicincitans* on the shoot yield, nutrient content and soil microbial parameters of barley and maize and to investigate the effect of the singular application of AMF and *E. radicincitans* on the shoot yield, nutrient content and soil microbial parameters of faba bean. Additionally, in 2008 experiments the combined application of mineral fertilizers with AMF and/or *E. radicincitans* were conducted to investigate the effect of the combined treatment on the performance of the microbial inoculants when they are applied to barley, maize and faba bean under greenhouse conditions.

4.1 Results

4.1.1 Maize Experiment

4.1.1.1 Shoot Growth and Nutrient Content

The combined application of the organic fertilizer with AMF and/or *E. radicincitans* had no significant effect on the dry shoot weight of the maize in the greenhouse experiments in 2007 and 2008 (Figs. 8 and 9). In addition, there was no significant effect of the combined application of AMF and *E.*

radicincitans on the shoot growth of the maize in comparison to the singular application of AMF or *E. radicincitans* in the first and second experiments.

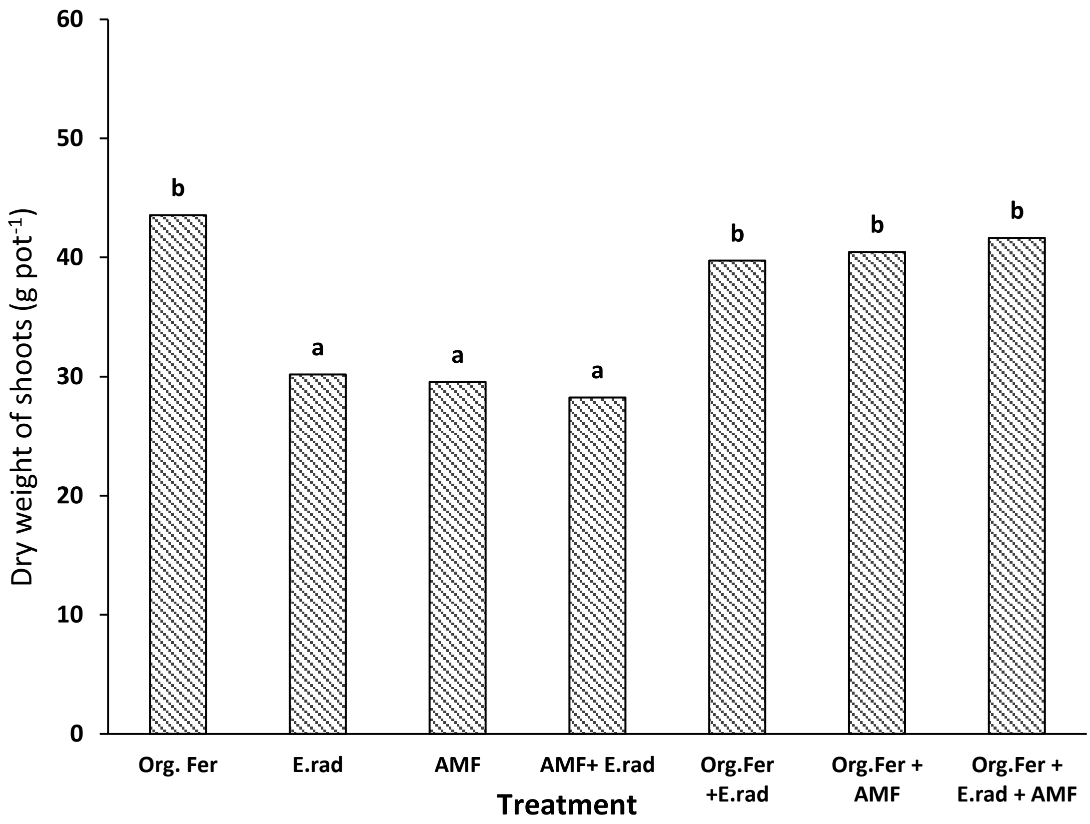

Fig. 8 Dry shoot weights of the maize (g pot^{-1}) in the greenhouse experiment in 2007, measured after the singular application of organic fertilizer (Org. Fer), AMF and *E. radicincitans* (E. rad), and the combined application of organic fertilizer with AMF and/or *E. radicincitans*; bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

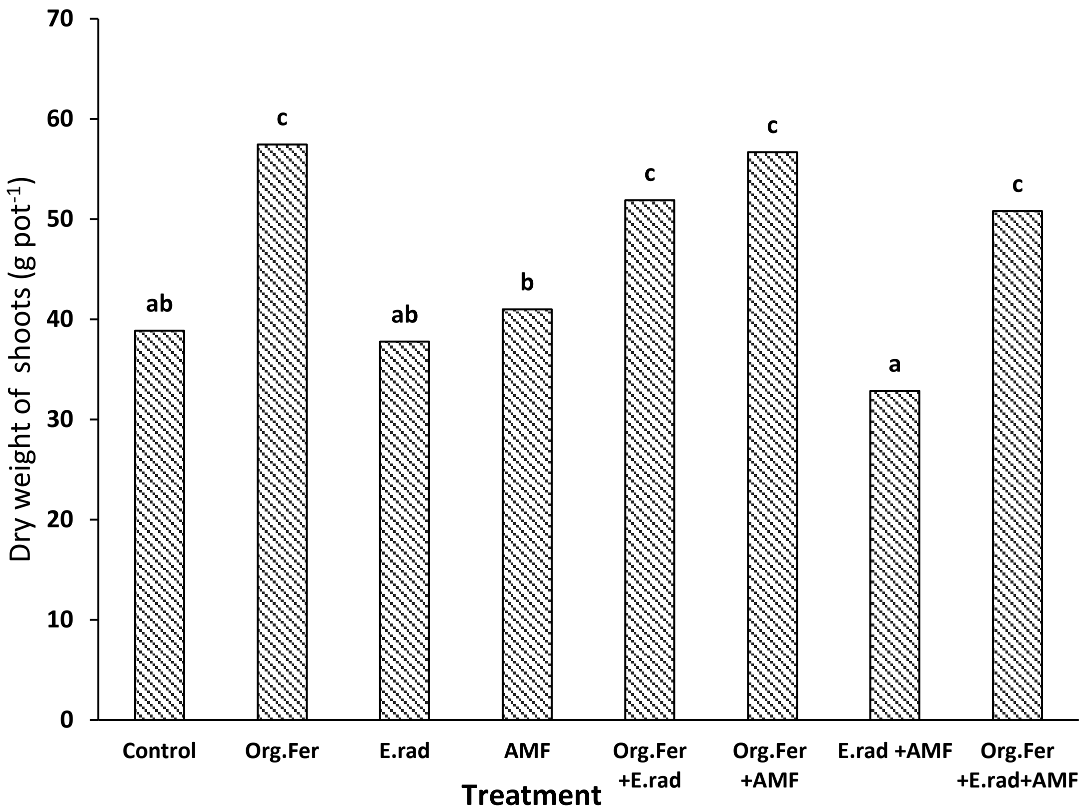

Fig. 9 Dry shoot weights of the maize (g pot^{-1}) in the greenhouse experiment in 2008 measured after the singular application of organic fertilizer (Org. Fer), AMF and *E. radicincitans* (E. rad), and the combined application of organic fertilizer with AMF and/or *E. radicincitans*; **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

Figure 10 shows that there was no significant effect of the application of AMF and *E. radicincitans* on the shoot growth of the maize when they were applied singularly in comparison to the control treatment. Furthermore, in the second experiment in 2008, the effect of the combined application of the microbial inoculants with the mineral fertilizer on the shoot growth of the maize was significantly lower than the effect of the singular applicatio0n of the mineral fertilization. However, the maize shoot growth in the combined application of AMF and *E. radicincitans* was significantly higher that the singular application of AMF or *E. radicincitans* or the control experiment.

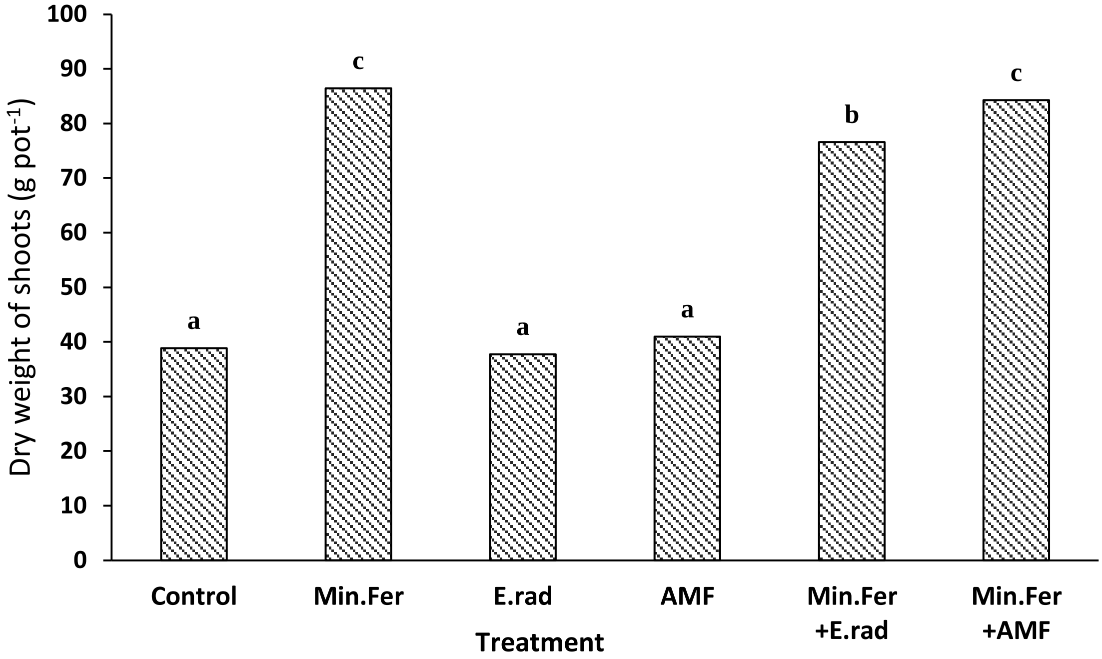

Fig. 10 Dry shoot weights of the maize (g pot^{-1}) in the greenhouse experiment in 2008 measured after the singular application of the mineral fertilizer (Min. Fer), AMF and *E. radicincitans* (E. rad), and the combined application of mineral fertilizer and AMF or *E. radicincitans*; **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The influence of the singular and combined treatments on the nutrient content in the maize plant is presented in Table 11. The application of the organic fertilizer in combination with AMF and/or *E. radicincitans* had no significant effect on the P, N, K and Mg content in the maize plants in comparison to the singular application of the manure or the microbial inoculants in 2007. Furthermore, in the same experiment, no significant effect of the combined application of AMF and *E. radicincitans* was detected in comparison to the singular application of AMF or *E. radicincitans*.

Table 11 Shoot content of N, P, K and Mg (g m^{-2}) in the different treatments in maize greenhouse experiment in 2007

Treatment	P	N	K	Mg
Organic fertilizer	0.10 a	0.28 b	0.61 ab	0.10 c
E. radicincitans	0.12 a	0.16 a	0.46 a	0.08 abc
AMF	0.11 a	0.16 a	0.44 a	0.07 ab
AMF + *E. radicincitans*	0.11 a	0.15 a	0.41 a	0.06 a
Organic fertilizer + *E. radicincitans*	0.11 a	0.2 b	0.75 b	0.09b c
Organic fertilizer + AMF	0.10 a	0.24 b	0.62 ab	0.09b c
Organic fertilizer + *E. radicincitans* + AMF	0.12 a	0.25 b	0.73 b	0.10 c

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$

Table 12 presents the content of N, P, K and Mg in the maize shoot after the singular and combined treatment in the greenhouse experiment conducted in 2008. The result showed that the application of the manure significantly increased the content of P, N and K but not Mg in comparison to the control treatment. On the other hand, there was no significant effect on the nutrient content of the maize by the combined application of the manure with AMF and/or *E. radicincitans* compared to the singular application of the manure. It was also observed that the nutrient uptake in the mineral fertilization treatment was significantly higher than in all other treatments. However, the combined application of the mineral fertilizer with AMF and/or *E. radicincitans* had no significant effect on the P, N, K and Mg content in comparison to the singular mineral fertilization treatment.

Table 12 Shoot content of N, P, K and Mg (g m^{-2}) in the different treatments in maize greenhouse experiment in 2008

Treatment	P	N	K	Mg
Control	0.05 a	0.32 a	0.47 a	0.11 ab
Mineral fertilizer	0.12 d	0.95 c	1.18 c	0.19 e
Organic fertilizer	0.07 c	0.47 b	0.77 b	0.14 bc
E. radicincitans	0.05 ab	0.31 a	0.47 a	0.1 abc
AMF	0.06 ab	0.34 a	0.52 a	0.11 abc
Mineral fertilizer + *E. radicincitans*	0.10 d	0.91 c	1.08 c	0.19 e
Mineral fertilizer + AMF	0.11 d	0.98 c	1.16 c	0.20 e
Organic fertilizer + *E. radicincitans*	0.07 c	0.50 b	0.68 b	0.14 bc
Organic fertilizer + AMF	0.06 ab	0.53 b	0.70 b	0.14 bc
E. radicincitans + AMF	0.04 a	0.29 a	0.42 a	0.09 a
Organic fertilizer + *E. radicincitans* + AMF	0.06 abc	0.53 b	0.73 b	0.14 bc

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$

4.1.1.2 Soil Microbial Parameters

The results presented in Table 13 show that in the 2007 experiment the number of P-solubilizing bacterial cells was increased by the application of the organic manure in comparison to the singular application of AMF or the combined application of AMF and *E. radicincitans*. However, there was no significant effect of the combination of the manure with AMF and/or *E. radicincitans* on the soil microbial parameters in comparison to the singular application of the manure. It was also observed that the application of AMF and *E. radicincitans* did not significantly affect the soil microbial parameters in comparison to the singular application of AMF or *E. radicincitans*.

Table 13 Soil microbial parameters in the different treatments in maize greenhouse experiment in 2007

Treatment	BR	SMB	PSB
Organic fertilizer	12.53 a	184.5 a	1.98E+07 a
E. radicincitans	15.53 a	186.3 a	3.12E+07 a
AMF	6.66 a	145.7 a	1.81E+07 a
AMF + *E. radicincitans*	14.61 a	143.4 a	5.58E+06 a
Organic fertilizer + *E. radicincitans*	12.01 a	177.1 a	2.42E+07 a
Organic fertilizer + AMF	13.02 a	157.6 a	1.73E+07 a
Organic fertilizer + *E. radicincitans* + AMF	12.75 a	203.6 a	1.58E+07 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], PSB: P-solubilizing Bacteria (Bacterial Cells g^{-1} soil) and SMB: soil microbial biomass µg C g^{-1} soil

The data in Table 14 show that basal respiration was significantly higher after the combined application of the organic manure with AMF and *E. radicincitans* in comparison to the control treatment or the singular application of *E. radicincitans.* However, it was not significant in comparison to the singular application of the organic manure or AMF in the maize experiment in 2008. Besides, the combined application of the mineral fertilizer or the organic fertilizer with AMF or *E. radicincitans* had no significant effect on either basal respiration or soil biomass in comparison to the control treatment or to the singular applications of the fertilizers or the bio-inoculants.

Table 14 Soil microbial parameters in the different treatments in maize greenhouse experiment in 2008

Treatment	BR	SMB
Control	11.96 a	353.0 ab
Mineral fertilizer	14.55 ab	310.4 a
Organic fertilizer	17.75 abc	364.4 ab
E. radicincitans	14.44 ab	401.5 ab
AMF	18.10 abc	424.9 ab
Mineral fertilizer + *E. radicincitans*	16.63 abc	309.9 a
Mineral fertilizer + AMF	20.53 abc	380.3 ab
Organic fertilizer + *E. radicincitans*	15.21 abc	380.2 ab
Organic fertilizer + AMF	22.55 bc	539.2 b
E. radicincitans + AMF	12.26 a	450.9 ab
Organic fertilizer + *E. radicincitans* + AMF	24.90 c	407.0 ab

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test ($p \leq 0.05$); BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], PSB: P-solubilizing Bacteria (Bacterial Cells g^{-1} soil) and SMB: soil microbial biomass µg C g^{-1} soil

4.1.2 Barley Experiment

4.1.2.1 Shoot Growth and Nutrient Content

The dry weight of barely shoots of greenhouse experiments in 2007 and 2008 after the singular and combined treatments are given in Figs. 11 and 12. The plot in Figs. 11 and 12 revealed that no significant effect was found of the combined application of the organic manure with AMF and/or *E. radicincitans* on the shoot growth of barley in comparison to the singular application of the manure, except in the treatment of organic fertilizer with AMF and *E. radicincitans* in 2008. In the 2008 experiment, the growth of barley shoot was significantly decreased compared to the singular application of the manure. The combined application of AMF and *E. radicincitans* had no significant effect on the shoot growth of the barley in comparison to the singular application of AMF or *E. radicincitans* in both the 2007 and 2008 experiments.

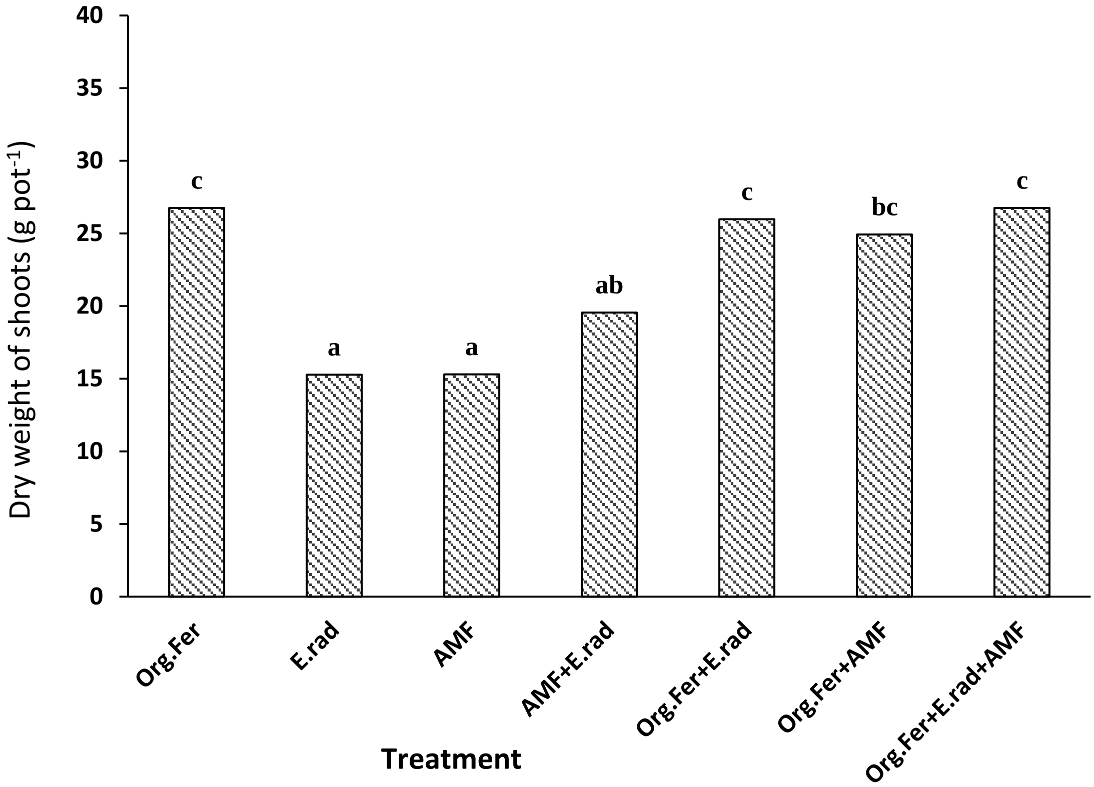

Fig. 11 Dry shoot weights of the barley (g pot^{-1}) in the greenhouse experiment in 2007, measured after the singular application of organic fertilizer (Org. Fer), AMF and *E. radicincitans* (E. rad), and the combined application of organic fertilizer (Org. Fer) with AMF and/or *E. radicincitans;* **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

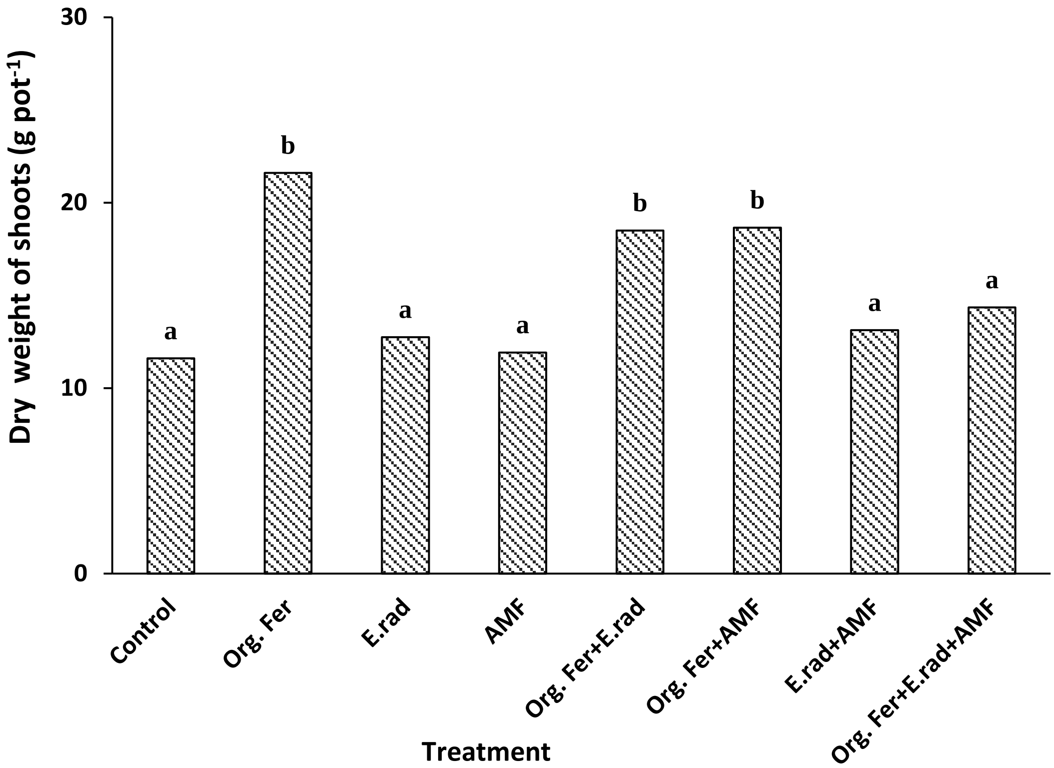

Fig. 12 Dry shoot weights of the barley (g pot^{-1}) in the greenhouse experiment in 2008, measured after the singular application of organic fertilizer (Org. Fer.), AMF and *E. radicincitans* (E. rad.), and the combined application of organic fertilizer with AMF and/or *E. radicincitans*; **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The application of mineral fertilizer to the barley in the 2008 experiment, whether singularly or in combination with AMF or *E. radicincitans*, significantly increased the shoot growth in comparison to the control treatment or to the singular application of AMF or *E. radicincitans*. However, the dry shoot weight was not significantly affected by the combined application of the mineral fertilizer with AMF or *E. radicincitans* in comparison to the singular mineral fertilization treatment (Fig. 13).

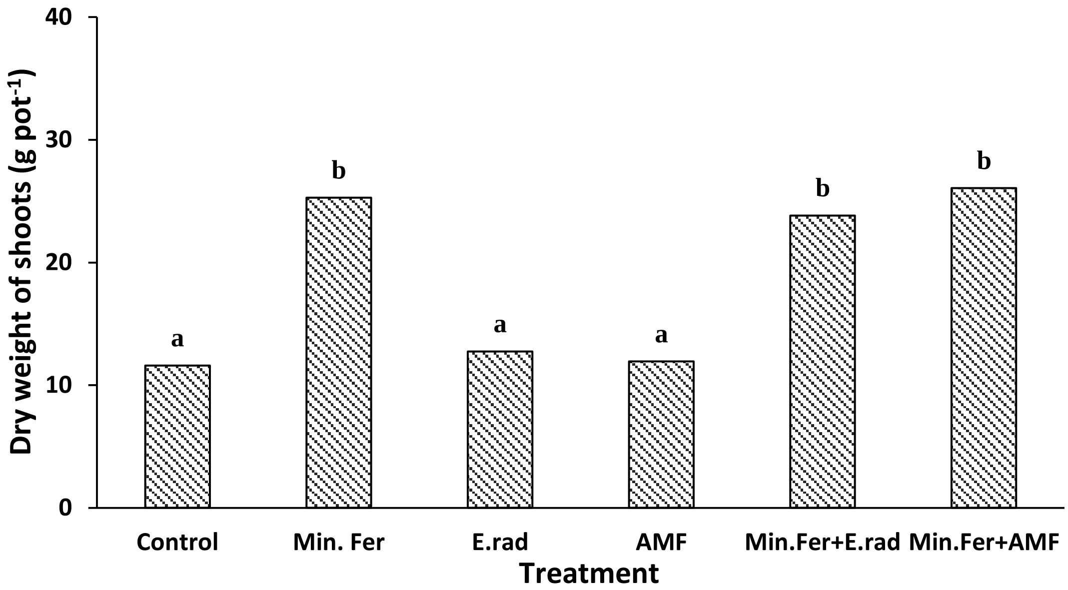

Fig. 13 Dry shoot weights of the barley (g pot^{-1}) in the greenhouse experiment in 2008, measured after the singular application of Min. Fer, AMF and *E. radicincitans* (E. rad.), and the combined application of Min. Fer with AMF or *E. radicincitans*; **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The analysed nutrient contents in the barley shoots after the singular and combined treatments in the greenhouse experiments of 2007 are presented in Table 15. According to the obtained results, the nutrient contents in the barley shoots in 2007 were not significantly affected by the combined application of manure with AMF and/or *E. radicincitans* in comparison to the singular application of the manure. However, the combined application of AMF and *E. radicincitans* significantly increased the content of N, P and Mg but not K in comparison to the singular application of AMF or *E. radicincitans*.

Table 15 Shoot content of N, P, K and Mg (g m^{-2}) in the different treatments in barley greenhouse experiment in 2007

Treatment	P	N	K	Mg
Org. Fer	0.072 b	0.027 b	0.536 bc	0.049 b
E. radicincitans	0.051 b	0.014 a	0.264 a	0.028 a
AMF	0.051 a	0.016 a	0.273 a	0.028 a
AMF + *E. radicincitans*	0.068 b	0.020 b	0.376 ab	0.044 b
Org. Fer + *E. radicincitans*	0.070 b	0.028 b	0.565 c	0.045 b
Org. Fer + AMF	0.064a b	0.030 b	0.500 bc	0.045 b
Org. Fer + *E. radicincitans* + AMF	0.071 b	0.030 b	0.571 c	0.052 b

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; Org. Fer: organic fertilizer

In the 2008 barley experiment, the application of the organic fertilizer significantly increased the nutrient contents of the barley shoots in comparison to the control treatment or to the singular application of AMF or *E. radicincitans* (Table 16). However, the Mg content was not significantly different between the treatment of the organic fertilizer and the treatment of *E. radicincitans*. The combined application of the manure and AMF or *E. radicincitans* did not significantly affect the nutrient content in comparison to the singular application of the manure. It was also observed that in the treatments with mineral fertilizer, the combined application of the mineral fertilizer with AMF or *E. radicincitans* significantly increased the nutrient content in comparison to the singular application of AMF or *E. radicincitans*, but there was no effect of the combination in comparison to the singular application of the mineral fertilizer.

Table 16 Shoot content of N, P, K and Mg (g m^{-2}) in the different treatments in barley greenhouse experiment in 2008

Treatment	P	N	K	Mg
Control	0.020 a	0.335 a	0.177 a	0.029 a
Mineral fertilizer	0.052 de	0.851 d	0.557 ef	0.055 c
Organic fertilizer	0.039 cd	0.524 c	0.449 de	0.049 bc
E. radicincitans	0.024 ab	0.337 a	0.218 abc	0.034 ab
AMF	0.020 a	0.336 a	0.175 a	0.028 a
Mineral fertilizer + *E. radicincitans*	0.067 e	0.877 d	0.629 f	0.060 c
Mineral fertilizer + AMF	0.067 e	0.926 d	0.691 f	0.057 c
Organic fertilizer + *E. radicincitans*	0.037 bcd	0.466 c	0.371 bcd	0.038 ab
Organic fertilizer + AMF	0.032 abc	0.548 c	0.396 cde	0.045 bc
E. radicincitans + AMF	0.025 abc	0.340 ab	0.216 ab	0.034 ab
Organic fertilizer + *E. radicincitans* + AMF	0.028 abc	0.457 bc	0.339 abcd	0.035 ab

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at p ≤ 0.05

4.1.2.2 Soil Microbial Parameters

The measured soil microbial parameters in soil of the barely greenhouse experiment of 2007 are summarized in Table 17. It was clear from the results presented in Table 17 that the basal respiration was significantly higher in the singular application of *E. radicincitans* than in the combined application of the bacteria with the organic manure. In addition, the soil microbial biomass was significantly higher in the treatment of the organic manure and AMF in comparison to the singular application of AMF. However, it was observed that there was no significant effect of the combined application of the manure with AMF and/or *E. radicincitans* on the soil microbial parameters in the barley experiment in comparison to the singular application of the manure.

Table 17 Soil microbial parameters in the different treatments in barely greenhouse experiment in 2007

Treatment	BR	SMB	PSB
Organic fertilizer	5.70 ab	147.6 b	1.96E+07 abc
E. radicincitans	9.23 c	134.3 b	1.18E+07 ab
AMF	6.5 abc	97.2 a	1.30E+07 abc
AMF + *E. radicincitans*	8.3 bc	121.5 ab	-
Organic fertilizer + *E. radicincitans*	5.7 ab	130.7 ab	2.88E+07 bc
Organic fertilizer + AMF	5.5 a	139.7 b	9.92E+06 a
Organic fertilizer + *E. radicincitans* + AMF	6.5 abc	149.6 b	2.97E+07 c

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], PSB: P-solubilizing Bacteria (Bacterial Cells g^{-1} soil) and SMB: soil microbial biomass µg C g^{-1} soil

Soil microbial parameters measured in the barely greenhouse experiment of 2008 are given in Table 18. The results revealed that there were no significant differences among the different treatments in the greenhouse barley experiment of 2008.

Table 18 Soil microbial parameters in the different treatments in barely greenhouse experiment in 2008

Treatment	BR	SMB
Control	7.07 a	188.9 a
Mineral fertilizer	9.75 a	247.6 a
Organic fertilizer	8.63 a	267.6 a
E. radicincitans	6.9 a	221.9 a
AMF	7.2 a	187.6 a
Mineral fertilizer + *E. radicincitans*	9.82 a	228.5 a
Mineral fertilizer + AMF	7.61 a	208.0 a
Organic fertilizer + *E. radicincitans*	9.75 a	253.5 a
Organic fertilizer + AMF	6.36 a	242.3 a
E. radicincitans + AMF	4.7 a	170.5 a
Organic fertilizer + *E. radicincitans* + AMF	8.23 a	249.2 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test ($p \leq 0.05$); BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], SMB: soil microbial biomass µg C g^{-1} soil

4.1.3 Faba Bean Experiment

4.1.3.1 Shoot Growth and Nutrient Content

In the 2007 experiment, the dry shoot weight of the faba bean was higher when AMF and *E. radicincitans* were applied singularly or in combination (AMF and *E. radicincitans*) in comparison to the control treatment (Fig. 14), but there were no significant differences between the treatments of the bio-inoculants and the control treatment.

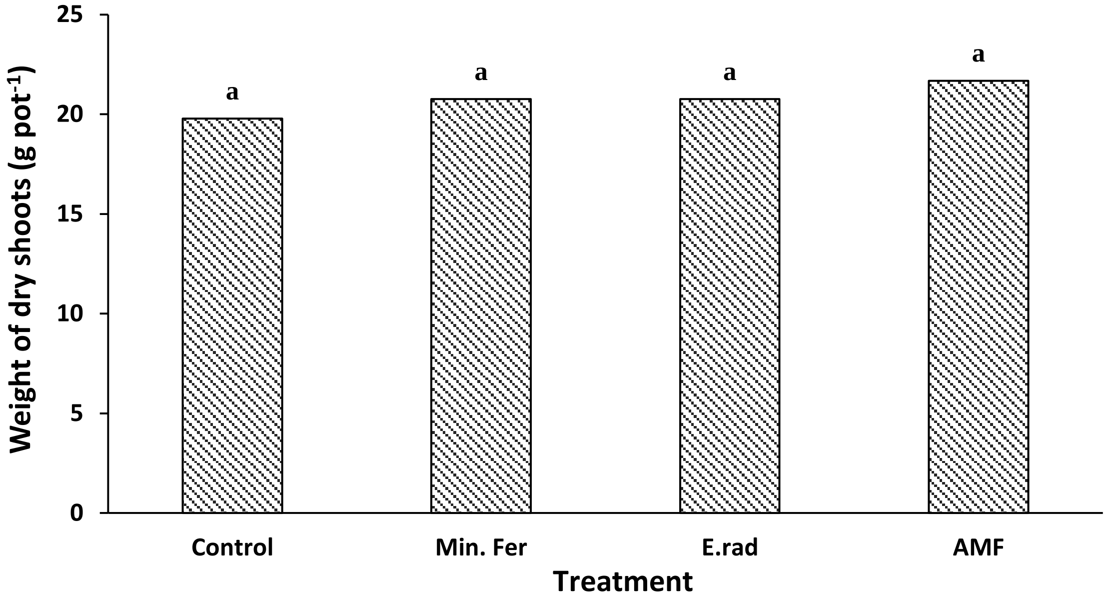

Fig. 14 Dry shoot weights of the faba bean (g pot^{-1}) in the greenhouse experiment in 2007, measured after applications of AMF, *E. radicincitans* (E. rad.) and mineral fertilizer (Min. Fer.); **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

In 2008, the shoot growth of the faba bean was increased when AMF was applied, but the increase was not statistically significant compared to the control treatment (Fig. 15). On the other hand, the combined application of mineral fertilizer with *E. radicincitans* or with AMF increased the shoot growth of the faba bean. However, the increase was significant only in the treatment of the combined application of AMF and the mineral fertilizer in comparison to the singular application of the mineral fertilizer or AMF.

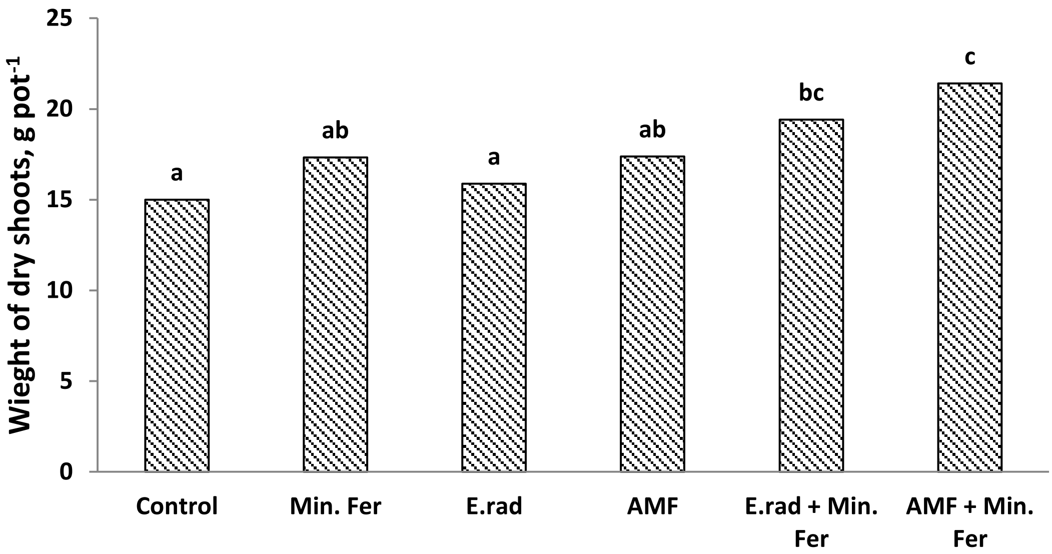

Fig. 15 Dry shoot weights of the faba bean (g pot^{-1}) in the greenhouse experiment in 2008, measured after the singular application of AMF, *E. radicincitans* (E. rad.) and mineral fertilizer (Min. Fer.), and the combination of mineral fertilizer and AMF or *E. radicincitans*; **Note:** bar graphs with different letters are significantly different according to Tukey's test at $p \leq 0.05$

The analysed nutrient contents in the shoots of the faba bean of the 2007 greenhouse experiment are shown in Table 19. The result showed that the application of AMF and *E. radicincitans* caused insignificant increase in the nutrient content measured in the faba bean shoots of 2007 greenhouse experiment.

Table 19 Shoot content of N, P, K and Mg (g m^{-2}) in the different treatments in faba bean greenhouse experiment in 2007

Treatment	P	N	K	Mg
Control	0.055 a	0.47 a	0.35 a	0.069 a
E. radicincitans	0.056 a	0.49 a	0.38 a	0.073 a
AMF	0.057 a	0.51 a	0.35 a	0.076 a
E. radicincitans + AMF	0.055 a	0.49 a	0.37 a	0.073 a

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$

In 2008, the application of *E. radicincitans* or AMF increased the content of P and N but only the content of P was significantly affected by the application of AMF compared to the control (Table 20). It was also observed that the combination of the mineral fertilizer and *E. radicincitans* or AMF slightly increased the P and N content compared to the individual application of the mineral fertilizer.

Table 20 Shoot content of P and N (g m^{-2}) in the different treatments in maize greenhouse experiment in 2008

Treatment	P	N
Control	0.031 a	0.40 a
Mineral fertilizer	0.049 bc	0.52 ab
E. radicincitans	0.034 a	0.43 a
AMF	0.047 b	0.52 ab
Mineral fertilizer + *E. radicincitans*	0.050 bc	0.59 b
Mineral fertilizer + AMF	0.057 c	0.63 b

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$

4.1.3.2 Soil Microbial Parameters

The soil microbial parameters of faba bean experiments are summarized in Tables 21 and 22.The highest values of microbial biomass and basal respiration were found after the application of AMF alone or with the mineral fertilizers, but the difference was only significant after the combined application of AMF with the mineral fertilizers. The single application of *E. radicincitans* did not affect the soil parameters, but the combined application of *E. radicincitans* with AMF resulted in higher soil biomass values in 2007 over the control. However, this increase was most probably due tothe AMF application. The number of P solubilizing bacteria was singnificantly higher than the control treatment in in 2007. Unexpectedly, the combined application of *E. radicincitans* and AMF resulted in lower number of P solubilizing bacteria in comparison to the single application of the bacteria.

Table 21 Soil microbial parameters in the different treatments in faba bean greenhouse experiment in 2007

Treatment	BR	SMB	PSB
Control	15.6 ab	148.7 a	1.44E+06 a
E. radicincitans	17.6 ab	186.1 ab	3.61E+06 b
AMF	19.1 b	193.2 b	2.69E+06 ab
E. radicincitans + AMF	14.4 a	192.2 b	1.74E+06 ab

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test at $p \leq 0.05$; BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], SMB: soil microbial biomass µg C g^{-1} soil and PSB: P-Solubilizing Bacteria (bacteria cells g^{-1} soil)

T**able 22** Soil microbial parameters in the different treatments in faba bean greenhouse experiment in 2008

Treatment	BR	SMB	PSB
Control	20.6 a	299.1 a	8.83E+06 a
Mineral fertilizer	20.7 a	338.0 a	1.03E+07 a
E. radicincitans	20.9 a	337.8 a	1.817E+07 a
AMF	26.1 ab	433.1 ab	1.84E+07 a
Mineral fertilizer + *E. radicincitans*	20.6 a	304.1 a	nd
Mineral fertilizer + AMF	29.5 b	494.9 b	nd

Note: Reported data are the mean of 4 replications, and values in each column with different letters are significantly different according to Tukey's test ($p \leq 0.05$); BR: basal respiration [µg CO_2-C (g^{-1} soil h^{-1})], SMB: soil microbial biomass µg C g^{-1} soil and PSB: P-Solubilizing Bacteria (bacteria cells g^{-1} soil)

4.2 Discussion

4.2.1 Shoot Growth and Content of Nutrient

Plant responses to the singular inoculation with AMF or with *E. radicincitans* were almost the same, since the singular application of the microbial inoculants to the plants had no significant effect on shoot growth or nutrient content, except the application of AMF to the faba bean plants in the second experiment significantly increased the P content. This effect could be because of the differences in the plant responses to microbial inoculants depending on plant species. A significant response in the growth and yield of crops to microbial inoculants (including AMF and PGPR) has been proved in many previous studies (Kucey and Janzen 1987; Khalid et al. 2006; Gravel et al. 2007; Kumar et al. 2007; Zhuang et al. 2007). However, others (Poi and Kabi 1979; Chanway and Holl 1992; Nowak 1998; Khalid et al. 2004; Hafeez et al. 2006) have discussed that the responses of plants to inoculation may fluctuate depending on many factors, such as plant species and the survival of the introduced microorganisms. Brimecombe et al. (2001) reported that soil microorganisms have been shown to respond to plant exudation and that plant species can have different root exudation patterns.

The combination of AMF and *E. radicincitans* either with each other or with the fertilizers was relatively different among the tested plants. The combined application of AMF and *E. radicincitans* increased shoot growth and significantly increased P, N and Mg content in the barley experiment, while it decreased or had no effect on the maize plants. The dry shoot weight of the faba bean plants was increased in treatments of the mineral fertilizer with AMF or with *E. radicincitans*, but it was significant only when the mineral fertilizer was combined with AMF, but the increase in nutrient content was not significant. In contrast to the faba bean experiment, the combined application of mineral fertilizer and AMF had no effect on the dry shoot weight and nutrient content of the barley and maize shoots, but the application of the mineral fertilizer and *E. radicincitans* significantly decreased the dry shoot weight of the maize plants.

The application of the organic fertilizer in combination with the microbial inoculants to barley and maize had mostly negative but not significant effects on dry shoot weight and nutrient content. The effect on maize could be due to the increased competition for energy resources and reduced mycorrhizal dependency. The low effect of the microbial inoculants in combination with fertilizers could be because of the soil conditions and the use of the fertilizers, since microbial inoculants can be useless when plants get their nutrient requirements directly from the soil (reducing the mycorrhizal dependency). Alternatively, it could be because of the time of sampling, since it has previously been proved that sampling time influences the effects of bio-inoculants. For instance, Canbolat et al. (2006) provided a basis for comparison of the impact of inoculants with fertilizer. The study was conducted with barley seedlings. It was shown that available P and N were significantly greater in the first harvest at 15 days after planting compared with 30 and 45 days after planting, which indicated that the impact of inoculants on nutrient content can depend on time or the stage of growth of the plant. Similarly, Adesemoye et al. (2009) observed that the time of sampling (i.e. the plant's stage of growth) significantly affected the effectiveness of the inoculants. Furthermore, Canbolat et al. (2006) reported increases in the N and P content of plant dry matter with each inoculated *Bacillus* strain compared with the control. It was also shown that the amounts of N and P in plants inoculated with *Bacillus* were lower than the plants that were fertilized with N, P or NP fertilizers. This is an indication that the inoculants were not able to replace fertilizer fully (Canbolat et al. 2006).

4.2.1 Soil Microbial Analyses

Soil microbial parameters values differed according to plant species, the differences between the soil basal respiration values in AMF or *E. radicincitans* treatments and the organic fertilizer treatments were correlated to a reverse deference in the soil microbial biomass values. This result could be due to the high content of microorganisms in soil of organic fertilizer treatments in comparison to the microbial inoculant treatments (Tables 17 and 18) and due to the competition on the nutrients because of the high content of microbial cells in the soil at the time when basal respiration was measured. However, the activity of the microbial communities increased after the addition of glucose to the soil. The effect of organic manure on soil microbial parameters has been reported in previous studies: Larkin et al. (2006) found that bacterial populations and microbial activity in soil were increased after the addition of organic manure (swine and dairy manure).

In faba bean experiment, the increase in soil basal respiration after the application of AMF and *E. radicincitans* compared to the control treatment was not correlated to the same ratio of differences in soil microbial biomass, but it was correlated to the number of P-solubilizing bacterial. The increased number of P-solubilizing bacteria could be due to the direct effect of the application of *E. radicincitans* or because of the indirect effect of the application of AMF, since the application of AMF stimulates the growth and activity of P-solubilizing bacteria in the rhizosphere. The synergistic effects of AMF and P-solubilizing bacteria have been indicated by different researches (Artusson et al. 2005). Also, Kim et al. (1998) found the same effect under the condition of limited P availability in soil.

Chapter 5

5 General Discussion

The previous chapters, Chapter 3 and Chapter 4, of this dissertation dealt with the results and discussion of the results obtained in several separate experiments. In Chapter 5, the general discussion of the main results of the study is given with a view on the hypotheses given in the general introduction. Finally, some perspectives on further research are also presented in this chapter.

5.1 Effects of AMF and *E. radicincitans* on Grain Yield, Shoot Growth and Nutrient Content in Plant Material

The often-observed positive effects of AMF and PGPR on plant growth could be due to the contribution of the microbial nutrient to the plant metabolism and the production of plant growth hormones. The application of beneficial microorganisms such as PGPR or AMF to soils in order to increase crop production is becoming an important issue as the cost of mineral fertilizers increases. For example, the total worldwide import costs of mineral fertilizers ($1,000) in 2009 for N, P and K fertilizers were respectively 16,575,075, 1,193,715 and 126,209,070 (FAO). Environmental issues such as water pollution and eutrophication are closely related to agricultural P fertilization management. The build-up of P in soils is a major concern in agricultural practice due to the low efficiency of P fertilizer in the field. The use of beneficial microorganisms could be an optional effort in order to be able to re-use the P build-up in agricultural land (Vessey 2003). The beneficial effects of these microorganisms may become apparent in agricultural soils that are deficient in certain nutrients.

The general beneficial effect on the barely grain yield in first field experiment in 2007 of the inoculation with AMF or with *E. radicincitans* could be due to the increase in the uptake of the nutrient as a result of the application of AMF or *E. radicincitans.* Some previous scholars observed similar results (Ruppel et al. 1989; Remus et al. 2000; Achatz et al. 2010). In contrast, there was no effect of either AMF or *E. radicincitans* on the grain yield or nutrient content of barley in the second field experiment in 2008 when they were singularly applied, which could be due to the unfavourable soil conditions. It can be assumed that the soil conditions (pH and nutrient availability, since the soil pH was less than 4.9 in most of the plots and the P content was 2.23 mg 100 g^{-1} soil; Chapter 2) and the seeds used that were not treated with fungicide (since the seeds in the first experiment were treated with fungicide but the seeds in the second experiment were treated by x-ray) could be the reasons for the disparity of the results of the measured parameters (grain yield and nutrient content in plant material) and the functions of the microbial inoculants in the first and second barley field experiments. The soil pH value is associated with chemical changes in the soil; these changes can restrict the availability of essential plant nutrients and increase the availability of toxic elements (Schroth et al. 2003). Essential plant nutrients can also be leached below the rooting zone. Biological processes favourable to plant growth may be adversely affected by acidity. However, low pH soils can inhibit the survival of useful bacteria (Hollier and Reid 2005).

The results from the faba bean field experiment in 2007 confirm the hypothesis that AMF inoculation has a positive effect on faba bean growth and yield. Previous studies conducted by Kucey and Paul (1983) reported similar results. The observation in the present study also showed that grain yield and P, N, K and Mg content were significantly increased by AMF inoculation. However, this assumption was not proved with *E. radicincitans* inoculation, since the data show that the increase was not significant when the faba bean plants were inoculated with the bacteria.

5.2 Effects of the Combined Application of Organic or Mineral Fertilizer with AMF and/or with *E. radicincitans* on Plant Performance

The continuous long-term use of fertilizers with low use efficiency is an important reason behind many environmental problems. Despite the negative environmental effects and high cost, the total amount of fertilizers used worldwide is expected to increase with the growing world population due to the need to produce more food through intensive agriculture. Microbial inoculants could be suggested as promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake as well microbial inoculants could increase the efficiency of fertilizers.

5.2.1 The Combined Application of AMF/*E. radicincitans* and Organic Fertilizer

Data from the barley field experiments reveal that the soil conditions (pH value and content of elements, Chapter 2) were important factors for the functions of the combined application of the organic fertilizer with the microbial inoculants to barley plants. In the first field experiment with barley (Chapter 3), the combined application of the organic manure with both AMF and *E. radicincitans* had no significant effect on grain yield. The decrease in the nutrient content in the plant material was little when the manure was combined either with AMF or with *E. radicincitans*. This little effect of the application of the mincrobial inoculants along with organic manure could be due to the increased microbial competition in the rhizosphere caused by the addition of the organic fertilizer. The observed result is in agreement with previous studies (St John et al. 1983; Nicolson 1959; Lockwood 1990). This suggests that the individual application of the microbial inoculants or the organic fertilizer was more efficient than the combined application under the field conditions and soil properties of the first experiment (Chapter 2).

The lower seed yield in the barley experiment in 2008 compared to the experiment in 2007 could be explained by the sub-optimal soil conditions (soil pH and available nutrients) in the field experiment in 2008. No significant effects of the singular applications of AMF, *E. radicincitans*, the mineral fertilizer or the organic fertilizer, or the combined application of AMF with *E. radicincitans*, were found. In contrast, grain yield and P, N, K and Mg content were significantly increased by the combined application of the organic fertilizer with AMF or with *E. radicincitans* in the second barley experiment in the field (Chapter 3). This effect could be due to the improvement of the sub-optimal soil conditions (pH value and available nutrients) and hence the improvement of the acting conditions of the microorganisms after the application of the organic fertilizer. Grain yield was significantly increased after the combined application of the organic manure with AMF or with *E. radicincitans* in comparison to the control and to the individual application of the microbial inoculants or the organic fertilizer. Grain yield was 60% higher than the control after the combined application of the organic fertilizer in combination with *E. radicincitans* and it was 70% higher than the control when the organic fertilizer was combined with AMF. The addition of organic fertilizer to soil can increase the pH value (when the soil pH is low) and hence can improve the functions of microbial inoculants as a result. Whalen et al. (2000) found that the pH value of soil was increased after an addition of organic manure.

The soil conditions in the second field experiment (low soil pH and nutrient availability) (Chapter 2) could be the reason for the failure of the faba bean experiment. This could be because the faba bean plants were sensitive to such conditions of growth in this experiment.

In the greenhouse experiments, the combined application of the organic fertilizer with AMF and/or with *E. radicincitans* to the maize plants had little effect on the dry shoot weight or the nutrient content of the shoots of the maize plants. The combined application of the manure with both AMF and *E. radicincitans* on the barley in the greenhouse experiment significantly decreased the dry shoot weight (Chapter 4). The reason for this effect could be the increase in microbial competition in the rhizosphere after the addition of the microbial inoculants and the stimulation effect of the organic fertilizer on these microbes. Similar observations have been made previously (Nicolson 1959; St John et al. 1983; Lockwood 1990).

5.2.2 The Combined Application of AMF/*E. radicincitans* and Mineral Fertilizer

Plant responses to the inoculations differed depending on the species of the plant and the type of bio-inoculant. There was no significant effect of the combined application of the mineral fertilizer either with AMF or with *E. radicincitans* on the dry shoot weight of the barley plants in comparison to the singular applications of the inoculants or the mineral fertilizer. In contrast, the dry shoot weight of the maize was significantly decreased when the mineral fertilizer was combined with *E. radicincitans* in comparison to singular treatment with the mineral fertilizer. There was no significant effect of the application of the mineral fertilizer in combination with AMF or with *E. radicincitans* on the nutrient content in the plant shoots (Chapter 4).

A different effect was observed in the faba bean experiment. The dry shoot weight of the faba bean was significantly increased by the combined application of the mineral fertilizer with AMF. The effect with maize could be due to the increase in competition for energy resources and the reduced mycorrhizal dependency. The low effect observed of the microbial inoculants in combination with the fertilizers could be because of the soil conditions and the use of the fertilizers, since microbial inoculants could have no effet when plants get their nutrient requirements directly from the soil. Koide and Mosse (2004) and Lerat et al. (2003) reported that when nutrient supply is abundant, AMF-colonized plants are less dependent on the fungus. Also, it has previously been proved that higher nutrient supply to a substrate can suppress fungal growth (Vierheilig, 2004; Pinior et al. 1999). Regarding the metabolic reason for the suppressive effect of high nutrient supply on AMF colonization, this may be due to partial C immobilization in the plant, because high P and N availability to the plant may reduce C flow to AMF fungal structures (Olsson et al. 2005). Additonal explanation for the results of the microbial inoculants with the mineral fertilizer on the plants in the greenhouse experiment could be because of the time of sampling, which was during plant growth. Canbolat et al. (2006) observed in a barley pot experiment with bacterial inoculants and mineral fertilizer that available P and N were significantly greater in the first harvest at 15 days after planting compared with 30 and 45 days after planting, which indicated that the impact of inoculants on nutrient uptake can depend on time or the stage of growth of the plant. Also, Adesemoye et al. (2009) observed that the effectiveness of inoculants can be significantly affected by the time of sampling.

In general, the result of combined application of mineral fertilizer with the microbial inoculants was different for the different plants: it had a positive effect with the faba bean, no effect with the barley and a significant negative effect with the maize. This suggests that the effect of mineral fertilizer on plant responses to microbial inoculants is highly correlated to the plant species.

5.3 Effects of AMF and *E. radicincitans* Inoculation on Soil Microbial Parameters

There was no significant effect of the microbial applications singularly or in combination with the organic fertilizer on the soil microbial parameters (basal respiration, soil biomass and the number of P-solubilizing bacteria) in field except in the treatment of the combined application of the organic manure with *E. radicincitans* in the first barley experiment. The number of P-solubilizing bacteria showed a significant increase compared to the singular application of the manure or the bacteria (Chapter 3). This low effect of the microbial inoculants on the soil microbial parameters could be explained by the time of soil sampling from the plots, since the samples were taken one week after harvesting. The soil was dry and the plant roots had already died before the harvest, since the plants were harvested in the mature stage. It is often difficult to interpret field measurements under field conditions. Sparling (1997) reported that soil respiration rates are characteristically variable and can show wide variation depending on such factors as soil water content, temperature and substrate availability. However, the microbial analyses showed increases in the treatments with the microbial inoculants and the organic fertilizer, but they were not correlated to the yield and nutrient content in the plant material. This could also be because of the low effect of the microbial inoculants and the fertilizers, as mentioned earlier.

Regardless of the non-significant differences in the results of the microbial parameter analyses, it was observed from the soil chemical analysis of the field experiments (Chapter 2) and the soil microbial analysis of the field experiments (Chapter 3) that the soil microbial parameters showed correlation to the soil chemical parameters. This implies that soil microbial parameters should be an indicator for the evaluation of soil fertility and quality, related to the nutrient availability in the soil.

5.4 Perspectives on Further Research

The present study has provided valueable information on the singular and combined application of AMF and *E. radicincitans* in the greenhouse and field experiments. For practical application of the microbial inoculants, further study will be required in the following area:

1- The development of a more efficient and successful method of inoculating plants with AMF preparation, to avoid the methodological problem of the first experiment (the seeds were treated with fungicide).

2- Further investigation of the effects of the microbial inoculants on the efficiency of the mineral fertilizers under field conditions in the region of study.

3- The effect of the combined application of AMF and *E. radicincitans* with organic manure in the second field experiment of barley suggests the need for further research on the effects of the different microbial inoculants with the manure with different crop plants, under the condition of low pH soils.

4- The evaluation of further parameters, such as root colonization with AMF and real-time polymerase chain reaction (PCR), as well as the measurement of further chemical elements in plant material, such as micro-nutrient content.

5- Since it was demonstrated that time of sampling (i.e. the plant's stage of growth) significantly affected the effectiveness of the inoculants, taking plant samples from experiments in different stages of growth would give a better understanding of the efficiency of the microbial inoculants.

6- The investigation of the effect of the time of soil sampling in a field experiment on soil microbial parameters, as well as the correlation of soil microbial analyses to plant growth and nutrient content.

7- There is an urgent need for integrated nutrient management that targets agricultural inputs and lowers the adverse environmental impacts of agricultural fertilizers and practices. A better understanding of the interactions between microbes, fertilizers and plants is very important. There is a need for more information along the models previously discussed.

8- The importance of the interactions among the host plant, AMF and the rhizosphere bacteria in the soil requires more research. These interactions must be clearly elucidated as they can have significant effects in agriculture and ecology. In addition to their individual functioning in soil, the combined effects of soil microbes are also very important, as the production of bio-inoculants and their enhancing effects on soil structure and plant nutrient uptake can increase plant growth and hence crop yield. So, future research may focus more precisely on the interactions among the host plant, AMF and soil bacteria for the more efficient use of soil microorganisms for the development of advanced agricultural strategies.

Chapter 6

6 Summary

AMF and PGPR can be beneficial to crop plants due to their nutrient acquisition properties and stimulation of plant growth. These characteristics of AMF and PGPR make it possible for them to be applied to agricultural production process.

The present work focuses on the prospects of AMF and PGPR to solve plant nutritional problems. The use of bio fertilizers improves the opportunity of sustainable plant production and hence reduces the negative effects of the chemical fertilizers on the environment due to the reduction of chemical inputs in agricultural production.

The contributions of AMF and PGPR to plant production and nutrition were investigated with barley and faba bean plants in field and greenhouse conditions and with maize in greenhouse conditions. The effects of the singular and combined applications of the microbial inoculants were investigated in field and greenhouse conditions. In addition, mineral and organic fertilizers were combined with AMF and/or with *E. radicincitans* to investigate the effects of these fertilizers on the functions of the microbial inoculants.

Grain yield, shoot yield and content of N, P, K and Mg in the plant material were measured. Also, the effects of the microbial inoculants singularly or in combination with mineral or organic fertilizers on soil microbial parameters (such as basal respiration, microbial biomass and the most probable number of P-solubilizing bacteria) were investigated, since the determination of soil microbial parameters may result in information about the soil fertility status. The application of PGPR and AMF to plants and soils is expected to promote plant growth and production and to enhance the nutritional status of soils, which in turn also affects soil microbial activities.

AMF and *E. radicincitans* showed a positive effect when they were applied singularly in the first field experiment: the grain yield of the barley was increased by 51% after AMF inoculation and by 55% after *E. radicincitans* inoculation in comparison to the control treatment; P and N content were significantly increased after inoculation with AMF or with *E. radicincitans*. However, there was no significant effect of the combined application of the manure with AMF or with *E. radicincitans* under the conditions of the field experiment in 2007 (Chapter 3). In the faba bean experiment, grain yield was increased by 40% after the plants were inoculated with AMF. Also, nutrient content was increased by AMF application in the faba bean field experiment in 2007, but grain yield and nutrient content did not increase significantly after *E. radicincitans* application (Chapter 3).

The singular application of AMF or *E. radicincitans* showed no effect on grain yield or nutrient content in the second barley field experiment in 2008. The extreme soil acidity could be the reason for the contradictory results with the first experiment. The combined application of the organic manure with the microbial inoculants significantly increased grain yield and nutrient content. The grain yield of the barley was increased by 95% after the application of organic fertilizer and *E. radicincitans*, and it was increased by 106% after the application of organic fertilizer and AMF in comparison to the control treatment (Chapter 3).

The singular application of AMF or *E. radicincitans* in the greenhouse had no significant effect on shoot yield. Also, the combined application of organic or mineral fertilizer with AMF and/or *E. radicincitans* generally had no significant effect on the dry shoot weight of the barley and maize, but shoot yield was significantly decreased in the treatment of organic fertilizer with AMF and *E. radicincitans* with the barley in the second experiment and in the treatment of mineral fertilizer with *E. radicincitans* with the maize in the second experiment. Nutrient content was not significantly affected either by the singular application of the microbial inoculants or by combination with the fertilizers (Chapter 4).

In the faba bean greenhouse experiment, inoculation with AMF or with *E. radicincitans* did not significantly affect the shoot yield or nutrient content; only P content was significantly increased by AMF inoculation. Faba bean shoot yield and P content were significantly increased after the application of mineral fertilizer in combination with AMF (Chapter 4).

In the field experiments, both microbial inoculants showed no significant effects on soil microbial parameters (basal respiration, microbial biomass and the most probable number of P-solubilizing bacteria) (Chapter 3). There was an increase in the microbial parameters after the application of both microbial inoculants, but this not significant with the barley and maize. Soil basal respiration and soil biomass in the faba bean plants were significantly increased after the application of AMF with mineral fertilizer.

Overall, the results of these experiments lead us to the conclusion that plant inoculation with both AMF and *E. radicincitans* improved grain yield and plant nutrient status under the described experimental conditions. However, plant responses were varied, depending on the plant species and the conditions of the experiment (field or greenhouse) with the same plant. The addition of organic fertilizer to microbial inoculants could be beneficial, but the beneficial effect of the organic fertilizer in combination with the microbial inoculants was highly related to the soil conditions in this study.

7 Zusammenfassung

Der Einsatz von mikrobiellen Impfmitteln, wie arbusculären Mykorrhizapilzen (AMF) und pflanzenwachstumsfördernden Rhizobakterien (PGPR) in der Landwirtschaft kann einen positiven Einfluss auf das Pflanzenwachstum von Kulturpflanzen haben. Daraus ergeben sich Möglichkeiten zur Reduzierung des Einsatzes von Düngemitteln.

In der vorliegenden Arbeit wurde die Wirkung von AMF und *E. radicincitans* auf die Nährstoffaufnahme und das Pflanzenwachstum von Gerste (*Hordeum vulgare*) und Ackerbohne (*Vicia faba*) in Feld- und Gefäßversuchen, sowie von Mais (*Zea mays*) im Gefäßversuch untersucht.

Dabei wurde die Wirkung der Einzelapplikation sowie der kombinierten Applikation der Mikroorganismen getestet. Weiterhin wurde der Einfluss unterschiedlicher Düngungsvarianten auf die Wirksamkeit der mikrobiellen Impfmittel untersucht. Es wurden Kombinationen von AMF und / oder *E. radicincitans* mit mineralischen oder organischen Düngemitteln in den Gefäßversuchen im Gewächshaus, sowie die Kombinationswirkung der mikrobiellen Impfmittel mit einer organischen Düngung im Feldversuch mit Gerste getestet.

Als Ertragsparameter wurden Kornertrag und oberirdische Biomasse (Trockenmasse) untersucht. Zudem wurde die Nährstoffaufnahme von N, P, K und Mg in die Pflanze ermittelt. Überdies wurde in dieser Arbeit der Einfluss der mikrobiellen Impfmittel alleine oder in Kombination mit mineralischen und organischen Düngemitteln auf die mikrobielle Aktivität im Boden untersucht. Es wurden die Basalatmung, der mikrobielle Biomasse-Kohlenstoff, sowie die wahrscheinlichste Anzahl der gesamten kultivierbaren Bakterien und der P-lösenden Bakterien bestimmt.Es wurde angenommen, dass es durch die Applikation der AMF und PGPR zu einer Erhöhung der mikrobiellen Aktivität und damit zur Förderung des Pflanzenwachstums sowie der Bodenfruchtbarkeit kommt.

Die Ergebnisse zeigen, dass sich nach der Applikation von AMF im Feldversuch bei Gerste der Kornertrag um 56 % im Vergleich zu den Kontroll-Behandlung erhöhte. Nach Beimpfung mit *E.radicincitans* kam es zu einer Steigerung um 51 %. Auch der P- und N-Gehalt des oberirdischen Pflanzenmaterials erhöhte sich signifikant nach der Impfung mit AMF und *E. radicincitans*. Die kombinierte Anwendung von AMF oder *E. radicincitans* mit Rindergülle zeigte im Feldversuch 2007 keine nachweisliche Ertragswirkung.

Der Kornertrag von Ackerbohne erhöhte sich im Feldversuch 2007 um 40 % im Vergleich zur Kontrolle, nachdem die Pflanzen mit AMF beimpft wurden. Auch die Nährstoffaufnahme war verbessert. Keine Wirkung konnte hingegen nach einer Ausbringung von *E. radicincitans* bezüglich Kornertrag und die Nährstoffaufnahme von Ackerbohne festgestellt werden.

Die Einzelanwendung von AMF oder *E. radicincitans* hatte im zweiten Feldversuch (2008) bei der Gerste keinen Effekt auf den Kornertrag und die Nährstoffaufnahmen. Die Ursache für die sehr unterschiedlichen Ergebnisse beider Jahre sind möglichereise auf die extremen Bodenbedingungen (Bodensäure und die Verfügbarkeit von Nährstoffen) im Jahr 2008 zurückzuführen (s. Kapitel 2). Nach der kombinierten Anwendung der mikrobiellen Impfmittel mit dem organischen Düngemittel waren bei Gerste der Kornertrag (AMF: 95 %; *E. radicincitans*: 106 %) und der Nährstoffgehalt im Vergleich zur Kontrolle erhöht.

Im ersten Gefäßversuchen mit Gerste und Mais im Gewächshaus konnte kein signifikanter Einfluss der AMF- oder *E. radicincitans*- Einzelanwendung bzw. der kombinierten Anwendung mit organischen auf den Ertrag nachgewiesen werden. Im zweiten Versuch wurde bei Gerste sogar eine Ertragsdepression in der Variante organische Düngung + AMF + *E. radicincitans*, und bei Mais in der Variante mineralischer Düngung + *E. radicincitans* festgestellt. Die Nährstoffaufnahme der Pflanzen wurde in den Gefäßversuchen weder durch die Einzelapplikation der Mikroorganismen noch durch die Kombination dieser mit den Düngemitteln beeinflusst.

Der Ertrag und die Nährstoffaufnahmen von Ackerbohne wurden im Gefäßversuch ebenfalls nicht durch die AMF - Impfung oder *E. radicincitans*-Applikation beeinflusst. Lediglich der P-Gehalt im Pflanzenmaterial stieg nach der AMF-Applikation signifikant an. Die Kombination von AMF mit mineralischem Dünger hatte hingegen einen Anstieg von TM-Ertrag und P-Aufnahme zur Folge.

Die bodenmikrobiologischen Parameter (Basalatmung, Kohlenstoff aus mikrobieller Biomasse, wahrscheinlichste Anzahl der gesamten kultivierbaren Bodenorganismen) wurden in den Feldversuchen größtenteils nicht von den mikrobiellen Applikationen beeinflusst. Bei Gerste und Mais zeigten sich lediglich tendenzielle Veränderungen der mikrobiellen Parameter. Bei Ackerbohne hingegen waren Basalatmung und Boden Biomasse nach der Applikation von AMF in Kombination mit mineralischem Dünger erhöht.

Die Ergebnisse aller Versuche zeigen, dass die Impfung von Pflanzen mit AMF und *E. radicincitans* unter den gegebenen Versuchsbedingungen zur Ertragssteigerung und Verbesserung des Nährstoffstatuses der Pflanzen führen kann. Die Wirkung der mikrobillen Impfstoffe variierte jedoch in Abhängigkeit von der Pflanzenart und den Versuchsbedingungen (Freiland/ Gewächshaus). Die Kombination der mikrobiellen Impfstoffe mit organischem Dünger kann sinnvoll sein, besonders bei niedrigen pH-Werten des Bodens.

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9 Appendix

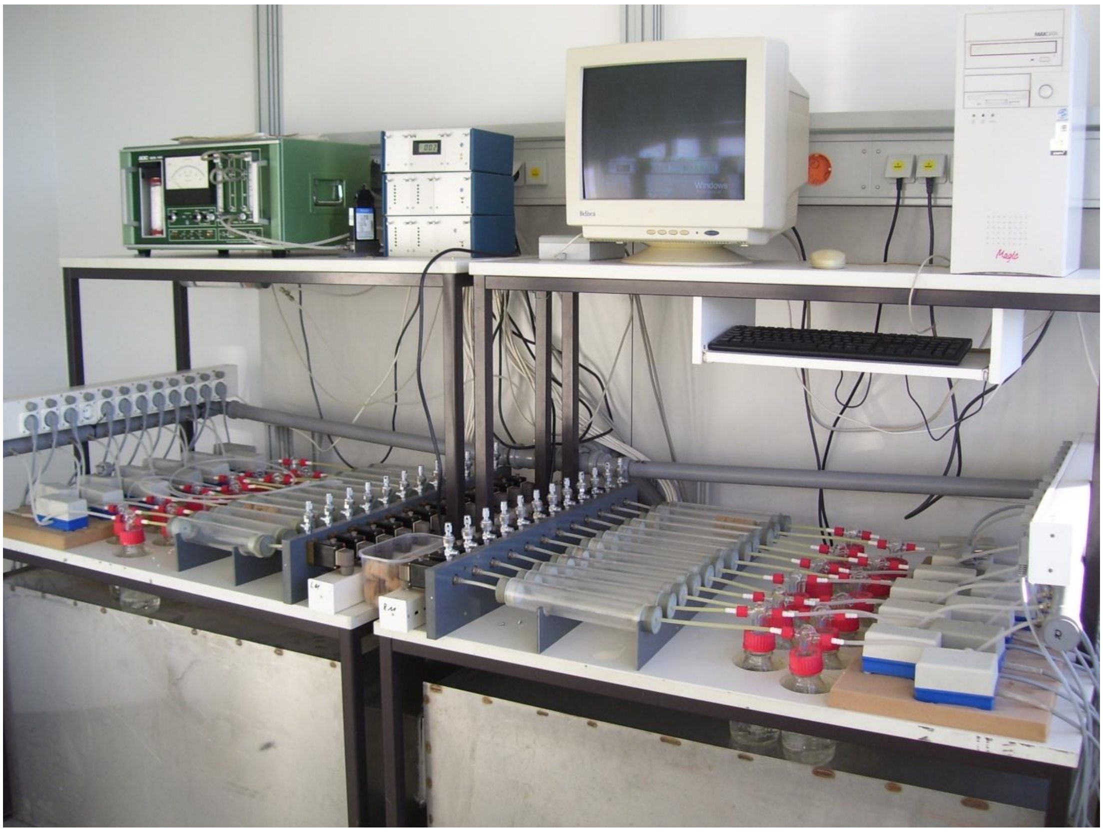
Individual or combined influence of some soil properties on the percentage of root colonization by AMF in different plants.

Soil treatments and interactions	Peanut	Sorghum	Maize
Plant × Soil texture ($P \leq 0.01$; l.s.d. 4.11)			
Field texture	47.4 Aa	37.9 Ab	48.1 Ba
Sandy texture	47.4 Ab		53.4 Aa
Clayey texture	46.1 Ab	35.8 Ac	51.1 Aba
		29.7 Bc	
Plant × Phosphorus ($P \leq 0.01$; l.s.d. 3.35)			
Phosphorus	44.7 Bb	34.7 Ac	51.6 Aa
No Phosphorus	49.2 Aa	34.2 Ab	50.2 Aa
Plant × Phosphorus × organic matter ($P \leq 0.01$; l.s.d. 4.74)			
Phosphorus × Organic matter	42.9 Bb	31.9 Bc	51.5 Aa
Phosphorus × no Organic matter	46.6 Ab	37.5 Ac	51.6 Aa
No phosphorus × Organic matter	45.8 Ba	35.3 Ab	50.4 Aa
No phosphorus × no Organic matter	51.6 Aa	33.1 Ab	49.9 Aa

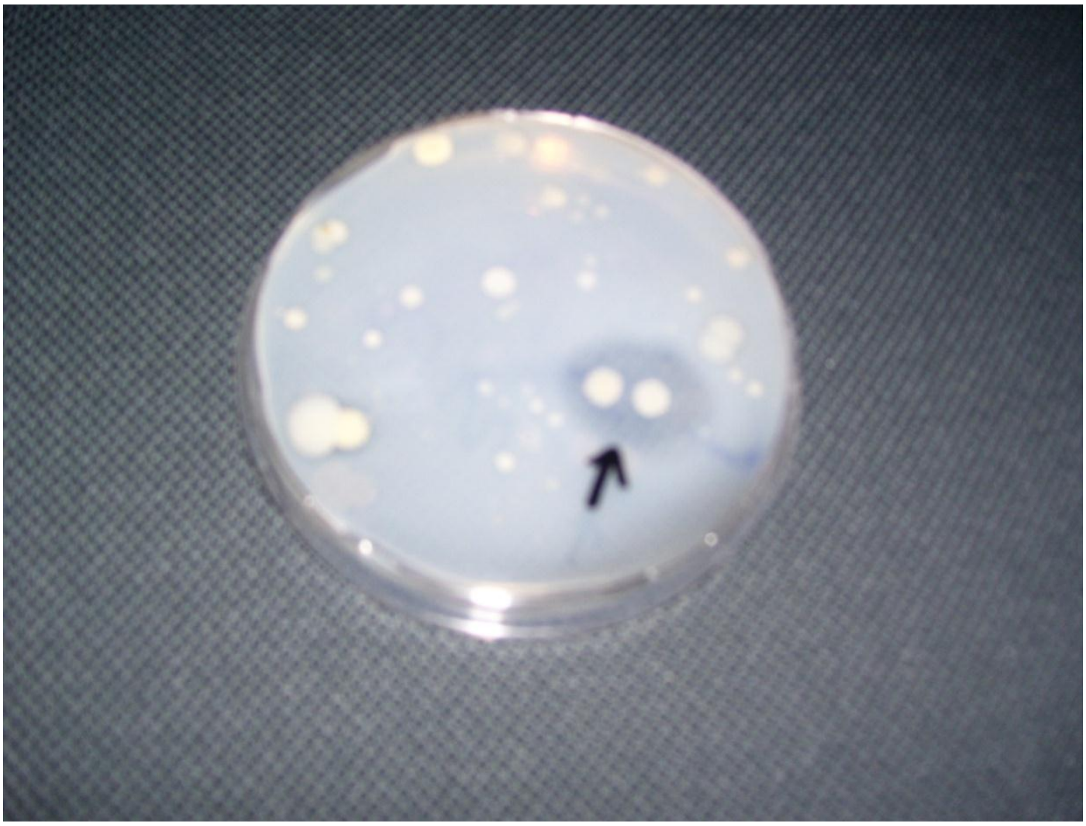
l.s.d. = least significant difference. Averages followed by the same letters do not differ from each other, at the 5% level, according to Tukey's Studentized Range Test; lower case letters refer to the lines, upper case letters refer to the columns (Carrenho et al 2007)

Properties of AMF product used in the experiments (INOQ 2007)

Test parameter	Value	Method
Carrier material	Expanded clay	
Grain size [mm]	2-4	
specific weight [$g\ l^{-1}$]	300	
pH	7.5	External analysis
Mycorrhizal Fungi	*Glomus etunicatum* *G. intraradices* *G. claroideum*	
Content of fertilizer of the substrate [$mg\ l^{-1}$] Salt content (NPK)	100 Not extractable	External analysis
Most probable number of propagules (MPN) (on Tagetes erecta plena [$n\ cm^{-3}$])	140 ± 29	MPN test
Germination inhibition	None	Bioassay
Fungal contaminants Bacterial contaminants	None	External analysis DNA multiscan
Pathogenity of contaminants	None	
Potential phytophageous faunistic Contaminants Diptera, Coleoptera - larva, Collembola, Acari Nematoda, Gastropoda	None None	
Botanical contaminants	None	
Storage	2 years, under cool and dry conditions	



Heinemeyer Infrared Gas Analyzer at the Leibniz Institute of Vegetables and Ornamental Crops in Großbeeren



Phosphorus solubilizing bacterial colonies on Muromcev solid medium

Field Experiment 2007

Maize, barley and faba bean plants in green house experiment

10 Lebenslauf

CURRICULUM VITAE

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CONFERENCES AND ACTIVITIES

1. Almethyeb, M.; Ruppel, S.; Eichler-Loebermann, B. (2009): Vesicular Arbuscular Mycorrhiza Fungi and plant growth promoting bacteria increase the yield, phosphorus and nitrogen uptake of Vicia Faba plants. In: AgrosNet: Doktorandentag, Berlin. , 2009.
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3. Almethyeb, M.; Ruppel, S.; Eichler-Loebermann, B. (2007); Gesellschaft für Pflanzenbauwissenschaften: Nutzung von Mikroorganismen für eine verbesserte P-Versorgung im Pflanzenbau. 19: 50. Jahrestagung in Bonn (18.-20. September 2007).

PUBLICATIONS

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Aus der Professur für Pflanzenbau
der Agrar- und Umweltwissenschaftlichen Fakultät

The Influence of Singular and Combined Applications of Arbuscular Mycorrhizal Fungi and *Enterobacter radicincitans* on Growth and Nutrition of Plants

Dissertation

zur Erlangung des akademischen Grades

Doktor der Agrarwissenschaften (doctor agriculturae)

an der Agrar- und Umweltwissenschaftlichen Fakultät

der Universität Rostock

vorgelegt von

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Rostock, April 2014

Theses

Problem and Research Approach

- As the world’s population grows, agricultural production needs to supply its higher demand in terms of nutrition.

- The excessive addition of fertilizers may increase crop productivity but at the same time can cause extensive damage to ecosystems. This is especially true for phosphorus (P), which represents an essential plant nutrient which cannot be substituted. Since mineral P resources are limited and prices for mineral P fertilizers rise, new approaches to ensure the P supply in crop husbandry have to be placed on top of the research agenda.

- The term biofertilizers covers a wide range of beneficial microorganisms such as bacteria and fungi which are able to potentially promote plant growth based upon their impact on the soil P cycle, especially in the rhizosphere. Biofertilizers could also be effective to improve fertilizers use efficiency. As a consequence to the application of the biofertilizers, not only costs may be reduced but also potential harm to ecosystems.

- The biofertilizers applied in former studies include arbuscular mycorrhiza fungi (AMF) the plant growth promoting rhizobacteria (PGPR). Promising results on plant P uptake stimulation and growth enhancements were reported.

- Arbuscular mycorrhiya fungi form beneficial relationships with plant roots (80 % of plant species), which could improve the nutrient uptake of their host plants.

- *Enterobacter radicincitans* DSM 16656 are one type of PGPR showing an ability to increase the growth and yield of different agricultural plants.

- A combined application of the bio inoculants with organic or mineral fertilizers could help to reduce the amount of fertilizers and to increase the fertilizer use efficiency.

- In pot experiments, the effects of the single application of AMF and *Enterobacter radicincitans* were investigated with three crop plants (barley (*Hordeum vulgare*), faba bean (*Vicia faba*) and maize (*Zea mays*)). The effects of combined application of AMF and *Enterobacter radicincitans* with mineral or organic fertilizers were investigated as well.

- The field experiments were established to have a practical evaluation of the effects of AMF and *Enterobacter radicincitans* applied alone or in combination with organic fertilizers on biomass production of barley (*Hordeum vulgare*) and faba bean (*Vicia faba*).

Main Results and Future Outlook

- The application of AMF showed the ability to increase grain yield and nutrient uptake of barley under field conditions (moderate pH), whereas the combined application of AMF and organic fertilizers did not show further adventages.

- *Enterobacter radicincitans* DSM 16656 also revealed the ability to increase grain yield, P and N content in the field experiment. The combined application of *Enterobacter radicincitans* and organic fertilizers did not affect barley growth.

- Applied on soil with low pH (pH = 4.9) neither AMF nor *Enterobacter radicincitans* affected the growth of barley.

- The application of organic fertilizers could improve the effects of AMF and *Enterobacter radicincitans* when applied to soils with low pH. In the field experiment with low soil pH a combined applications of organic fertilizer with AMF or with *Enterobacter radicincitans* have shown the ability to increase yield and nutrient uptake of barley

- AMF application can improve growth and nutrient uptake of faba beans, as it was shown in a field experiment conditions (moderate pH).

- In pot experiments under greenhouse conditions, single and combined applications of AMF and *Enterobacter radicincitans* have shown different effects on plant growth in dependence on the tested plant.

- Microbial inoculants could be ineffective or even detrimental under certain conditions. The ineffectiveness could be due to a high microbial activity as a result of organic fertilization, or due to nutrient competition with the native soil micorflora.

- AMF application in presence of the organic fertilizers reduced P uptake of maize plants, while AMF single inoculation increased shoot yield of maize and P uptake of faba bean plants in a pot experiment.

- Combined application of AMF and mineral fertilizers increased faba bean shoot yield in a greenhouse experiment. At the same time the application of *Enterobacter radicincitans* decreased shoot yield of maize plants when combined with mineral fertilizers.

- The application of microbial inoculants could affect soil microbial parameters. The effect of AMF and *Enterobacter radicincitans* on basal respiration, soil biomass and the number of P solubilizing bacteria was different and depended on plant species and experimental conditions (field or greenhouse).

- The main results of this work indicate the ability of *Arbuscular mycorrhiza fungi* and *Enterobacter radicincitans* to increase plant growth and plant nutrition, and hence crop plant production under climatic conditions of Middle Europe. For the future research, there is a need to improve application methods for AMF.

- The effect of the combined application of *Arbuscular mycorrhiza fungi* or *Enterobacter radicincitans* with organic fertilizers was investigated under field conditions, a similar study should be carried out to investigate the impacts of a combined application of the microbial inoculants with mineral fertilizers.

Spelle, April 2014

Malek Almethyeb