INTERACTIONS OF CROP PLANTS AND ARBUSCULAR MYCORRHIZAL FUNGI

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FOREWORD

This dissertation is a cumulative work of manuscripts, either published or submitted, selected from my publication list. Therefore, this thesis is based on the following papers which are referred by their Roman numerals. The bibliographic references cited through all chapters are listed together after **Chapter 6**.

Antunes PM, Lehmann A, Hart MM, Baumecker M, Rillig MC (2012) Long-term effects of soil nutrient deficiency on arbuscular mycorrhizal communities. Functional Ecology 26: 532-540.

I. Lehmann A, Barto EK, Powell JR, Rillig MC (2012) Mycorrhizal responsiveness trends in annual crop plants and their wild relatives - a meta-analysis on studies from 1981 to 2010. Plant and Soil 355: 231-250.

II. Lehmann A, Rillig MC. 201X. Are there temporal trends in root architecture and soil aggregation for *Hordeum vulgare* breeding lines? Applied Soil Ecology 65: 31– 34.

Leifheit EF, Veresoglou SD, Lehmann A, Rillig MC (2013) Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation - a meta-analysis. Plant and Soil. DOI 10.1007/s11104-013-1899-2.

III. Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2013) Arbuscular mycorrhizal influence on Zinc nutrition in crop plants - a meta-analysis. S*ubmitted to Soil Biology and Biochemistry.*

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CHAPTER 1

General Introduction

Agriculture

The demands on agriculture change with time. Once, the major goal was providing humans and livestock with food and resources by cultivating suitable land. However, with the ever-growing human population, increasing aspirations and environmental awareness, new demands become increasingly important.

The intensification of agriculture and breeding of new, disease tolerant and productive crop plants caused an unprecedented maximizing of yield (Matson et al. 1997). In such a highly productive system, the application of synthetic fertilizers, pesticides, herbicides and the usage of tillage and intensified irrigation are necessary for the achievement of optimal output. However, the side effects are severe and appear over time; high input of fertilizer and pesticides led to pollution of groundwater, the maximized output exhausted the soil, while other management practices amplified erosion and water consumption (Matson et al. 1997; Stoate et al. 2001).

Despite these already existing problems, the world population is still increasing. In the year 2050, about 10 billion people will live on our planet and need to be supplied with food and other agricultural products (www.unfpa.org). An additional obstacle is the reduction of agricultural areas due to losses to urbanization and desertification (Tan et al. 2005; Verburg et al. 1999). Hence, more food has to be grown on increasingly sub-optimal soils. The agriculture of today and tomorrow has to accomplish higher outputs and stress resistance towards climatic and edaphic factors, e.g. salt, drought and deficient essential soil nutrient concentrations. Furthermore, the future agriculture has to focus on enhanced nutrient uptake efficiency and bioavailability in crop plants and improve environmental parameters, like soil

health, soil stability, nutrient and fertilizer retention and maintenance of biodiversity (Brummer et al. 2011). In essence, the agriculture of tomorrow has to fulfil one aspect: sustainability.

In sustainable, organic agriculture soil health and support of ecological processes are the main focus and thus they can profit from ecosystem services provided by the rhizosphere (Pimentel et al. 2005). The rhizosphere describes the sphere of influence of root, soil and soil inhabiting biota (Hiltner 1904). For improvement of soil quality and health, agrochemicals are not allowed in sustainable agriculture. Furthermore, problematic management practices like tillage are reduced or replaced by new techniques (Phillips et al. 1980; Triplett and Dick 2008). More promising advantages of sustainable, organic agriculture in comparison to conventional agriculture are reduced nutrient and agrochemical- leaching, higher carbon storage, reduced erosion, improved water conservation, higher soil organic matter and improved biodiversity (Bengtsson et al. 2005; Drinkwater et al. 1995; Kreuger et al. 1999; Mäder et al. 2002; Pimentel and Edwards 1982; Pimentel et al. 2005; Reganold et al. 1987). These potential positive impacts on soil and thus our livelihood are essential for a future concept of agricultural sustainability. The reduction or elimination of management practices impairing the soil physically or chemically influence soil organisms as well; both pests and beneficial organisms are affected (Matson et al. 1997; Oehl et al. 2003; Tonhasca and Byrne 1994). Among the group of beneficial organisms, arbuscular mycorrhizal fungi (AMF) are a well researched and important group of soil microorganisms influencing ecological processes (Rillig 2004; Smith and Read 2008; van der Heijden et al. 2008; Wardle et al. 2004) and thus are of paramount interest for sustainable agriculture (Hart and Trevors 2005).

Arbuscular mycorrhizal fungi

AMF are members of the *Glomeromycota* and are ubiquitously present in terrestrial ecosystems. They can be found in both natural ecosystems and agricultural sites; thus they are

an integral root component of plants capable of forming this symbiotic interaction purportedly since the advent of land plants over 400 million years ago (Remy et al. 1994; Smith and Read 2008).

They are obligate biotrophic symbionts and are capable of interacting with the majority of land plants, and therefore many crop plants (Smith and Read 2008). However, plant species and even genotypes vary in their responsiveness to AMF; responsiveness describes the difference in plant growth of colonized compared to non-colonized control plants (Janos 2007). An additional important factor influencing responsiveness to AMF is the soil nutrient status. High concentrations, especially of P, often lead to reduced responsiveness due to a reduced role of AMF in mediating nutrient uptake (Cavagnaro and Martin 2011; Marschner and Dell 1994; Paszkowski 2006). Besides enhanced nutrient uptake via the additional mycorrhizal pathway, AMF are known for additional beneficial functions and hence effects on associated plants, e.g. increased stress tolerance against biotic (pathogens, soil herbivory), abiotic factors (salt, drought, high or low soil pH) and improvement of soil quality (stability and reduced leaching) (Borowicz 2001; Evelin et al. 2009; Garg and Chandel 2010; Six et al. 2004; Strauss and Irwin 2004; van der Heijden 2010; Veresoglou and Rillig 2012). This multifunctionality is strongly influenced not only by plant traits but by fungal traits as well (Newsham et al. 1995; Smith et al. 2003). Different AMF species diverge functionally, i.e. different AMF species or even isolates show a heterogeneous performance, like hyphal growth, fungal P uptake and fungal root colonization (Allen et al. 1995; Munkvold et al. 2004). A diverse AMF community comprises more potentially beneficial or complementary species showing a range of functions and grades of compatibility with different plants (Hoeksema et al. 2010; van der Heijden et al. 1998). Agricultural sites with reduced plant diversity can also harbor a diverse AMF assemblage; in particular, sustainable, organic management practices seem to positively influence AMF communities resulting in

higher propagule density, root colonization and AMF species richness (Hijri et al. 2006; Oehl et al. 2004).

Crop plants

Crops are cultivated plants selected for desired traits. About 12.000 to 5.000 years ago, domestication of all major crops (soja, maize, barley, wheat and rice) began; via natural selection wild plant species were transformed into cultivars with increased yield production (Doebley et al. 2006). In general, modern genotypes differ in several traits from their wild ancestors: they have fewer but bigger fruits, a more pronounced apical dominance and alterations in seed dormancy, stress tolerance and timing of flowering (Doebley et al. 2006). These changes are commonly known as the domestication syndrome.

During the 19th century, the rules of plant breeding were significantly changing. The first artificial fertilizer, superphosphate, was used for yield increase. The Mendelian theory laid the foundation for the targeted use of hybridization and the heterosis effect (Palladino 1993); hybrid genotypes exhibited higher yield as compared to their inbred parental lines. Plant breeding gave rise to new varieties responding strongly to fertilizer with increased yield and reduced longitudinal growth, e.g. dwarf wheat genotypes. Norin 10 is the famous progenitor of modern high-yielding wheat cultivars produced by Norman Borlaug and colleagues (Dalrymple 1985; Reitz and Salmon 1968). These and other high-yielding crop varieties and improved agricultural techniques (high water irrigation, pesticide and fertilizer use) increased food production around the world (Wissuwa et al. 2009); this period with its set of management practices and high yielding cultivars was called the Green Revolution. However, the development of the modern crop species with their long history of trait selection led to limited genetic material (Doebley 1989). Comparisons of allele richness of landraces (old, local domesticated varieties) and modern genotypes revealed a reduction in genetic diversity, a genetic bottleneck (Fu et al. 2005; Nersting et al. 2006).

Interaction of AMF and crops in agriculture

AMF-mediated services depend on several factors, e.g. plant and AM fungal traits, edaphic and direct or indirect effects affecting plant, fungi or both simultaneously (Smith and Smith 2011; Tawaraya 2003). Besides these factors often addressed in research, the breeding under high fertilizer conditions of potential host plants for AMF, especially of high yielding varieties, might have inadvertently led to reduced responsiveness of crop plants to AMF and changes in root architecture. Root architecture is defined as the spatial configuration of the root system over time (Lynch 2007). It is essential for the plant to explore soil, acquire nutrients and interact with soil organisms. Screening of cereal genotypes revealed that modern cultivars tend to have reduced length of primary roots in corn (Sanguineti et al. 2006), decreased total root length density and reduced total seminal root length in barley (Bertholdsson and Kolodinska-Brantestam 2009; Zhu et al. 2003).

The breeding conditions and techniques changed fundamentally since the 19th century. The system of natural selection, that produced local adapted varieties (landraces), was replaced by new breeding techniques to produce highly productive cultivars (Harlan 1975). The connection between genotype age (expressed with release year) and responsiveness to AMF was and still is the objective of numerous studies whose results are inconsistent. Some studies show that genotypes with a release year before 1950 consistently exhibit a positive AMF-mediated growth response compared to cultivars released after 1950 (Hetrick et al. 1992, 1993; Zhu et al. 2001), while other studies provided evidence for an opposing trend (Bryla and Koide 1998; Koide et al. 1988). However, there are also studies refuting a possible effect of the release year on mycorrhizal responsiveness (Galvan et al. 2011; Sawers et al. 2010). Different approaches (experiments under controlled conditions or in the field, statistical modelling) were used to find general patterns for this varying responsiveness of modern cultivars compared to older accession or landraces. If there would be any difference

in responsiveness between cultivars and landraces, this should affect breeding strategies for sustainable, organic agriculture.

An additional impact of plant breeding could be mediated indirectly via the contribution of plant root architecture and associated AMF to soil stability and thus soil health, the basis for any productive agriculture. Soil structure is characterized by the spatial arrangement of particles and pores of different size and shape (Bronick and Lal 2005). These particles "cohere to each other more strongly than to other surrounding particles" (Soil Science Society of America 1997) and are called soil aggregates. Soil aggregate formation is mediated by physical and chemical binding agents and the activity of soil microorganisms, e.g. AMF (Bronick and Lal 2005; Jastrow et al. 1998; Rillig and Mummey 2006). In combination with plant root systems, an extensive hyphal network is formed leading to soil entanglement, and their subsequent stabilization by the production of exudates by both AM fungi and associated plants (Jastrow et al. 1998; Miller and Jastrow 1990). Aggregate stability and with that soil stability is a decisive factor for erosion prevention, and the promotion and maintenance of soil health (Barto et al. 2010; Elliott 1986; Gianinazzi et al. 2010).

Breeding for high output had other verifiable side effects on plant performance; while grain biomass increased due to introduction of dwarfing alleles, the micronutrient content (Zn, Fe and Cu) remained more or less constant resulting in a reduced nutrient concentration. This dilution effect is evident in wheat germplasms (Fan et al. 2008; Garvin et al. 2006; Gooding et al. 2012); germplasms are collections of genetic material like seeds and plants. Consequently, the question arose if the diverse and maintained soil biota in sustainable, organic agriculture are capable of compensating for this negative effect and increase micronutrient concentration in plant tissues. AMF are known for the uptake and transfer of immobile nutrients such as P and Zn (e.g. Marschner and Dell 1994; Bolan 1991; Jansa et al. 2003), especially when they are limiting. In this context, AMF were suggested as potential biofortification agent (He and Nara 2007); the term biofortification describes a technique that permits increasing

bioavailable concentrations of essential minerals in the edible portions of crops (White and Broadley 2005). Inedible plant portions, e.g. shoots and roots, also gain increased Zn concentrations and can be further processed to green manure or compost and used as a sustainable and organic Zn fertilizer resource (Mishra et al., 2006).

As illustrated, the interaction of plants and soil microorganisms is of problematic and complex nature in agricultural systems. Plants and soil microorganisms have to cope with conditions that are not natural and appeared relatively recently in relation to their evolutionary history. Sustainable, organic agriculture makes no use of high input management but, nevertheless, has to produce high output to be profitable and competitive on the market. Therefore, if any detrimental alterations due to plant breeding practices are present in germplasms applied in sustainable, organic agriculture they will become manifest and reduce yield amount and quality. This has to be prevented to meet the needs and demands of a future-oriented sustainable agriculture (Gianinazzi et al. 2010).

Thesis outline

The interaction framework of AMF, crop plant and influencing biotic and abiotic factors in the range of sustainable, organic agriculture is complex and hence the aim of the present dissertation is to focus on three topics identified in the introduction:

I. Effect of breeding history on AMF-mediated growth promotion (Chapter 2)

II. Effect of breeding history on root architecture and hence soil stability (Chapter 3)

III. Role of AMF for Zn nutrition of crops (Chapter 4).

The topics identified were addressed in the following three chapters. For chapter 3 a greenhouse experiment was performed, while chapter 2 and 4 are meta-analyses. Meta-

analysis is as statistical tool for quantitative data synthesis (Borenstein et al. 2009). They have the advantage of objective analysis of data collections with effect size weighing and specific and well-defined inclusion criteria to answer questions of broad interest. In contrast, approaches which qualitatively analyze data obtained from primary literature (review articles) are potentially biased by subjective quality assessment and may be affected by potentially flawed weighing of data.

Chapter outline

The release year of a crop plant cultivar reflects the agricultural and breeding practices of its time. Therefore, in *chapter 2*, we conducted a meta-analysis on 39 publications working on 320 different crop plant genotypes with an identified release year or a distinct attribution to one of the three defined release year groups (ancestor, old or new).

We hypothesized that there are differences in mycorrhizal responsiveness of new, old and ancestral genotypes. Furthermore, we analyzed the effect of experimental treatments, P efficiency (P acquisition and P utilization efficiency) and AMF root colonization on a potential mycorrhizal responsiveness trend for the release year.

In *chapter 3*, we tested for a potential impact of plant breeding on root architecture and hence possible negative effects on soil aggregation in a German barley germplasm. The experiment was performed under greenhouse conditions.

We hypothesized that the emphasis on yield in breeding programs had a deleterious effect on root length in barley, especially on very fine and fine root length, and that there are ripple-on effects on soil aggregation, a process that is strongly mediated by root length.

Zinc (Zn) deficiency in soil and subsequently in crop plants is a major challenge of modern agriculture. In *chapter 4*, we quantitatively analyzed the potential role of arbuscular

mycorrhizal fungi (AMF) for plant Zn nutrition over a variety of crops and soils. Therefore, we performed a random-effects meta-analysis on 104 articles comprising 263 trials.

We hypothesized that application of AMF positively affects plant tissue Zn concentration for root, shoot and fruit across all crops examined. Additionally, we hypothesized that the positive mycorrhizal effect is dependent upon environmental and experimental factors, especially soil parameters, like soil type, soil pH and nutrient concentrations.

CHAPTER 2

Mycorrhizal responsiveness trends in annual crop plants and their wild relatives- A meta-analysis on studies from 1981 to 2010

Abstract

Year of release of a cultivar reflects the agricultural and breeding practices of its time; we hypothesize that there are differences in mycorrhizal responsiveness of new high yielding and old crop plants and landraces. We evaluated the importance of the year of release on mycorrhizal responsiveness, arbuscular mycorrhizal (AM) fungal root colonization and P efficiency. We also analyzed the effect of experimental treatments, P efficiency and AM fungal root colonization on a potential mycorrhizal responsiveness trend for year of release.

We conducted a meta-analysis on 39 publications working on 320 different crop plant genotypes. New cultivars were less intensely colonized but were more mycorrhiza-responsive compared to ancestral genotypes. This trend was potentially influenced by the moderator variables density, pre-germination, plant, plant type and AMF species. AM root colonization was also important for the mycorrhizal responsiveness trend for year of release, but P efficiency was not. With the data available we could find no evidence that new crop plant genotypes lost their ability to respond to mycorrhiza due to agricultural and breeding practices.

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Introduction

Arbuscular mycorrhizal (AM) fungi are members of the *Glomeromycota* (Schüßler et al. 2001) and form symbiotic associations with the majority of land plant species (Fitter and Moyersoen 1996; Wang and Qiu 2006). AMF can offer various benefits that potentially result in host biomass increase; these include improved P acquisition (Bolan 1991; Koide 1991), defense against pathogens (Borowicz 2001; Harrier and Watson 2004), improvement of water relations (Auge 2001), and stress tolerance (Al-Karaki et al. 2001; Rouphael et al. 2010; Smith et al. 2010).

The increase in biomass mediated by AMF is often expressed as mycorrhizal responsiveness. This is defined as the effect of mycorrhizal fungi on plant growth given a specific plant-available soil P concentration compared to non-mycorrhizal control plants (Janos 2007). The effect can be positive (Yao et al. 2001b; Yücel et al. 2009), neutral or negative (Hao et al. 2008; Hetrick et al. 1992). The extent of mycorrhizal responsiveness varies widely between plant species and even between plant genotypes. In order to find a pattern in this variability for crop plants, Hetrick et al. (1992 and 1993) suggested that the cultivar year of release could be a decisive factor. The study of 20 wheat cultivars under greenhouse conditions revealed that cultivars released before 1950 profited more consistently from AM fungal inoculation in terms of biomass, while the response of cultivars released after 1950 was more variable. Additional greenhouse studies confirmed this general pattern (Hetrick et al. 1996; Zhu et al. 2001).

However, a study by Galvan et al. (2011) on onion cultivars and hybrids found no evidence that modern breeding practices changed growth responses, at least in onion. Sawers et al. (2010) also challenged the suggestion by Hetrick et al. (1992 und 1993) by using linear regression models. The analysis of plant growth response to mycorrhiza in subsets of the publications of Hetrick et al. (1992) and Kaeppler et al. (2000) revealed that the trends (for

plant biomass and year of release) were biased by non-linearity of the used response ratio (R`= (M-NC)/NC) and thus suggested that new, old and ancestral genotypes have the same potential for an increase in mycorrhiza benefit (increase in biomass). As a result, it is currently difficult to make general statements regarding the effects of crop breeding on mycorrhizal responsiveness.

Breeding conditions have certainly changed over time, since the early beginnings of human agriculture to the present, and we suggest that the cultivar's year of release represents the breeding practices of its time. 4000 years ago, humans finished the domestication of the major crops essential for their survival (Doebley et al. 2006). Throughout the millennia, genotypes were selected for positive traits like bigger fruits and more seeds. In the 19th century, the first artificial fertilizer, superphosphate, was used to improve yield. The re-discovery of the Mendelian theory in 1900 led to new technique of hybridization (Palladino 1993). Hybrid genotypes exhibited higher yield as compared to their inbred parental lines (heterosis effect). From then on, crop plants were bred to maximize yield and to respond better to fertilizer. In 1935, a dwarf wheat genotype, Norin 10, was bred in Japan (Reitz and Salmon 1968). After 1950, this genotype was used by Norman Borlaug and colleagues to produce semi-dwarf varieties (Dalrymple 1985). Their characteristics were lower shoot biomass, but higher yield output and a reduced snapping of their shorter shoots. Besides wheat and rice, other crop plants were improved to high-yielding varieties in the following decades all over the world. The breeding of these new varieties in addition to improved agricultural techniques and management practices (already established in most parts of North America and Europe) increased food production around the world (Wissuwa et al. 2009). The increased food production is linked to higher water irrigation, pesticide and fertilizer use.

High fertilizer application means high concentrations of plant-available P in the soil. High P concentrations often cause a reduction in mycorrhizal responsiveness (Hao et al. 2008; Kaeppler et al. 2000). Additionally, breeding under high P input can influence the P

efficiency of a cultivar (Huang et al. 2007; Manske et al. 2001; Wissuwa et al. 2009). P efficiency is defined as the ability of a plant "to produce yield under a certain available P supply condition and/or to utilize it in the production of biomass or the harvestable organ" (Fernandez et al. 2009) and has a direct impact on mycorrhizal responsiveness, since P-efficient cultivars generally have lower mycorrhizal responsiveness than P-inefficient ones (Baon et al. 1993; Khalil et al. 1994; Tawaraya et al. 2001; Yao et al. 2001b). An improved P efficiency reduces the effectiveness of the interaction of plant and fungus, at least concerning the increased P supply by the fungus (Li et al. 2008b).

The effectiveness of the plant and fungus interaction is also influenced by the host plant. In the literature, mycorrhizal responsiveness trends based on the year of release differed by crop plant. Negative trends over time were found for members of the genus *Triticum* (Hetrick et al. 1992; Hetrick et al. 1996; Zhu et al. 2001) and positive trends for representatives of the genera *Solanum* and *Avena* (Bryla and Koide 1998; Koide et al. 1988). Not only is the identity of the plant host important but the identity of the colonizing fungus as well. The right plant-fungus combination is critical for promoting optimal plant growth. AMF species are diverse in their effects on plant growth ranging from both extremes along a mutualism-parasitism continuum (Johnson et al. 1997; Sensoy et al. 2007), e.g. they can differ with their degree of P supply via the mycorrhizal pathway (Smith et al. 2003). Besides the intensity of root colonization, biomass increase, or P acquisition AMF species also have other influences on plant physiology, e.g. reducing expression of P_i-transporter and starvation-inducible genes (Burleigh et al. 2002). Despite the co-evolution of plant and AM fungi and the conservation of symbiosis-related features, it is rather astonishing that mycorrhizal P_i-transporter genes diverged between, e.g. rice and potato (Paszkowski et al. 2002). Thus AMF need to be flexible in their interaction with different host plants, making it possible that physiological incompatibility can occur; this can result in a suboptimal plant growth reaction and mycorrhizal responsiveness, respectively.

Other factors can also decrease the mycorrhizal responsiveness of crop plant genotypes, e.g. plant density (Schroeder and Janos 2004), substrate volume (Daft 1991), type of growth substrate (Vierheilig and Ocampo 1991b), experimental duration and country of origin of a cultivar (An et al. 2010). Year of publication is another, typically ignored factor and could also be indicative of changing scientific practices as demonstrated for herbivory and mycorrhizal colonization (Barto and Rillig 2010). Given the number of factors that contribute to mycorrhizal responsiveness, it is important to evaluate their effects on a potential mycorrhizal responsiveness trend for the year of release of crop plants.

To our knowledge only one study (An et al. 2010) tried to test multiple factors for their effect on AM fungal root colonization (but not biomass response) of different maize germplasms (inbred lines released between 1960 and 1999, hybrids and landraces) from different countries and with different pathogen resistances. Since this study contained no analysis on plant biomass performance, a synthesis of data on mycorrhizal responsiveness of plant genotypes with different year of release has not been performed to date.

Thus, we conducted a meta-analysis to quantitatively synthesize the data for mycorrhizal responsiveness in annual crop plants for different years of release and to test three hypotheses.

(i) Due to changes in agricultural and breeding practices over time, we expect differences in mycorrhizal responsiveness between new high yielding and old crop plants and landraces. Although landraces were bred into parental lineages of old and new cultivars, they themselves are the product of mainly natural selection, are adapted to their local and natural environment, are more genetically and phenotypically diverse, but produce less yield than their hybrid offspring (Harlan 1975). In old genotypes, the hybridization was used actively to profit from the heterosis effect in the F1-generation of parental inbred lines resulting in higher yield and C translocation into shoot and ears causing higher nutrient demand. Finally, the dwarfing gave rise to genotypes with reduced shoot length but enhanced yield.

(ii) For many abiotic and biotic factors, an influence on mycorrhizal responsiveness has been detected; thus we hypothesize that these factors also have an effect on any mycorrhizal responsiveness trend for the year of release of crop plants. Besides validation of the influence of factors (as reported in literature used in this meta-analysis) on mycorrhizal responsiveness in our dataset, in particular we need to test important factors for their effect on any mycorrhizal responsiveness trend for the year of release of crop plants. The flexibility in reaction to abiotic or biotic factors, respectively, was eventually co-influenced by changes in agricultural and breeding practices over time. Besides general biotic and abiotic factors such as plant density, soil volume, pH of growth substrate, seed pre-germination, duration of experiment, setting, P treatment and year of publication, we focus on the specific biotic factors AMF and plant species because of their importance for the quality of the symbiosis.

(iii) We hypothesize that P efficiency and AM fungal root colonization affect mycorrhizal responsiveness. Furthermore, since these two factors were likely affected by breeding practices, we expect the year of release to influence both P efficiency and AM fungal root colonization.

Materials and Methods

The focus of this meta-analysis was on publications dealing with AM fungi and multiple cultivars, genotypes or varieties of annual crop plants with different years of release.

The literature search started on 28 June 2010 and was performed with the Web of Science Citation Index Expanded database. The search strings used were mycorrhiza* AND cultiva*, mycorrhiza* AND genotyp*, mycorrhiza* AND variet*, mycorrhiza* AND accession*, and generated 969, 383, 319 and 26 publications, respectively.

Papers were screened for studies testing at least two different annual crop plant cultivars, genotypes or varieties under the same experimental conditions and using AMF as a treatment; thus a direct comparison of mycorrhizal and non-mycorrhizal plant growth performance was possible. We chose annual crop plants because most of the major food crops were annual (e.g. maize, wheat, barley, tomato, potato and soybean; see www.fao.org), and the greatest number of year of release dates were available for these major food crops.

To guarantee the independence of the extracted data, the plant genotypes were not allowed to be clones. Furthermore, root or shoot cultures were also not considered because of their highly artificial character and the low comparability with pot cultures or field trials. Therefore, experiments had to be performed in a soil substrate. In addition, the shoot, root or total dry weight biomass and the sample size (N) had to be reported.

Publications fitting these first criteria were further screened for the availability of the genotype's year of release date, because only studies with at least one genotype with a YOR or YORgroup (for definitions of these terms see section "Effect size and moderator variables") were considered.

Determination of the year of release

For the determination of a crop plant's year of release several sources were utilized: (i) Crop plant registration papers published by the Crop Science Journal (Crop Science Society of America) were searched for cultivar names via the online publication search function. (ii) The Germplasm Resources Information Network (GRIN) of the United States Department of Agriculture (USDA) provided information not only on a crop plant's year of release but pedigrees and country of origin as well. (iii) Information about the year of release, the pedigree and the country of origin specifically for barley (*Hordeum vulgare* L.) was obtained by the lineage catalogue of barley cultivars of the Bayrische Landesanstalt für Landwirtschaft (LfL) and specifically for wheat (*Triticum aestivum* L.) by the online database "Wheat Pedigree and Identified Alleles of Genes" (http://genbank.vurv.cz/wheat/pedigree/). (iv) The crop plant's name was searched using the GOOGLE™ search engine or ISI Web of Science for publications about pedigrees. Studies analyzing pedigrees were a good source of information for the year of release. (v) Several papers contained information about year of release directly, but these dates were sometimes not reliable. However, if no data were available using other options (points i to iv), the data directly from the paper were used. If no year of release was available and the crop genotype was not a landrace, wild accession or wild crop relative, then the study was not included.

This final screening returned 39 papers fitting the above mentioned criteria and reporting YOR or YORgroup, respectively, for at least one annual crop plant genotype. The crop plants belonged to the families of *Poaceae*, *Fabaceae*, *Pedaliaceae*, *Asteraceae* and *Cucurbitaceae*. The 39 publications reported on 320 different crop plant genotypes (Table I.S1) and for 120 genotypes a year of release could be determined. 270 of the 320 genotypes could be sorted into one of three year of release groups (ancestor, old or new).

Data recording

As in other meta-analyses (Curtis and Wang 1998; Lekberg and Koide 2005), several trials were extracted from each of the 39 publications. Multiple trials within each publication were treated as independent when they were drawn from systems differing in at least one of the following criteria: (i) setting (lab or field), (ii) Phosphorus treatment (yes or no), (iii) AMF species used as inoculum or (iv) plant genus used as experimental host plant. When systems only differed in duration of experiment, only the last harvest was included in the dataset.

Besides plant dry weight, AM fungal root colonization and P efficiency data were extracted from each publication. Biomass was recorded as mg of total, root and/ or shoot dry weight excluding fruits, fruit seeds or flower dry weight. If the data were only available in graphs, the freeware Digitizeit 1.5.8a (by I. Bormann 2001-2006, http://www.digitizeit.de/de/) was used for data collection.

Effect sizes and moderator variables

The principal dependent variable (effect size) in this meta-analysis was mycorrhizal responsiveness (MR). The effect size was calculated by taking the natural logarithm of the response ratio of mycorrhizal to non-mycorrhizal plant biomass ($MR = \ln(\text{biomass}_{myc}/\text{biomass}_{non\text{-}myc})$). MR was calculated from total dry weight data. When available shoot or root dry weight data were used for the calculation.

The usage of response ratios can be problematic (Righetti et al. 2007). As demonstrated (Online Resource 1), our response ratio fitted best the assumption of linearity and thus was reliable for interpretation of mycorrhiza effects.

P efficiency and AM fungal root colonization data were used to calculate the supplementary effect sizes to test for their role on mycorrhizal responsiveness. According to Wang et al. (2010) P efficiency can be divided into P utilization efficiency (PUE) and P acquisition efficiency (PAE). Additionally, for PUE (g shoot biomass/ mg P) and PAE (mg P/ g root

biomass) standardized response ratios were calculated; resulting in the effect sizes mycorrhizal PAE ($\ln(PAE_{myc}/ PAE_{non\text{-}myc})$) and mycorrhizal PUE ($\ln(PUE_{myc}/ PUE_{non\text{-}myc})$). Data for PAE and PUE were reported only in 17 of the 39 papers. Therefore, the power of tests with these two effect sizes was low and results should be interpreted with caution.

For AM fungal root colonization (%AM), the percent of root length colonized by AMF was used to calculate the corresponding effect size by the mean difference of mycorrhizal and control plants (%AM = % root colonization $_{myc}$ - % root colonization $_{non\text{-}myc}$). For one study only, the controls were contaminated with AM fungi. 35 of the 39 studies used a gridline intersect method, while only 4 studies randomly selected root fragments. Data of both methods were combined in our dataset in agreement with Lekberg et al. (2005) who found no statistically significant source of error in doing so.

The moderators used were year of release (YOR), year of release group (YORgroup), density (number of plants/ kg soil), plant (e.g. *Hordeum*, *Zea* or *Triticum*), plant type (cereals, vegetables or legumes), pre-germination of seeds (yes or no), duration of experiment, setting (lab or field), year of publication, and experimental conditions such as AMF species used as inoculum, addition of P fertilizer (treatment P, yes or no), the applied P amount (treatment P concentration, in mg P/ kg soil) and pH of growth substrate.

YOR and YORgroup were the principal independent variables (moderators) for answering questions in this meta-analysis. The YOR denoted the date when a crop plant became available on the market; it is not exactly the date when a crop plant was bred. YORgroup was related to the YOR moderator. This categorical moderator included three levels: ancestor, old and new. The "new" YORgroup contained all cultivars released after 1950, the "old" YORgroup were all released after 1900 and before 1950. The "Ancestor" YORgroup included all cultivars released before 1900 as well as the wild crop relatives and landraces, for which no YOR exist. This separation was made according to the studies of Hetrick et al. (1992 and 1993) and to account for changes in plant breeding practices, i.e.

cultivars bred before 1900 were more likely products of anthropogenic selection events (for criteria like size and taste), while cultivars bred after 1900 arose mainly from hybridization of inbred lines. Cultivars bred after 1950 were comprised of the high yielding varieties and Norin-10-based semi-dwarfs.

The moderator "plant" was dominated by members of the family *Poaceae* (*Poaceae* trials= 463, other plant trials= 113). Species of the *Poaceae* often have a fine and dense root system and thus are hypothesized to be less dependent on AMF (Newsham et al. 1995). To detect growth differences between *Poaceae* and non-*Poaceace* species, the moderator plant type was introduced. The moderator level "cereals" contained all study plants belonging to the family of the *Poacecae*, the level "legumes" all members of the family *Fabaceae* and the final level "vegetables" was formed by the remaining fruit and leaf vegetables. Trials for YORgroup "old" were only present in the plant type level "cereals", i.e. for "legumes" and "vegetable" only data for "new" and "ancestral" genotypes were available.

The moderator setting was influenced by the high number of studies performed under controlled greenhouse conditions (lab trials= 562, field trials= 14). Therefore, the dataset is dominated by artificial growing systems. The moderator P treatment (addition of P fertilizer, yes or no) was also dominated by the high number of P-deficient studies (P treatment no= 497, P treatment yes= 79). Thus, the dataset is also dominated by potentially P-deficient growth substrates. The moderator soil pH covered a range of acidic (5.5) to alkaline (8.7) pH levels.

Statistics

Only a small number of studies reported standard errors. Therefore, the sample size (N) was used to perform a non-parametric weighting of studies (Hedges et al. 1999). This non-parametric weight w_{ij} was calculated as follows:

For experiment *j* within study *i*, w_{ij}= ($N_{ij}E$* $N_{ij}C$)/ ($N_{ij}E$ + $N_{ij}C$), where $N_{ij}E$ is the sample size of mycorrhizal plants and $N_{ij}C$ is the sample size of non-mycorrhizal control plants. If $N_{ij}E = N_{ij}C$, then the formula was reduced to $w_{ij} = N^2/ 2*N$. This method has been widely used in the meta-analysis literature (Adams et al. 1997; Hoeksema and Forde 2008; Lekberg and Koide 2005).

The statistical analyses were performed with R version 2.12.1 (R Development Core Team 2010). The packages "meta" (Schwarzer 2007), "metafor" v. 1.6-0 (Viechtbauer 2010), and a non-parametric bootstrap code were used. The code for the non-parametric bootstrap was based on the "error" bootstrap by van den Noortgate and Onghena (2005). The bootstrap samples were simulated via a hierarchical system with two levels: vectors of level 1 residuals were nested within vectors of level 2 residuals. The R code is accessible in the electronic supplementary material (Online Resource 2).The "metafor" function was used for creating a random effects model testing the effect of a moderator on one effect size. The calculation of the *P*-value and 95% confidence interval was performed by using the non-parametric "error" bootstrap. To test for significance of moderator effects, a two-tailed test was used. The bootstrap was used to evaluate the influence of the moderators on the effect. The metagen function ("meta" package in R) was used for calculation of the mean effect size for each moderator level.

To deal with hypothesis (i) we tested the effect size MR against the moderator variables YOR and YORgroup. Additionally, we tested the effect of the moderators YOR and YORgroup on both mycorrhizal (lnM) and non-myorrhizal biomass (lnNC) to be able to interpret the moderator effect on MR (being a response ratio) correctly due to the problematic nature of response ratios (see above).

To address hypothesis (ii), we evaluated first the influence of the abiotic (density, pre-germination, duration of experiment, setting, year of publication, treatment P, treatment P concentration and soil pH) and the biotic moderator variables (plant, plant type, AMF species)

on MR. Second, Pearson's Chi-squared test was performed on moderators to test for their independence. Specific subsets were produced to test non-independent moderators for their influence on the effect size MR and their importance for the MR trend for the year of release of crop plants. Only moderators with a sufficient number of trials could be tested by the bootstrap. The effects of chosen moderators on MR were examined in the subsets "Before 1950" and "After 1950". The subset "Before 1950" contained all cultivars with the YORgroup levels "ancestor" and "old". The "After 1950" subset included all "new" cultivars. Third, subsets for the biotic moderator variables plant, plant type and AMF species were produced for moderator levels with the highest number of trials: "Barley", "Maize" and "Wheat" for plant, "Cereals", "Legumes" and "Vegetables" for plant type and "Gl. mosseae" and "Gl. intraradices" for AMF species. In these subset populations, the effect of YOR and YORgroup on MR was re-evaluated.

Fourth, the plant genera, AMF species or experimental practices may change over time and may be detectable via correlation with the year of publication. Therefore, the method used by Barto and Rillig (2010) was used. The levels of the tested moderator were ranked by their mean year of publication. The level with the lowest mean received the first rank, the level with the second lowest mean rank two and so on. This modified moderator was correlated with the year of publication to determine whether or not there were temporal shifts in the moderator. If a moderator does not change over time, then there will be no correlation.

For the last hypothesis (iii), we tested first the correlation of mycorrhizal PAE (mPAE), mycorrhizal PUE (mPUE) and root colonization (%AM), respectively against MR. Additionally to the bootstrap, we used a weighted regression with a ranked dependent variable (following Kendall's Tau rank correlation) for evaluation of potential relationships between the different effect sizes. Although both methods are based on regressions, the weighted, rank modified regression reported useful parameters, like R^2 and residual error, while these pieces of information were not delivered by the bootstrap. However, the bootstrap *P*-value was more

trustworthy and was preferred. The correlation analysis was performed on the complete dataset. Second, we analyzed the effect of the moderator variables YOR and YORgroup on the effect sizes mPAE, mPUE and %AM by using the bootstrap.

Results

Is there a mycorrhizal responsiveness trend for the year of release of crop plants?

We found a significant effect of YORgroup on MR in crop plants. Old and new cultivars were more responsive than ancestral accessions (Table I.1). No effect was detectable for the moderator YOR.

Table I.1 Effect of moderators "YORgroup" and "YOR" on mycorrhizal responsiveness. The mean and 95% confidence interval (CI) for moderator levels (ancestor, old and new) were calculated with the "metagen" function in R. The moderator effect on mycorrhizal responsiveness is represented by the 95% confidence interval calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Moderator	Level	Mean	Trials	CI
YORgroup			463	[0.0699; 0.1692]***
YORgroup	ancestor	0.268	171	
YORgroup	old	0.634	33	
YORgroup	new	0.480	259	
YOR			262	[-0.0028; 0.0036]

Due to difficulties in interpretation of results of response ratios, we tested the influence of the year of release moderators on both lnM and lnNC (Table I.2). Moderator YOR reported only non-significant effects thus we only presented results for moderator YORgroup. Moderator YORgroup had a negative effect on both lnM and lnNC, but the effect on lnM was not significant.

Table I.2 Effect of moderator "YORgroup" on mycorrhizal (lnM) and non-mycorrhizal biomass (lnNC) for the complete dataset and for the P-deficient (Treatment P(No)) and P-sufficient (Treatment P (Yes)) subset. The moderator effect on the dependent variables is represented by the 95% confidence interval (CI) calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Subset	Dependent variable	Trials	CI
Complete dataset	lnM	579	[-0.2194; 0.0013]
	lnNC	579	[-0.3067; -0.0587]**
Treatment P (No)	lnM	499	[-0.2191; -0.0002]*
	lnNC	499	[-0.3064; -0.0615]**
Treatment P (Yes)	lnM	80	[-0.4394;1.3456]
	lnNC	80	[-0.5404; 1.9213]

One of the major constraints of the dataset was the dominance of studies working with a potentially P-deficient soil substrate. Therefore, we tested the differences in the effect of the moderator YORgroup and both lnM and lnNC for P-deficient and sufficient studies (Table I.2). In studies with potentially P-deficient soil substrate, the same negative effect of YORgroup on lnM and lnNC was detectable as for the complete dataset, but in this subset the effect on lnM was marginally significant. There were no significant differences for P-sufficient soil substrates, neither for lnM nor for lnNC.

What factors influence mycorrhizal responsiveness and the mycorrhizal responsiveness trend for the year of release in crop plants?

Testing for the importance of a variety of moderators revealed that MR was influenced by several factors (Table I.3). For the moderator variables pre-germination and AMF species the effect on %AM was tested as well. The data are available in Table I.S2. The pre-germination of seeds and the subsequent transplantation as seedlings caused a decrease in MR, as did a high plant density per soil weight (density).

Table I.3 Effect of moderators on mycorrhizal responsiveness. The mean and 95% confidence interval for moderator levels were calculated with the "metagen" function in R. The moderator effect on mycorrhizal responsiveness is represented by the 95% confidence interval (CI) calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Moderator	Level	Mean	Trials	CI
Density			431	[-0.2355; -0.1587]***
Treatment P			576	[-0.1722; 0.1137]
Treatment P	yes	0.287	79	
Treatment P	no	0.475	497	
Treatment P conc			572	[-0.0006; 0.0074]
Soil pH			408	[-0.2531; -0.1068]***
Pre-germination			500	[-0.4354; -0.2447]***
Pre-germination	yes	0.301	303	
Pre-germination	no	0.670	197	
Duration			471	[-0.0046; -0.0005]*
Year of publication			576	[0.0146; 0.0282]***
Setting			576	[-0.1746; 0.3704]
Setting	field	0.355	14	
Setting	lab	0.449	562	
Plant			576	[-0.1063; -0.0843]***
Plant type			576	[-0.2188; -0.0572]***
AMFspec			345	[-0.0805; -0.0403]***

For the moderators treatment P (application of phosphorus as a factor, yes or no) and treatment P concentration (applied P-level, when P was an experimental factor), no effect was observed on MR; neither in the complete dataset nor in the subsets "Before 1950" and "After 1950". The moderator soil pH had a negative effect on MR: the more alkaline the soil the less plant biomass increased under AMF influence. However the relationship between MR and soil pH was more complex as was detectable by this simple model. Therefore, three subsets were produced: "Acidic" with soil pH levels smaller than 6, "Neutral" with soil pH levels between 6 and 7, and "Alkaline" with soil pH levels higher than 7. In the subset "Acidic", soil pH had a positive effect on MR, in "Neutral" a weak negative effect was present, and in the

subset “Alkaline”, soil pH had a negative effect on MR (data not presented). This indicated that the closer the soil pH was in the neutral pH range, the better plants were growing.

The duration of experiment also had a negative effect but a very flat slope (-0.0025). In addition, the more recently a paper was published the more positive was MR. The moderators plant, plant type and AMF species also had an influence on MR. The moderator plant type was more important than the moderator plant, and plant was more important than AMF species. The moderator setting (lab or field) was imbalanced by the low number of trials for the level “field” and of no use for interpretation.

Pearson's Chi-squared test showed that none of the moderators were independent (Table I.S3). Therefore, it was not possible to interpret the influence of one moderator on the effect size separately from the others. However, by analyzing moderators of interest in specific subsets, the extent of their importance could be evaluated.

To test for the impact of moderators on MR in old and ancestral accessions as well as in new cultivars, moderators were analyzed in the two subsets “Before 1950” and “After 1950” containing all genotypes of the YORgroup “ancestor”, “old”, and “new”, as appropriate. The results of the subset tests were similar to the overall analysis with two exceptions. In the subset “Before 1950”, soil pH was no longer significant (Table I.4). In the subset “After 1950”, duration of experiment was no longer significant compared to the whole dataset. For the subset “After 1950” and the moderator soil pH, we tested if the same trend for acidic, neutral and alkaline pH is detectable as for the complete dataset. Therefore, the subset “After 1950” was subdivided into three subsets just like for the complete dataset. For the “After 1950- acidic”, soil pH had a positive effect on MR, but the number of trials was exceptionally low (15). For the other two pH subsets, no influence on MR was found (data not presented). Thus, the hump-shaped relationship present in the complete dataset was not detectable in the “After 1950” subset.

Table I.4 Effect of moderators on mycorrhizal responsiveness for subsets "Before 1950", including ancestral and old genotypes, and "After 1950", including new genotypes. The moderator effect on mycorrhizal responsiveness is represented by the 95% confidence interval (CI) calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Subset	Moderator	Trials	CI
Before 1950	Density	176	[-0.2936; -0.0973]***
	Treatment P	204	[-0.3685; 0.5426]
	Treatment P conc	204	[-0.0684; 0.1637]
	Soil pH	139	[-0.1374; 0.0681]
	Pre-germination	200	[-0.6439; -0.3671]***
	Duration	139	[-0.0086; -0.0017]**
	Plant	204	[-0.3137; -0.1766]***
	AMFspec	105	[-0.1669; -0.0975]***
After 1950	Density	183	[-0.2636; -0.1607]***
	Treatment P	259	[-0.2598; 0.1640]
	Treatment P conc	257	[-0.0003; 0.0116]
	Soil pH	181	[-0.3413; -0.1516]***
	Pre-germination	213	[-0.4669; -0.1311]***
	Duration	230	[-0.0040; 0.0030]
	Plant	259	[-0.1127; -0.0815]***
	AMFspec	163	[-0.0656; -0.0149]*

The moderator pre-germination was further analyzed in separate subsets to gain more insight into its effects. For this, the effect of plant type on MR was tested in the subsets "Preger YES" and "Preger NO". For the first subset, the level "cereals" (monocots) had a lower MR that the levels "legumes" and "vegetables" (both dicots). For the latter subset, the opposite was true (Table I.S4).

To test the specific influence of AMF species on MR trend for the year of release in crop plants, the importance of YOR and YORgroup was tested separately within the two subsets "Gl. intraradices" and "Gl. mosseae", the two most often used AMF species in single cultures for this meta-analysis. Two opposing trends were found: The moderator YORgroup had a positive effect on MR of plants inoculated with *Glomus intraradices* isolates, but a negative effect on plants inoculated with *Glomus mosseae* isolates (Table I.5). In other words,

ancestral genotypes growing in *Glomus mosseae* single culture had a higher MR than new cultivars and the opposite was true for *Glomus intraradices*. No trends were detectable for the moderator YOR.

Table I.5 Effect of moderators "YORgroup" and "YOR" on mycorrhizal responsiveness (MR) for (i) plant type subsets "cereals", "vegetables" and "legumes" and plant subsets "barley", "maize" and "wheat" as well as for (ii) AMF species subsets "Glomus intraradices" and "Glomus mosseae". The moderator effect on MR is represented by the 95% confidence interval (CI) calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Subset	Moderator	Trials	CI
Cereals	YORgroup	389	[0.0151; 0.1131]*
	YOR	224	[-0.0044; 0.0008]
Legumes	YORgroup	41	[0.5311; 1.4091]***
	YOR	26	[-0.0216; 0.0319]
Vegetables	YORgroup	29	[-0.0294; 0.7798]
	YOR	8	[-0.0991; 0.1684]
Barley	YORgroup	53	[-0.2072; 0.3354]
	YOR	49	[-0.0074; 0.0090]
Maize	YORgroup	74	[-0.7245; -0.2098]***
	YOR	70	[-0.0117; 0.0067]
Wheat	YORgroup	242	[-0.0411; 0.0825]
	YOR	94	[-0.0053; 0.0006]
Gl. intraradices	YORgr	78	[0.0980; 0.3498]***
	YOR	41	[-0.0025; 0.0151]
Gl. mosseae	YORgr	86	[-0.3611; -0.1528]***
	YOR	39	[-0.0101; 0.0193]

Furthermore, the moderators plant and plant type were also tested for influence on the MR trend for the year of release in crop plants. A positive influence of YORgroup on MR was detectable in subsets "Cereals" and "Legumes", but no trend could be found for the moderator YOR (Table I.5). For the complete dataset and the subset "Cereals" and "Legmues", the same trend was present. For the plant subsets "Barley", "Maize" and "Wheat", an effect was

observed only for the "Maize" subset and the moderator YORgroup. This trend had low statistical support due to low power of YORgroup levels ("ancestor"-trials= 2, "old"-trials= 10, "new"-trials= 62).

MR increased with year of publication (Table I.3, Fig. I.1a). This effect was mainly driven by studies published in the years 2000 to 2010 and 1990 to 1995. No clear shift in usage of AMF species was detected (Fig. I.1b); *Glomus mosseae*, *Glomus intraradices* and *Glomus etunicatum* were all used in studies from 1990 to 1995 as well as from 2000 to 2010. The experimental plants shifted over time (Fig. I.1c). In the years 1990 to 1995 legumes were most often used, while in the years 2000 to 2010 vegetables were the preferred study objects. Cereals were used regularly throughout the research history. The usage of pre-germinated and then transplanted seedlings was mainly found before 1995 (Fig. I.1d). In studies published after 2000, plants were more often directly seeded into the substrate. There was no clear shift in YORgroup detectable; ancestral, old and new genotypes were used both in the years 1990 to 1995 and the years 2000 to 2010 (Fig. I.1e). In contrast, YOR shifted clearly over time (Fig. I.1f). Studies published before 1995 used older genotypes than studies published after 1995 indicating that researchers used more recently released cultivars as experimental plants in more recently published studies. This temporal shift may be biased by the availability of year of release dates for old genotypes because the moderator YOR contained 227 new and only 28 old genotypes.

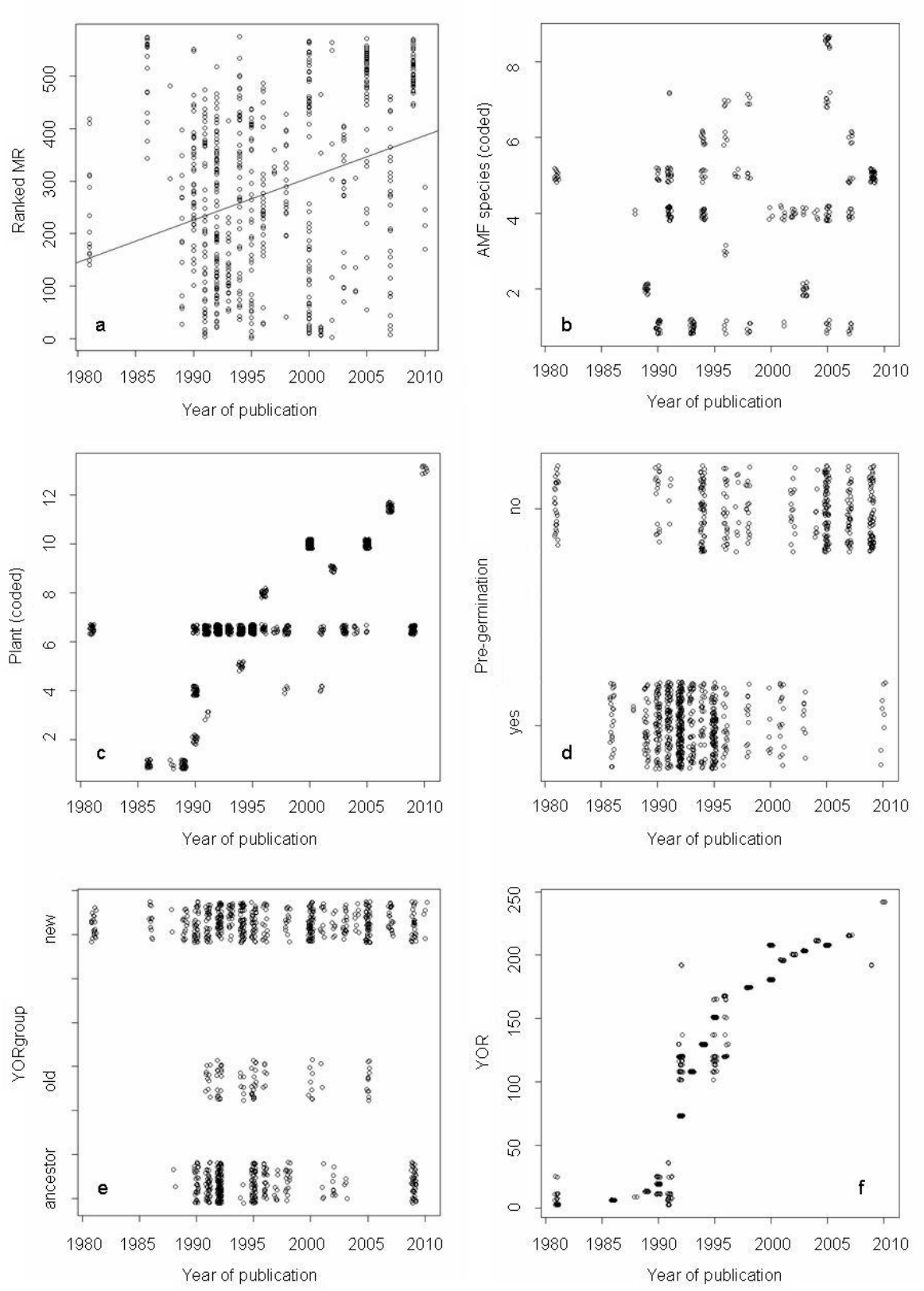

Fig. I.1 For figure legend, see next page.

Fig. I.1 Weighted correlation of mycorrhizal responsiveness (MR) and five moderators (AMF species, plant, pre-germination, YORgroup and YOR) and year of publication. Variables on the y-axis were ranked and sorted by their mean year of publication with the lowest mean located at the bottom of the figure. For better visualisation of overlapping data points, the data were jittered on the x- and y-axes. Relationship between year of publication and a) MR (R^2= 0.1042, df= 574, P< 0.0001), b) AMF species used as single cultures (R^2= 0.0807, df= 343, P< 0.0001, code: 1= *Gl. etunicatum*, 2= *Gl. fasciculatum*, 3= *Gl. manihotis*, 4= *Gl. intraradices*, 5= *Gl. mosseae*, 6= *Gi. margarita*, 7= *Gl. clarum*, 8.5= *Ac. morrowiae*/ *Gi. rosae*), c) plants used as study object (R^2= 0.3255, df= 574, P< 0.0001, code: 1= Alfalfa, 2= Oat, 3= Pea, 4= Sorghum, 5= Groundnut, 6= Tomato, 7= Soybean, 8.5= Wheat/ Barley, 10= Bean, 11= Lettuce, 12= Maize, 13.5= Rice/ Pepper, 15= Cucumber), d) pre-germination of seeds (R^2= 0.3781, df= 498, P < 0.0001), e) YORgroup (R^2< 0.0001, df= 461, P= 0.954) and f) YOR (R^2= 0.8356, df= 259, P < 0.0001)

What is the role of mycorrhizal responsiveness and the year of release for AM fungal root colonization and P efficiency in crop plants?

The correlation between MR and %AM, mPAE and mPUE was tested. %AM was positively correlated with MR (R^2=0.30, df= 395, P< 0.0001; Fig. I.2).

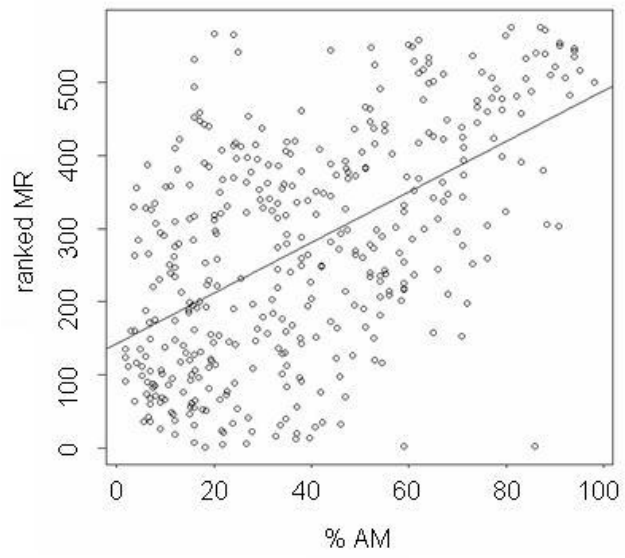

Fig. I.2 Weighted correlation of mycorrhizal responsiveness (MR) and AM fungal root colonization (%AM). Due to a non-normal distribution of the data, MR was ranked-transformed. R^2= 0.2979, df= 395, P < 0.0001.

The effect size mPUE correlated negatively with MR (R^2=0.11, df= 122, P<0.0001) and mPAE tended to correlate positively with MR (R^2=0.04, df= 122, P= 0.072). The correlation of mPUE and MR was mainly driven by the study of Khalil et al. (1994). After excluding this study from the dataset, the relationship was no longer significant (R^2= 0.007, df=100, P= 0.612). The same study also had a strong influence on mPAE. After the exclusion of this study, the correlation was negative but still weak (R^2= 0.07, df= 90, P=0.006).

Table I.6 Effect of moderators "YORgroup" and "YOR" on AM fungal root colonization (%AM) and mycorrhizal P acquisition efficiency (mPAE) and mycorrhizal P utilization efficiency (mPUE). The mean and 95% confidence interval (CI) for moderator levels (ancestor, old and new) were calculated with the "metagen" function in R. The moderator effect on AM fungal root colonization is represented by the 95% confidence interval calculated with the "error" bootstrap (van den Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Effect size	Moderator	Level	Mean	Trials	CI
%AM	YORgroup			410	[-6.7918; -1.8346]***
	YORgroup	ancestor	40.826	146	
	YORgroup	old	30.468	37	
	YORgroup	new	31.996	227	
%AM	YOR			264	[-0.0909; 0.1356]
mPAE	YORgroup			157	[-0.1274; 0.1015]
	YORgroup	ancestor	0.0780	39	
	YORgroup	old	0.2777	2	
	YORgroup	new	0.2047	116	
mPAE	YOR			121	[-0.0076; 0.0033]
mPUE	YORgroup			163	[-0.1406; 0.0876]
	YORgroup	ancestor	-0.1927	41	
	YORgroup	old	-0.2642	2	
	YORgroup	new	-0.2323	120	
mPUE	YOR			119	[-0.0036; 0.0068]

A negative association between %AM and YORgroup could be detected. Ancestral genotypes showed a colonization of about 41%, old of 30% and new cultivars of about 32%

root length (Table I.6). Again, the number of trials for "old" genotypes was very low. No trend could be found for YOR.

For P efficiency, no trend could be detected for either YORgroup or for YOR (Table I.6). However, the means of the YORgroup levels (ancestor, old, new) for mPAE were always positive, while those of mPUE were always negative. Overall, this indicated that the tested genotypes, when mycorrhizal, were efficient in P acquisition and inefficient in P utilization.

Discussion

Is there a mycorrhizal responsiveness trend for the year of release of crop plants?

The analysis of MR trends in plant biomass revealed that new genotypes released after 1950 were more mycorrhiza-responsive than ancestral genotypes. The phenomenon where new genotypes had a higher MR than ancestral accessions was not related to a higher biomass of new cultivars when mycorrhizal. New cultivars grew less when mycorrhizal or non-mycorrhizal as compared to ancestral accessions, but this trend was more pronounced for non-mycorrhizal biomass by a steeper, negative slope.

New cultivars were bred to grow fast under high fertilizer input, but the majority of studies used in this meta-analysis grew their plants on potentially P-deficient soil substrate. The low P availability could have been responsible for the reduced biomass of new cultivars as compared to ancestral accessions. There were not enough trials to detect an effect of YORgroup on mycorrhizal or non-mycorrhizal biomass in P-sufficient soil. Thus, it could not be convincingly tested whether the effect of YORgroup on biomass (for the complete dataset) was mainly driven by the P deficiency or other factors (as demonstrated for MR; see Table I.3).

Our findings for the effect of YORgroup on MR contradicted the hypothesis by Hetrick et al. (1992 and 1993) but were supported by the findings of Koide et al. (1988) and Bryla and Koide (1998). However, this effect was not detectable for the moderator YOR. The lack of an effect of YOR could be explained by the low number of trials for old cultivars released between 1900 and 1950.

The positive relationship between MR and YORgroup would suggest that there was no negative effect of breeding under high fertilizer conditions on MR of modern crop plants compared to their ancestral relatives (An et al. 2010; Galvan et al. 2011; Jackson et al. 2002; Sawers et al. 2010; Wright et al. 2005). This hypothesis was further supported by the analysis

of the moderator plant type. In cereals and legumes, new cultivars had higher MR than ancestral ones. This relation was also present in vegetables as a non-significant trend. Even if the focus was only on a specific plant type (cereals, legumes, vegetables) there was a positive effect of YORgroup on MR.

But why were ancestral accessions less mycorrhiza-responsive? Plants growing under nutrient limitation adapt to this condition (Chapin et al. 1986). Therefore, Koide et al. (1988) suggested that wild plant genotypes growing on natural, nutrient poor soil are better adapted to this nutrient limitation compared to new cultivars bred under high fertilizer input, and thus are less responsive to AM fungi. The adaptation could result in a reduced biomass due to a lower nutrient demand (Chapin et al. 1986). A main effect of AMF is the increase of biomass due to an increased nutrient supply. Because of the lower nutrient demand, wild and ancestral accessions should respond less to AM fungal nutrient supply.

One way to adapt to nutrient limitation is to increase nutrient efficiency. Old genotypes and ancient accessions have greater root lengths, higher root to shoot ratios, and a more branched root system compared to their younger relatives produced under higher fertilizer input (Koide et al. 1988; Zhu et al. 2003; Zhu et al. 2001). Although these root traits are genetically highly variable (Hao et al. 2008), there is no doubt that changes in root architecture and morphology can improve P efficiency (Gahoonia and Nielsen 2004). A large root system with long root hairs increases the root surface and thus P acquisition (Gahoonia et al. 1999). Plants also decrease the soil pH around their roots to dissolve immobile P via exudation of protons, organic acids or phosphatases (Asmar et al. 1995; Dalal 1977; Gahoonia et al. 2000; Schjorring 1986).

However, new cultivars could be more mycorrhiza-responsive because of an increased nutrient demand. New cultivars were bred to grow faster and produce more yield under fertilizer input. In a P-deficient soil this selection for fast growth and high yield promoted the

interaction of plant and AM fungi to satisfy the higher needs for nutrients, and thus resulted in an increased MR (as demonstrated in Table I.2).

What factors influence mycorrhizal responsiveness and the mycorrhizal responsiveness trend for the year of release in crop plants?

The moderators density, soil pH, seed pre-germination, duration of experiment, year of publication, plant and AMF species all had an effect on MR.

As expected, a high plant density per soil weight had a negative effect on MR (Schroeder and Janos 2004; Schroeder-Moreno and Janos 2008). A small substrate volume and high plant density are factors causing reductions in plant biomass and P acquisition due to nutrient and space limitations. In a large soil volume with high P concentration, root density correlates with P acquisition, but this is not the case in low soil volumes or soils with low P concentrations (Otani and Ae 1996). Furthermore, there are several possible explanations of why AMF did not ameliorate this reduction in biomass caused mainly by nutrient deficiency. (i) AMF might have reduced or disturbed the P acquisition pathway of the plant (Li et al. 2008a). (ii) The low P concentration in the growth substrate led to a conflict in plant and fungal P acquisition and to an overlap of the P depletion zones (Hayman 1983). (iii) Abbott and Robson (1984) reported that intraspecific density affected the development of intraradical AM fungal structures: higher density caused lower amounts of arbuscules per length of root colonized. Arbuscules are the P-exchange organs of AM fungi. Thus in P-deficient soil, plant and AMF are competing for nutrients and this might have caused conflicts in P exchange and/ or plant PAE. Therefore, the plant might down-regulate C-translocation to the fungi and cause reduction in %AM (as demonstrated for our dataset; see Table I.S2). MR and %AM are positively correlated (Fig. I.1a) and thus with decreasing %AM and increasing density, the MR decreases.

The effect of soil pH on MR was not surprising: the closer the soil pH was to the neutral pH range the better plants were growing. The negative effect of soil pH on MR was generated by genotypes released after 1950 (Table I.4), although the hump-shaped relationship was no longer detectable in this subset. For genotypes released before 1950, no effect of soil pH on MR could be detected. AMF can support their host plants with nutrients and water and therefore reduce the stress of immobilized P caused by a strong acidic or alkaline pH (Cardarelli et al. 2010; Cartmill et al. 2007; Cartmill et al. 2008). For ancestral accessions and genotypes released before 1950 the lack of an influence of the soil pH moderator may be due to the better adaptation to P immobilization and a lower dependence on AM fungi compared with genotypes released after 1950. New cultivars would be more susceptible to alkalinity stress because of their higher nutrient demand due to higher yield production.

The moderator duration had a negative but weak effect on MR. This would mean that mycorrhizal plants grew less in long lasting experiments as compared to their non-mycorrhizal controls resulting in a smaller response ratio. This effect was statistically weak as compared to the other moderator variables; this moderator had a nearly flat slope (-0.0025). The fact that there was still a significant effect (P= 0.0164) was likely due to the high number of trials (471) and thus exceptionally high statistical power. The moderator duration lost its influence in the subset “After 1950”. Although the duration of experiments had a negative effect on plant growth of cultivars released before 1950, this effect was weak. The slope was again very flat (-0.0051) and the significance (P= 0.004) likely attributable to the large number of trials (139). Taking this fact into account we could state that the duration of experiments was not a strong factor influencing the MR trend for the year of release of crop plants.

Pre-germination and transplantation of seedlings caused a decrease in MR. During transplantation of seedlings, fine roots and root hairs can be damaged, and then plants

experience stress due to new biotic and abiotic factors. This transplant shock can reduce overall plant biomass, leaf area and canopy photosynthesis as demonstrated in rice (Dingkuhn et al. 1990; Dingkuhn et al. 1991; Kotera et al. 2004) and could make the plant more susceptible to pathogens. In our dataset, pre-germination caused a reduction in MR of about 50 % (Table I.3). This leads to the assumption that pre-germination affected the plant and not the fungal symbiosis partner. Additionally, this is supported by the fact that %AM was not influenced by pre-germination (Table I.S2). The importance of the moderator variable plant type on pre-germination (tested in the subsets "Preger Yes" and "Preger NO") revealed that monocots (*Poaceae*) grew better when not pre-germinated while the opposite was true for dicots (*Fabaceae*, *Pedaliaceae*, *Asteraceae* and *Cucurbitaceae*). Thus, the negative effect of pre-germination can be explained partially by the dominance of the family *Poaceae* in our dataset, causing the high MR values for the pre-germination level "no".

Treatment P (yes or no) and treatment P concentration unexpectedly had no influence on mycorrhizal responsiveness, even though a reduction in %AM and MR with increasing P input is often reported (Jackson et al. 2002; Rajapakse et al. 1989; Raju et al. 1990). An explanation may be the low number of trials for these moderators because only 8 of 39 studies worked with P application as a factor. Additionally, P application does not necessarily translate to P availability due to leaching or binding to soil ions.

The same problem existed for the moderator variable setting (lab or field): only one study reported data from field experiments causing a tremendous imbalance of the moderator levels (lab trials = 562, field trials= 14).

The strong positive effect of year of publication on MR, meaning that there was a tendency towards reporting increasing MR with newer publication date, was likely caused by the moderator variable plant and pre-germination, but not by the AMF species used as inoculum (Fig. I.1). Pre-germination and plant type were moderators with strong effects on

MR, thus the positive correlation of year of publication and the effect size can be explained by the positive impact of direct seeding and usage of specific plant genera.

There was a strong effect of AMF species on the MR trend for the year of release of crop plants. In the subset "Gl. mosseae", YORgroup had a negative effect on MR and in the subset "Gl. intraradices" a positive effect (Table I.5), i.e. new cultivars had a higher MR when growing with *Glomus intraradices*. Old and ancestral accessions grew more when colonized by *Glomus mosseae*. Although YORgroup had an effect on %AM, there was no significant difference between the YORgroups (ancestor, old, new) in the subsets "Gl. mossaeae" and "Gl. intraradices", i.e. in the two AMF species subsets, there were no differences in biomass between ancestral, old and new genotypes (Table I.S2). *Glomus mosseae* is an early-stage colonizer (Sykorova et al. 2007) and well adapted to highly disturbed systems like agricultural soils (Hijri et al. 2006; Oehl et al. 2004) or likewise pots inoculated with mixed soil or colonized root fragments. New cultivars were bred to grow fast, and therefore they need to quickly acquire nutrients. Most studies incorporated in this meta-analysis used a potentially P-deficient growth substrate and thus promoted the symbiosis. The lower MR of new cultivars growing with *Glomus intraradices* might indicate some physiological incompatibility between AMF and plant, e.g. the plant can down-regulate AMF colonization by reduced C translocation to the fungus (Ercolin and Reinhardt 2011) or the fungus can influence the level of gene transcription in the host plant as demonstrated for segregated lines (Angelard et al. 2010).

In plant subsets, the effect of YORgroup and YOR was tested on MR for the family *Poaceae* (the group with the highest number of trials). No trend was detectable for wheat and barley, but a negative effect for maize, i.e. new maize cultivars had lower MR as compared to ancestral maize accessions. This negative trend contradicted the finding that the plant type level "cereals" produced a positive effect for YORgroup on MR.

However, the statistical power of the moderator YORgroup in the maize subpopulation was very low and thus the reliability of this trend is not high. For barley, the number of trials was even smaller and the variability likely too high for a significant trend. The "Wheat" subset had a sufficient number of trials but no trend for MR and YORgroup was detectable either. The high variability in the wheat subpopulation might be due to the fact that plants (also being members of the same genus *Triticum*) differ dramatically in their physiological traits, like P efficiency, pathogen resistance and tolerance against influences like P deficiency or intraspecific density.

Summarized, the moderator variables density, pre-germination, plant, plant type and AMF species had an effect on both subsets "Before 1950" and "After 1950" thus possessing the potential to influence a MR trend for year of release in crop plants. In contrast, the moderator variables duration and soil pH were only important for genotypes released before or after 1950, respectively.

The analysis of the effect of AMF species and plant on MR revealed that the AM fungal genotype was more important than the plant identity; although this was only testable for three *Poaceae* genera (barley, maize, wheat). The analysis of *Poaceae* ("cereals") and *Fabaceae* ("legumes") as a subset showed that on a larger scale plant identity gained importance on the MR trend for year of release in crop plants.

What is the role of mycorrhizal responsiveness and the year of release for AM fungal root colonization and P efficiency in crop plants?

In our dataset, MR was positively correlated with %AM (Fig. I.2) and this finding is consistent with those of Lekberg et al. (2005). However, in the literature the opposite has also been reported (Hetrick et al. 1993; Kaeppler et al. 2000; Yücel et al. 2009). Each of these contradicting studies used about 30 trials, while our analysis and the meta-analysis of Lekberg

et al. used about 400 and 290 trials, respectively. This large number of studies (containing even those reporting the opposite effect) likely helped uncover the positive relationship of MR and %AM, although the relationship was not that strong (R^2= 0.30).

The relationship between MR and P efficiency was inconsistent. Most of the studies used for the analysis of P efficiency worked with potentially low P soil. For this soil fertility level, it was suggested that PAE is more important than PUE (Wang et al. 2010). However, in our dataset mPAE had no significant effect on MR, but the negative effect of mPUE was highly significant. Therefore, plant genotypes with high MR acquired more P when mycorrhizal and utilized more efficiently the acquired P when non-mycorrhizal. This was notably the case for the maize and soybean genotypes of the Khalil et al. (1994) study. The exclusion of this study was able to turn the correlation of mPAE with MR from positive to negative, and additionally to nullify the effect of mPUE on MR. Some of the plant genotypes used in that study were those with the highest mPAE and lowest mPUE of the whole dataset, i.e. when those genotypes were mycorrhizal, they took up more P than non-inoculated control plants. They were highly inefficient in P acquisition, while non-mycorrhizal genotypes utilized P to a higher degree, i.e. they were P utilization efficient. These P acquisition inefficient and P utilization efficient genotypes were all highly mycorrhizal responsive.

The other genotypes in the dataset had a higher mPAE and a lower mPUE, but showed a high variability in MR. High P efficiency may cause an increased P supply and thus an increased plant P level. The high plant P level reduces the intensity of the AMF and plant interaction, as in %AM and biomass accumulation (Baon et al. 1993; Gao et al. 2007). For single studies and genotypes this might be true, but in general, variability in MR was too high and too dependent on other factors, like soil pH, plant density and substrate volume, plant species and AMF species, to expect a direct relationship between MR and P efficiency.

Analyzing the influence of the moderator YORgroup on %AM revealed that ancestral accessions were more intensely colonized than new cultivars (Table I.6). This decrease in

colonization from ancestral to new genotypes is consistent with the literature (Hetrick et al. 1993; Kaeppler et al. 2000; Zhu et al. 2001). An explanation for a reduction in %AM in new cultivars could be an increase in pathogen resistance. Toth et al. (1990) suggested that genotypes with a reduction in pathogen susceptibility tend to be less colonized by mycorrhizal fungi as well. However, no correlation between genotype age and pathogen susceptibility was evident (An et al. 2010; Steinkellner et al. 2012).

The negative effect of YORgroup on %AM and the positive effect on MR suggested that new cultivars were less colonized but had a higher MR, and thus were able to compensate for the low biomass of non-mycorrhizal plants, as compared to ancestral accessions. The importance of the correlation between %AM and MR is quite conflicting under these circumstances. %AM and MR were positively correlated, i.e. with a higher percent root length colonized plant genotypes profit more in terms of biomass from AMF, but that is not the case for e.g. new cultivars. The correlation of %AM and MR was highly significant but explained a moderate portion of variability (R^2= 0.30, P< 0.0001); therefore we suggest that the importance of this relationship is inferior to that of the moderator variable YORgroup and MR or %AM, respectively. Furthermore, %AM is also under the influence of different abiotic and biotic factors (An et al. 2010) and thus a correlation of MR and %AM cannot be used solely for any predictions concerning the outcome of either one or the other variable.

The moderator variables YORgroup and YOR had no influence on P efficiency, neither on mPAE nor on mPUE (Table I.6). New cultivars were not more P-efficient or inefficient than old or ancestral accessions. This result is supported by the inconsistent findings in the literature. Thus, P-efficient cultivars can be found among old varieties and landraces (Wissuwa and Ae 2001) as well as among new cultivars (Wright et al. 2005; Zhu et al. 2003). The ability of a genotype to acquire and utilize P is not related to any changes in agricultural and breeding practices (at least for this dataset) but is influenced by other factors such as root parameters (Gahoonia et al. 1999), nutrient supply (Wang et al. 2010),

pathogenic state, plant species (Fernandez et al. 2009) and associated AMF species (Khalil et al. 1994).

Summarized, %AM was important for the MR trend for the year of release of crop plants but P efficiency was not (for our dataset). A possible re-evaluation of the influence of P efficiency on this trend would need a higher number of trials for PAE and/ or PUE. It would be of great interest if agricultural and breeding practices had an influence on cultivars over time and thus on their potential to respond to AMF. Breeding for higher yield by introducing valuable traits of landraces into parental inbred lines is a one-way street, and limited by nutrient availability. Breeding for higher responsiveness without higher dependence (Galvan et al. 2011; Janos 2007) and/ or breeding for higher P efficiency (Wissuwa et al. 2009), and thus better P acquisition and/ or better conversion of P into yield, is of greater importance for future agriculture.

Conclusions

In general, new cultivars were less intensely colonized but were more mycorrhiza-responsive compared to ancestral genotypes, although the response was not always consistent across all conditions. This MR trend for year of release in crop plants was confirmed by the moderator plant type and potentially influenced by the moderator variables density, pre-germination, plant, plant type and AMF species, while duration and soil pH were only important for genotypes released before or after 1950, respectively. %AM was also important for the MR trend for year of release but P efficiency was not (at least in our dataset). Therefore, we state that new crop plant genotypes did not lose their ability to respond positively to AMF for plant growth due to agricultural and breeding practices, but this statement is only true under certain conditions; plants need to grow on P-deficient soil, with AMF species like *Glomus mosseae*, and the comparison needs to be done between ancestral and new genotypes.

Additionally, the MR trend for year of release was detected in a dataset dominated by lab studies, i.e. studies performed under controlled and mostly artificial greenhouse environments and thus an extrapolation of the results of this meta-analysis to the field situation is not recommended. More field studies testing the effect of AMF inoculation on new, old and ancestral genotypes need to be done before more reliable predictions can be made. The fact that this MR trend for the year of release was present under P-deficient conditions highlighted the potential of the combined use of new cultivars and specific AMF for sustainable agriculture.

The low impact of the moderator variable YOR (representing the year of release dates) was due to the fact that year of release dates were only available for new and old cultivars, and the latter ones were under-represented in our dataset. Although old genotypes hold the potential to outperform new cultivars in terms of MR, additional work needs to be done with this year of release class. Most studies focused on the comparison of ancestral and new

genotypes and thus the number of old genotypes released between 1900 and 1950 was quite low, which is problematic in terms of establishing clear patterns.

Additionally, it is highly recommended that in future studies a measure of the variance of sample means, like standard error, is included to permit parametric weighting methods. Then it would be possible to test with higher statistical power the influence of agricultural and breeding practices on plant growth promotion by AM fungi.

For this study and under these data constraints, new crop plant genotypes did not lose their ability to respond to mycorrhiza due to agricultural and breeding practices. Therefore, plant breeders focusing on sustainable, organic agriculture can include new cultivars in their germplasms.

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1007/s11104-011-1095-1.

CHAPTER 3

Are there temporal trends in root architecture and soil aggregation for *Hordeum vulgare* breeding lines?

Abstract

The crucial role of roots in mediating agricultural sustainability and food security is becoming more widely appreciated. Here we tested the potential impact of barley (*Hordeum vulgare* L.) breeding (German germplasm) on root architecture and possible ripple-on effects on soil aggregation. In a greenhouse study, we tested two barley breeding lines. We focused on very fine (<0.2 mm) and fine (0.2–1 mm) roots. Soil structure was measured as percentage of water-stable macroaggregates and aggregate size distribution from dry-sieving. Breeding of barley reduced very fine root length of one of the tested lines but had no effect on our measures of soil structure. Our results indicate that breeding practices need not lead to an overall decline in root length. While we did not find that reduced very fine root length propagated to negative effects on soil structure parameters, additional studies should address this important aspect in other crop lineages and soils.

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Introduction

In the last century, plant breeders focused on yield and shoot biomass to improve food security at the expense of the belowground part: the root. Now, the 'hidden half' comes to the fore and researchers realize its importance for the Second Green Revolution (Gewin 2010; Lynch 2007). The root and its architecture, the spatial configuration of the root system over time (Lynch 2007), is essential for soil exploration, nutrient acquisition, interaction with symbiotic soil organisms, e.g. rhizobia, arbuscular mycorrhizal fungi (AMF) (Fisher and Long 1992; Parniske 2008), pathogen defence, and soil stabilization (den Herder et al. 2010). The ever growing world population and the development of new, but suboptimal agricultural sites, with low fertility, drought and salinity stress, and the increasing soil degradation make it inevitable to include the factor root in germplasm screenings.

The screening of cereal genotypes revealed that the focus on yield improvement in modern plant breeding programs unintentionally caused changes in root architecture over time. Tests on time-series of genotypes (e.g. in corn (*Zea mays* L.); Sanguineti et al. 2006) and comparisons between a landrace and a modern genotype (e.g. in barley (*Hordeum vulgare* L.); Zhu et al. 2003) showed a negative trend for length of the primary root, and total root length density, respectively. Bertholdsson and Kolodinska-Brantestam (2009) went one step further and screened Nordic barley germplasm for changes in the longest seminal root and detected the same negative trend in a breeding line; we use this term here to describe a time line of cultivars connected through crossing and selection over a distinct time period.

However, to our knowledge, there is no study combining parameters of both soil ecology (see Zhu et al. 2003) and plant breeding (see Sanguineti et al. 2006) to examine root architecture traits important for soil stability in barley lines; the trait of interest would be root length with a focus on very fine root (<0.2 mm) and fine root (0.2–1 mm) length (Jastrow et al. 1998; Miller and Jastrow 1990). In the present study, we hypothesize that the emphasis on

yield in breeding programs had a deleterious effect on root length in barley, especially on very fine and fine root length, and that there are ripple-on effects on soil aggregation, a process that is strongly mediated by root length (e.g., Six et al., 2004).

Materials and methods

Experimental design and set-up

Two barley breeding lines, with Germany as country of origin, were selected (Fig. II.S1): line A (Groninger, Friedrichswerther Berg, Schladener I, Herfordia, Birgit, Monika) and line B (Kalkreuther Früh, Mahnsdorfer Viktoria, Dea, Senta, Franka, Carola). The cultivars belonged to six-rowed winter type with hooded seeds and were bred by local breeders under Central European climate conditions. The 12 genotypes were chosen from the 'barley lineage catalogue' of the Bayrische Landesanstalt für Landwirtschaft and were provided by IPK Gatersleben (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany).

The soil used was an Albic Luvisol collected from a meadow at Freie Universität Berlin with the following properties: 74% sand, 18% silt and 8% clay; 6.9 mg/100 g P (calcium-acetate–lactate); 5.0 mg/100 g K (calcium-acetate–lactate); 0.12% N (total); 1.87% C (total) and soil pH was 7.1. Before use, the soil was sieved to 4 mm to exclude coarse organic matter.

Plants were grown in 2 L pots and each genotype was represented by five replicates. Three seeds were sown directly in the soil. After 5 days, seedlings were thinned to one plant per pot. The experiment was conducted in a greenhouse of the Botanical Garden Berlin, Germany, during the time of October 2010 to January 2011. Plants were watered with deionized water as needed, and, after 5 weeks, fertilized with low-P Hoagland solution.

The chosen cultivars were bred in Germany and tested in a German soil under low nutrient conditions to favor the soil stabilizing AMF.

After 12 weeks of growth, soil samples and plant material were harvested.

Analysis

At harvest, shoots were cut off directly above the roots. Soil (100 g per pot) was carefully detached from the root system (the top 1 cm of soil in the pot was discarded). Roots were gently washed off to clean them of any attached material. Plant material was dried at 40°C and afterwards weighed.

For determination of root length, we used WinRHIZO (Win-RHIZO Pro v. 2007d, Regent Instrument Inc., Quebec, Canada; scanner: Epson Perfection V700 PHOTO) an automated root measuring system (Arsenault et al. 1995; Himmelbauer et al. 2004). We worked with 300 dpi resolution and the automatic threshold.

Soil samples were dried at 80 °C and sieved to 4 mm. To test for any effects of root architecture on soil stability, we determined the percentage of water-stable macroaggregates and, separately, the size distribution of macro- and microaggregates in the soil samples.

The stability determination of wet aggregates was performed following a modified protocol of Kemper and Rosenau (1986), resulting in the percentage of water-stable aggregates in the soil samples. Soil (4.0 g) was placed into a sieving machine (Agrisearch Equipment, Eijkelkamp, Giesbeek, Netherlands) and agitated for 5 min. Aggregates, water-stable and water-instable, and coarse matter > 250 _m (e.g. stones, organic debris) were separated during the process. The weight of water-stable aggregates was corrected for coarse matter.

A stack of sieves (2 mm, 1 mm, 250 _m, 53 _m) was used to determine the aggregate size distribution based on dry-sieving. The soil (50 g) was loaded on the top sieve, subsequently the stack was shaken (10-times in 10 s), and finally the aggregates size classes were weighed. For the mean weight diameter (MWD), we calculated the sum of the proportions of the weight and mean diameter of aggregates of all five size classes. MWD was calculated as:

MWD = (SC2 mm × 3 mm) + (SC2−1 mm × 1.5 mm) + (SC1 mm−212 _m × 0.606 mm)

+ (SC212−53 _m × 0.1325 mm) + (SC<53 _m × 0.0265 mm).

Statistics

Despite the fact that genotypes used were of the winter-type, plants of three genotypes flowered (Schladener I, Friedrichswerther Berg, Mahnsdorfer Viktoria) and thus were excluded from the statistical analysis due to reduced carbon allocation to root growth.

We used R v.2.15.0 (R Development Core Team 2010) for calculation of Pearson's product moment linear regressions and to test for normality and homogeneity of variances. No transformation was needed.

Results

Plant breeding and root architecture

Breeding programs had no significant effect on plant dry weight, neither for shoots nor for roots (Table II.1).

Table II.1 Shoot and root biomass dry weight of two barley breeding lines. Data are means of five replicates ± standard error.

Line	Cultivar [a]		Shoot DW [b]	Root DW
A	Groninger	(1894)	5.10 (0.35)	2.67 (0.13)
	Herfordia	(1950)	4.34 (0.14)	2.89 (0.12)
	Birgit	(1976)	4.50 (0.36)	2.77 (0.25)
	Monika	(1991)	4.55 (0.30)	2.56 (0.06)
B	Kalk	(1914)	5.49 (0.56)	2.80 (0.18)
	Dea	(1953)	5.66 (0.30)	2.77 (0.18)
	Senta	(1963)	4.86 (0.11)	2.48 (0.12)
	Franka	(1980)	4.95 (0.16)	2.84 (0.09)
	Carola	(1998)	4.79 (0.35)	2.87 (014)
	Regression [c]		R^2 P	R^2 P
A			0.12 0.15	0.003 0.8
B			0.12 0.08	0.001 0.8

[a] Cultivar name. Values in brackets are release year dates

[b] Shoot and root dry weight in g $(plant)^{-1}$

[c] R^2 and *P*-value are derived from simple linear regression.

The analysis of the root architecture traits revealed that total root length consisted of about 90% very fine roots (consisting of 40–60% with diameter < 0.05 mm) and 10% fine roots. The positive correlation of very fine and fine root length, respectively, with root dry weight was more pronounced in breeding line B compared to line A (Fig. II.S2). Additionally, very fine root length decreased over time for line A but not line B. In line B, the initially negative trend was interrupted with cultivar "Franka (1980)". No significant effect was detected for fine root length for either breeding line (Fig. II.1).

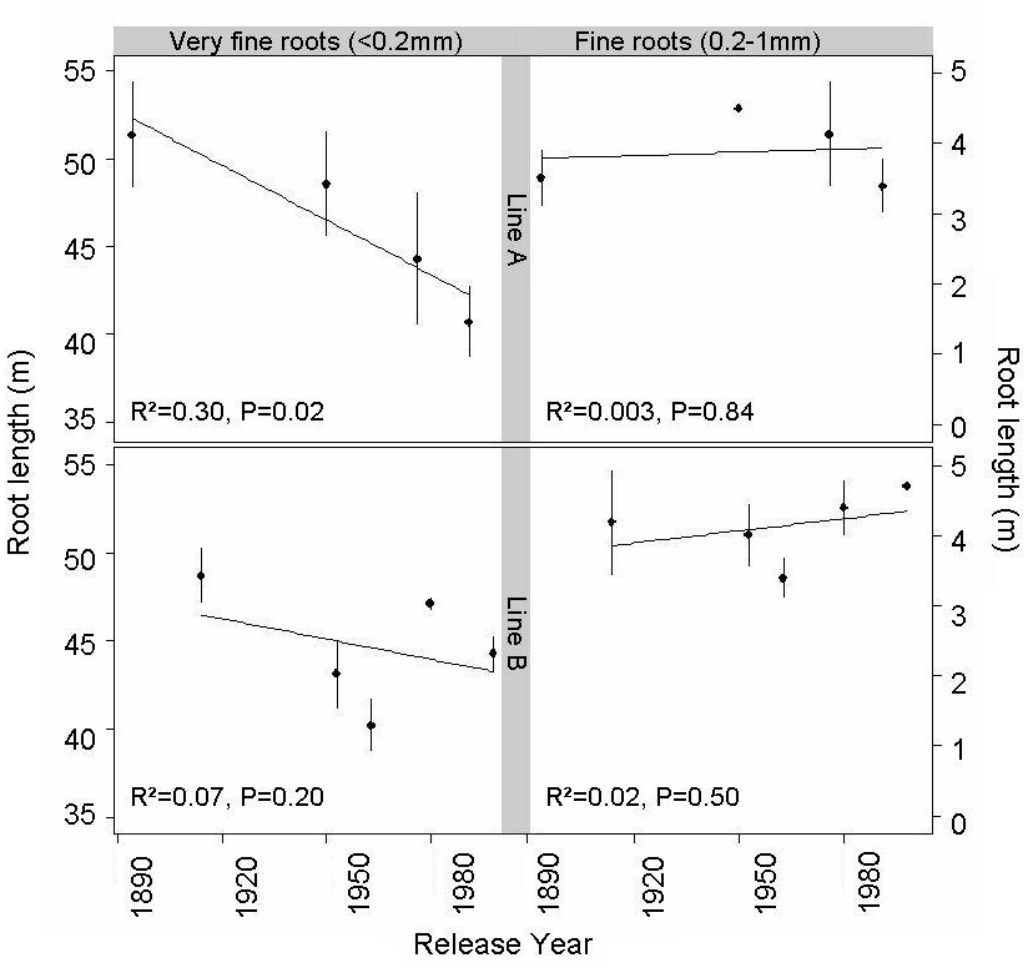

Fig. II.1 Root length (in m) of fine and very fine roots (columns) for two barley breeding lines A and B (rows) are presented. Bars indicate standard error. R^2 and P-value are derived from simple linear regression.

Plant breeding and soil stability

The percentage of water-stable macroaggregates did not change significantly in any breeding line and, additionally, length of fine (line A: R2 = 0.08, P = 0.23; line B: R2 = 0.001, P = 0.71) and very fine roots (line A: R2 = 0.02, P = 0.52; line B: R2 = 0.03, P = 0.41) had also no impact. The used soil consisted initially of 80% water-stable aggregates and thus is a highly aggregated soil. For line A there was a negative trend, which was not significant, while the opposite was true for line B (Fig. II.S3). This latter trend was also not significant. For MWD,

no obvious pattern was detectable for either release year of tested cultivars (Table II.S1; Fig. II.S4) and root length, fine (line A: R2 = 0.08, P = 0.23; line B: R2 = 0.01, P = 0.71) and very fine root length (line A: R2 = 0.02, P = 0.52; line B: R2 = 0.03, P = 0.41), respectively.

Discussion

The improvement or maintenance of soil quality is of major importance for sustainable agricultural systems. Several common production agriculture processes can lead to a decrease in soil aggregation, e.g. fungicides and fertilizer reduce indirectly soil stability via negative impact on arbuscular mycorrhizal fungi (e.g., Rillig 2004) and techniques like tillage directly destroy soil aggregates (Six et al. 2000; Tebrugge and During 1999). Thus, the improvement of soil quality and structure as well as the reduction of erosion and nutrient and water run-off needs to be the goal for plant breeders (Brummer et al. 2011). In order to achieve beneficial effects on soil aggregation, an important target for plant breeders is root length, which we examined here.

Plant breeding and root architecture

In our study, the reduction of root length over time could only be found in breeding line A and for the very fine roots (<0.2 mm). In contrast, in line B cultivar "Franka" had a higher very fine root length, shoot and root biomass than its direct progenitor and descendent. The increased application of fertilizer, during the last century, may have inadvertently selected for genotypes with reduced root system, especially the very fine roots important for water and nutrient uptake (Eissenstat 1992; Jackson et al. 1997; Sanguineti et al. 2006). The decrease of this root architecture trait should be eliminated in plant breeding programs, especially for situations under suboptimal growth condition (e.g. low plant-available phosphorus or drought). This is possible with the selection of the right cultivars from a germplasm as demonstrated for cultivar "Franka".

Plant breeding and soil aggregation

The negative effects of plant breeding on root length we observed did not propagate to effects on soil stability in our study system. Additionally, there was neither a correlation of soil stability and very fine and fine root length, respectively, nor a trend over time. However, roots are generally important for soil stability: roots reinforce soil by increasing shear-strength and in-plane tensile-strength (Reubens et al. 2007). Furthermore, the typical grass root architecture with a large number of very fine roots is able to stabilize soil more effectively than a coarse root system.

Roots are just one factor, but an important one, in the complex network of interactions influencing soil stability and aggregate formation that is best represented by a hierarchical model (Tisdall and Oades 1982). Fine and very fine roots directly or indirectly contribute to the formation of micro- and small macroaggregates, by mechanisms including association with arbuscular mycorrhizal fungi which can mediate soil aggregation (Rillig and Mummey 2006), soil entanglement, and production of exudates that can serve as binding agents for aggregates (Jastrow et al. 1998; Miller and Jastrow 1990). The soil we used was highly aggregated (80% WSA) and thus an increase in stability could be possible for plant species forming more intense associations with AMF (Barea 1991). Barley is a crop with low mycorrhizal responsiveness (Baon et al. 1993; Boyetchko and Tewari 1995; Chen et al. 2005). Therefore, it would be of interest to test also breeding lines of mycorrhiza responsive crops, e.g. onion (Galvan et al. 2011) and lettuce (Jackson 1995), for their influence on soil structure.

Conclusion

The negative trend for very fine root length was present in German barley germplasm, but this was not the case for all the tested lines, which is encouraging in terms of plant breeding effects. We found no evidence that the reduction of very fine root length translated to decreased soil stability. However, we suggest that further tests like this be carried out in other crops and in soils with different properties to gain further insights in this important research field.

Acknowledgements

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Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/ j.apsoil.2013.01.003.

CHAPTER 4

Arbuscular mycorrhizal influence on Zinc nutrition in crop plants - a meta-analysis

Abstract

The effects of soil Zinc (Zn) deficiency on human health and productivity of livestock and crops are severe and thus increasing the bioavailable concentrations of Zn in plant tissue has to be the goal of modern, sustainable agriculture. In this meta-analysis, we quantitatively analyzed the potential role of arbuscular mycorrhizal fungi (AMF) in improving Zn concentrations in plant tissues for a variety of crops and soils. We performed a random-effects meta-analysis on 104 articles comprising 263 trials to test the influence of 10 independent variables on AMF-mediated Zn uptake in comparison to non-mycorrhizal control plants for above-, belowground, fruit and seed tissue. AMF had a positive overall impact on Zn concentration in all tissue types and this positive effect was modulated primarily by soil texture. Soil pH and soil Zn concentration affected AMF-mediated Zn uptake in shoots whereas soil P concentration influenced fruit Zn concentration. For our dataset, we concluded that AMF positively affected Zn concentration in various crop plant tissues under distinct environmental conditions.

http://dx.doi.org/10.1016/j.soilbio.2013.11.001

Introduction

Zinc (Zn) is an essential micronutrient for plants, animals and humans and it is an integral component of hundreds of enzymes and thus obligate for metabolism (e.g. Alloway 2009; Coleman 1992; Vallee and Falchuk 1993). Therefore, Zn is relevant for development, reproduction and signalling due to its structural, catalytic and activating functions (e.g. Bedwal and Bahuguna 1994; Broadley et al. 2007; Cavagnaro 2008; Roohani et al. 2013). Due to its vital role, Zn deficiency generally causes impairments in physical development and fertility (Abdelrahman et al. 1998; Alloway 2009; Cakmak 2000; Prasad 2010). Zn deficiency usually appears simultaneously in humans, livestock and crops as a consequence of low soil Zn concentrations (Alloway 2009; Cakmak 2008; Prasad 2010; White and Zasoski 1999). Hence, any sustainable attempt to improve human Zn nutrition needs to focus on crops as the primary producers of the abovementioned food chain, and more specifically on the plant-soil continuum.

Zn has a low mobility in soil solution and its uptake is diffusion-limited. Reduced phytoavailability is a widespread problem in arid and semiarid regions mainly present in calcareous soils (Cakmak et al. 1999; Broadley et al. 2007) affecting about 50% of agricultural area used for cereal cropping world wide (Alloway 2009; Cakmak et al. 1999). One solution for preventing Zn deficiencies in plants irrespective of soil Zn status is through biofortification; a technique that permits increasing bioavailable concentrations of essential minerals in the consumable portions of crops (White and Broadley 2005). By this technique, food for humans and fodder for livestock can be improved in target crops. Additionally, not consumable plant portions (mostly shoots and roots) gain increased Zn concentrations and can be processed to green manure or compost and used as a sustainable and organic Zn fertilizer resource (Mishra et al. 2006).

Biofortification comprises two major approaches: genetic and agronomic biofortification (Cakmak 2008). Genetically increased plant Zn tissue concentration is achieved by breeding and selection for improved Zn efficiency in plants; a plant trait comprising Zn acquisition, translocation and utilization (Hacisalihoglu and Kochian 2003). However, this is a long-term process and can only be successful when focal soil is suitable for plant cropping (Cakmak 2008; Hacisalihoglu and Kochian 2003). By contrast, agronomical tools for enhanced Zn tissue concentration such as application of Zn fertilizers are readily usable and have been approved (Cakmak 2008; Zhang et al. 2012). Zn fertilizer can be applied via soil and leaves (Rengel et al. 1999), but the resulting Zn concentration in edible tissues varies depending on factors such as soil properties (pH, organic matter content and cation exchange capacity) and fertilizer form (chemical or organic fertilizer) (Phattarakul et al. 2012; Rashid and Fox 1992; Zou et al. 2012).

So far, these genetic and agronomic approaches have proven successful (Stein et al. 2007), but involve high investment costs for genetic engineering or fertilizer application. An additional, sustainable tool to improve micronutrient concentrations in crops could be arbuscular mycorrhizal fungi (AMF) (Cavagnaro 2008; He and Nara 2007); AMF are ubiquitous, symbiotic fungi from the phylum *Glomeromycota* (Schüßler et al. 2001). They are an integral root component (Smith and Smith 2011) of crops capable of forming this symbiotic interaction; some crop species do not form mycorrhizae, such as members of the *Brassicaceae* (Wang and Qiu 2006). The AMF-related services can result in better plant performance and soil quality (e.g. Auge 2001; Borowicz 2001; Newsham et al. 1995; Parniske 2008; Smith and Read 2008) but the most prominent facet of the range of services provided by AMF is the uptake of immobile nutrients such as P and Zn (e.g. Bolan 1991; Bürkert and Robson 1994; Jansa et al. 2003; Marschner and Dell 1994).

Association with AMF allows an alternative nutrient assimilation pathway through extraradical and intraradical hyphae, arbuscules and the root apoplast interface (Parniske

2008; Smith and Read 2008). In *Glomus intraradices*, a Zn transporter has been identified (GintZnT1) (Gonzalez-Guerrero et al. 2005) and its putative function includes transport of Zn through hyphae or even Zn loading in the apoplastic space between fungi and plant plasma membrane (Anton et al. 1999; Cavagnaro 2008; MacDiarmid et al. 2002; Palmiter and Findley 1995). The connection of the plant root system with the AMF external hyphal network increases the surface area beyond the nutrient depletion zones of roots (Leake et al. 2004; Smith and Read 2008)– a simple but effective step in the diffusion-limited process of Zn uptake. Additionally, AMF can acquire Zn in soil pores and nutrient patches not reachable for plant roots or root hairs (Bolan 1991). Overall, the additional AMF-mediated pathway allows for an increased Zn uptake of up to 25% in shoot and roots (Cooper and Tinker 1978; Marschner and Dell 1994).

However, the application of AMF as a sustainable management approach, primarily for improved plant growth, and also for improved Zn concentration in crops is not straightforward due to the high variability of plant responses to AMF; a vast amount of published literature testing the impact of different environmental and biological factors on AMF-mediated Zn concentration in plant tissues can be found. Soil texture not solely determines solubility and mobility of Zn in soil but simultaneously influences performance of AMF (Karagiannidis and Hadjisavva-Zinoviadi 1998). Soils with high cation exchange capacity (CEC), pH, clay and organic matter content exhibit reduced Zn phytoavailability and allow for improved Zn acquisition by AMF (Alloway 2009; Armour et al. 1990).

Besides soil texture, identity of plant and AM fungi is the dominating key source of variability in AMF-mediated Zn tissue concentrations. Species-level and even intraspecific variation in Zn-efficiency exists in both AM fungi and associated plants (Cakmak et al. 1997; Ciftci et al., 2010; Graham and Rengel 1993; Kafkas and Ortas 2009; White and Broadley 2009). For plants, variability in Zn efficiency can be mediated by morphological and physiological root traits. Cereals, for example, have fine, thin and highly branched root

systems that can result in improved nutrient uptake but reduced AM fungal root colonization, and AM nutritive and growth responsiveness (Newsham et al. 1995; Tawaraya 2003). For AMF, different species diverge functionally, i.e. AMF species or even isolates perform differently, for example in terms of hyphal growth, nutrient uptake and root colonization (Allen et al. 1995; Mehravaran et al. 2000; Munkvold et al. 2004) due to their diverse functional traits and life strategies (Chagnon et al. 2013). In addition, the composition of AM fungal inoculum also determines plant responses. A more diverse assemblage of AMF species increases the probability of the presence of beneficial or complementary species being more effective in providing beneficial services to associated plants compared to single species inocula (Hart and Forsythe 2012; Hart and Reader 2002; Hoeksema et al. 2010; Maherali and Klironomos 2007; Vogelsang et al. 2006).

Furthermore, the duration of an experiment may be of exceptional importance in determining AMF-mediated Zn tissue concentrations. Longer experiments can permit better development of the symbiosis (Subramanian et al. 2008; Vierheilig and Ocampo 1991a), while resources as rooting space and nutrients are decreasing (Daft 1991; Schroeder and Janos 2004). Additionally, the developmental stage of both AMF and associated plant is an influencing factor. In longer experiments, physiological changes resulting from the switch of vegetative to reproductive growth causes altered nutrient translocation, compartmentation and utilization (White and Broadley 2009). So far, there has been no evidence that AMF can directly influence the xylem and phloem loading steps for Zn transport from root to shoot and grains; the major bottlenecks of Zn translocation occurring during the vegetative and reproductive phase (for a detailed review see Palmgren et al. 2008; Stomph et al. 2009).

Interpreting the impact of AMF on plant Zn nutrition can be complicated due to the simultaneous effects of AMF-mediated plant P nutrition (Cardoso and Kuyper 2006). Enhanced P acquisition often results in plant growth promotion. In a Zn deficient soil, the increased biomass, as a result of improved P nutrition by AMF, can dilute the Zn tissue

concentrations aggravating Zn-deficiency (Cavagnaro 2008). Additionally, Zn-deficiency can up-regulate the expression of P affinity transporters and lead to an intensification of the deficiency symptoms (Huang et al. 2000). Furthermore, an improved P nutrition can cause increased concentrations of phytate in seeds (Erdal et al. 2002); phytate is an anti-nutrient chelating essential nutrients like Zn and thus reduces their bioavailability for humans and livestock except for ruminants. On the other hand, AMF are able to reduce phytate concentration while enhancing Zn concentrations in maize seeds (Subramanian et al. 2013).

As a consequence of this complex interaction framework of edaphic, environmental and biological factors affecting the AMF-mediated Zn nutrition, examples of positive, neutral and negative effects of mycorrhizal inoculation on crop tissue Zn concentrations are present in the literature (e.g. Alloush and Clark 2001; Bagayoko et al. 2000; Cavagnaro et al. 2008; Karagiannidis et al. 2007; Mohandas 1992; Rouphael et al. 2010). There have been a few qualitative syntheses (literature reviews) addressing this important issue (Cavagnaro 2008; He and Nara 2007; Impa and Johnson-Beebout 2012; Rehman et al. 2012). However, to our knowledge, no meta-analysis has yet been conducted to quantitatively synthesize and evaluate the potential role of AMF for plant Zn nutrition across a range of crop species grown under various conditions. Thus, it is unknown whether AMF are a viable option for alleviating Zn deficiency in humans and livestock consuming Zn-deficient plant portions. Therefore, we aimed at filling this gap by performing a meta-analysis to address the following hypotheses: (i) AMF increase Zn concentration for root, shoot and fruit tissue across different crops. (ii) The AMF-mediated Zn concentration in different crop tissues is influenced by edaphic factors (soil texture, soil pH and nutrient concentrations) limiting mobility of Zn in soil solution and thus plant and AM fungal bioavailability, respectively. (iii) Studies performed under controlled environmental conditions in pots result in higher AMF-mediated Zn tissue concentration than field studies due to exclusion of influential variables. This allows disentangling complex interaction frameworks but leads to overestimation of effects. (iv)

Environmental factors optimizing plant growth conditions positively influence the AMF-mediated Zn tissue concentration; thus plants grown in adequate soil volume for longer than 2 month with a diverse assemblage of AM fungi as inoculum perform best.

Materials and Methods

Literature search

We conducted a literature search using Web of Knowledge[SM] by Thomson Reuters on 18 October 2012 with the search strings '*mycorrhiza** AND *zn*' and '*mycorrhiza**AND *zinc*' which retrieved a total of 802 publications.

We screened titles and abstracts of these publications for use of crop species as test plants and presentation of Zn tissue concentration data. When such a potentially suitable publication was detected, we further checked those articles for meeting our inclusion criteria: The articles needed to report data about (i) Zn tissue concentrations, (ii) crop plants (annuals and perennials, with tissue suitable for human nutrition, e.g. leafs, root, grain or fruit, respectively) with (iii) a control for AMF inoculation to distinguish between mycorrhizal and non-mycorrhizal plants. To include field studies in the dataset, a control did not need to be free of intra- or extraradical AM fungal structures but control plant roots needed to be colonized significantly less than those from the AMF treatment. Furthermore, the experiments had to be performed in (iv) soil or at least sand-soil-mixture. (v) The experimental plants and soils were not stressed by heavy metals, salt, drought or soil compaction. Zn fertilization as treatment was always applied via soil. We could find no suitable studies reporting data about leaf fertilization. Thus in studies presenting data for a Zn fertilizer treatment, we recorded control data only to avoid a potential Zn stress as confounding factor. (vi) The influence of AMF was tested in the absence of *Rhizobium*, to eliminate the influence of N-fixing symbionts, and (vii) a measure of variance (standard error, standard variance) or at least Anova tables, Tukey HSD, LSD, t-test had to be reported.

Following screening, 104 studies complied with the inclusion criteria and were integrated in our analysis. We extracted information on P and Zn tissue (shoot, root, fruit and grain) concentrations and biomass in the presence and absence of AMF; we used

concentration as measure of tissue quality. For the majority of studies, Zn concentration data was not the main focus but rather a by-product. We further extracted information on variance, sample size (*N*) and 10 relevant independent variables that are described below.

We were only able to directly retrieve variance information from a handful of studies. In the instances when standard error (SE) was reported, standard deviation (SD) was calculated as follows: SD = SE * sqrt(*N*). When only *P*-values following an analysis of variance were reported, we calculated SD by making the assumption that the distribution of the original data originated from an ideal normal distribution with the means reported in the paper.

Datasets

The collected data were split into three datasets corresponding to above-, belowground and fruit tissue (target tissue of experiments), thus we created three datasets: *shoot* (101 studies), *root* (28 studies) and *fruit* (13 studies). These three datasets were used for three separate univariate meta-analysis. With the exception of a single study (Subramanian et al. 2008), which reported data for all three tissues, all other studies presented data only for either one (*shoot* or *fruit*) or two types of plant tissue (*shoot* and *root*, *fruit* and *shoot*). The *root* dataset included information for plant species with tissue not suitable for humans (e.g. tomato, corn and beans), while for the *shoot* dataset at least 8 studies with comestible tissue (e.g. leek and garlic) were present. However, the *fruit* dataset comprised exclusively data for edible fruits (e.g. tomato, melon and cucumber) and edible seeds (e.g. wheat, corn and rice) (see supplementary information III.1). Our datasets included information for a broad range of crop species grown in different kinds of soil with variable pH and Zn concentrations. Additionally, we collected data on soil organic matter (OM) and classified it following Baldock and Skjemstad (1999). We did not use OM as independent variable because it was only applicable

to the *shoot* dataset. However, the majority of studies used soil with low OM (sandy soils: OM < 1.2 %, silty soils: OM < 1.4%, clayey soils: OM < 1.6%; 54 of 79 trials in *shoot*).

Effect size

The effect size was calculated as the natural log response ratio (rr) of mycorrhizal and non-mycorrhizal nutrient concentration:

$$rrZn = \ln\left(\frac{Zn_M}{Zn_C}\right),$$

where Zn_M represents the Zn tissue nutrient concentration (mg * kg DWT^{-1}) of mycorrhizal plants and Zn_C that of control plants. We were aware of the fact that results deriving from *root* dataset could overestimate any AMF-mediated effect. Zn in intraradical AM fungal structures (hyphae, vesicles and arbuscles) could confound the actual root Zn concentrations in mycorrhizal plants and thus, results from *root* dataset had to be interpreted with caution. In addition, Zn could also adhere to the outside of roots, irrespective of AMF treatment, but this would not bias our results since we use a response ratio.

We calculated the additional effect sizes rrP and rrbiomass to evaluate the impact of AMF mediated P uptake and potential growth promotion on rrZn. The calculation of these effect sizes was equivalent to that of rrZn. The results of rrP and rrbiomass are presented in the supplementary information 2 (Table S2 to S4).

Effect sizes were calculated in Metawin v.2.1 (Rosenberg et al. 2000) by using control and treatment mean and variance (SD), respectively and sample size (*N*).

Scatterplots of effect size vs. sample size or variance, respectively, were produced to test for potential publication bias. No obvious bias could be detected that could not be explained by true heterogeneity (Fig. S1) (Nagakawa and Santos 2012).

Categorical independent variables

Plant tissue had three levels: fruit, shoot and root corresponding to the three datasets. That way, we were able to test for any significant differences of the AMF-mediated Zn concentration between the different plant tissues.

Soil texture had three levels: *sandy*, *silty* and *clayey* soil. The soil texture data reported was used to classify the soil by using the USDA Natural Resources Conservation Service soil taxonomy (soils.usda.gov). The level *sandy* soil contained sand, loamy sand, sandy loam, sandy loam clay and sandy clay. The level *silty* soil included silt, silty loam, silty clay loam, silty clay. Clay, clay loam and loam formed the level *clayey* soil.

Soil pH had three levels following the USDA criteria (soils.usda.gov): *acidic*< 6.5, *neutral*= 6.6 to 7.3, *alkaline*< 7.4.

For the independent variables soil Zn and soil P (soil Zn and P concentration, respectively), we only used data derived from the most frequently applied extraction methods: DTPA-extractable Zn and Olsen-extractable P. Thus, we could ensure that the data were comparable among studies. Furthermore, we grouped the range of concentrations in either *deficient* or *non-deficient* categories. The level *deficient* contained critically low soil concentrations. For soil Zn, any concentration up to 0.5 mg Zn* kg soil^{-1} described soils with 'very low' Zn concentrations causing deficiency symptoms in a variety of crops (Alloway 2009 and references therein). For soil P, concentrations up to 9 mg P* kg soil^{-1} were classified as P deficient and P fertilization would enhance crop plant biomass and yield (Johnston and Poulton 2011; Rowell 1994). The *non-deficient* levels comprised soil Zn and P concentrations causing light or no nutrient deficiency symptoms in a broad range of crop species (soil Zn: 0.51to 8.3 mg Zn* kg soil^{-1}; soil P: 9.1to 135 mg P* kg soil^{-1}). With the exception of four studies, no additional nutrient solution containing Zn or P was applied during the experiments. We tested for the impact of these studies on the effect size and found that the

exclusion of these studies did not alter the results; therefore we retained them for all our analyses (Table S1).

Setting had two levels: *lab*, containing all studies performed in pots under controlled environmental conditions, and *field*.

Fertilization had two levels: *no* and *yes* with regard to P fertilizer application.

Experimental *duration* had three levels: *short* studies lasted up to 2 month, *intermediate* studies 2 to 4 month (56-112 days) and *long*-time studies 4 month and more. The level intermediate represented the level that was expected to have resulted in optimal growth time for AMF in pot experiments. Growth time was adequate to ensure colonization by AMF and to detect any mycorrhiza-mediated effects (Hart and Reader 2002) without exceeding a threshold after which plants could get pot bound and severely limited for nutrients.

Rooting space was used as a measure for adequate pot size for experimental plants to evaluate the impact of growth substrate volume and plant density per pot on the effect size and had two levels: *adequate* and *inadequate*. Rooting space is the product of the ratio of root biomass and potting space, as proposed by Poorter et al. (2012) and the pot internal competition:

$$rooting\ space = \left[\frac{biomass\ (g)}{volume\ of\ growing\ space\ (L)} \right] * number\ of\ plants\ per\ pot\ .$$

Values smaller than 1 g* L^{-1} were classified as *adequate* and values bigger than 1 g* L^{-1} as *inadequate* rooting space (Poorter et al. 2012). Field studies were placed in level *adequate*. If soil volume was not reported as liter, weight was used instead.

Plant type had four levels: *grass*, *annual herb*, *perennial herb* and *woody*. These levels were chosen in respect to different growth strategies.

AMF inoculum had two levels: *single* and *mix*. Single species inocula were dominated by *Glomus* species. The mixed species inocula comprised more than one AMF species and were either extracted from field soil or obtained from commercial suppliers.

Statistics

We conducted random-effects meta-analyses in Metawin v.2.1 to test for the influence of the 10 categorical independent variables on the impact of AMF on Zn tissue concentration for shoot, root and fruit plant tissue.

We used a permutation procedure with 3999 iterations (Adams et al. 1997) because our effect sizes violated the criterion of normality. Confidence intervals were then estimated through a bootstrap procedure that implemented bias-correction.

The majority of studies included in our analyses contained more than one trial due to experimental setups. This is a common issue in ecological meta-analyses and a severe violation of the assumption of independence of studies (Gelman and Hill 2007; Stevens and Taylor 2009). To handle the issues of non-independence in our datasets we implemented the following two corrections: (i) Whenever multiple trials shared the same control we corrected for the underlying dependence of the trials using the methodology presented in Lajeunesse (2011). (ii) Multiple trials originating from the same study were reduced to a single effect size through a fixed-effects meta-analytical procedure. This approach ensured that the random effects component of the meta-analysis was restricted to trials that belonged to different studies. However, the reduction of effect sizes per study was limited to preserve information of the independent variables, i.e. trials of one study were not reduced if effect sizes originated from different experimental systems represented by the independent variables e.g. different soil textures.

Validation

A sensitivity analysis was conducted to test for any disproportional impact of single studies (Copas and Shi, 2000). We tested significant results and only robust or corrected results were presented in the results section (for further information consult supplementary information III.2).

Results

Overall AMF effect on Zn uptake in different target tissues

AMF had a positive overall effect on Zn tissue concentration (Fig. III.1). In *fruit*, we found a 13%, in *shoot* a 18% and in *root* a 32% increase in Zn concentration in mycorrhizal compared to non-mycorrhizal plants. There was a non-significant trend for rrZn to decrease from *root* to *fruit*.

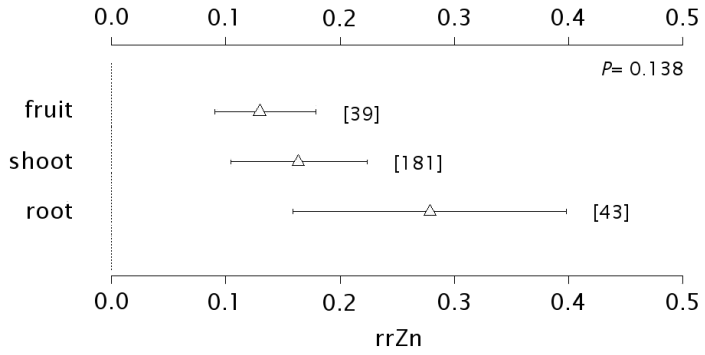

Fig. III.1. Effect of plant tissue type on rrZn. Effects are represented as means and bias corrected CIs. The means and CIs were positive and were not overlapping zero thus indicating that AMF had a beneficial impact on Zn tissue concentration. Values in parentheses were numbers of trials included in the analysis. Significance test for between-level differences was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

Edaphic factors affecting AMF-mediated crop Zn nutrition

Soil texture was the only independent variable that significantly influenced the effect size in all plant tissues (*fruit*, *shoot* and *root*) but the variable affected rrZn differently for *fruit*, *shoot* and *root*, respectively (Fig. 2). In *fruit*, plants grown in sandy soil yielded higher rrZn as plants grown in silty or clayey soils. The opposite was true for *shoot* and *root*.

Soil pH also had a significant effect on rrZn in *shoot*: AMF mediated a higher Zn concentration in shoot biomass in soil with neutral and alkaline pH as in acidic substrates. In

root and *fruit*, acidic substrates tended to lead to the lowest rrZn, but the effect was not significant (Fig. III.2).

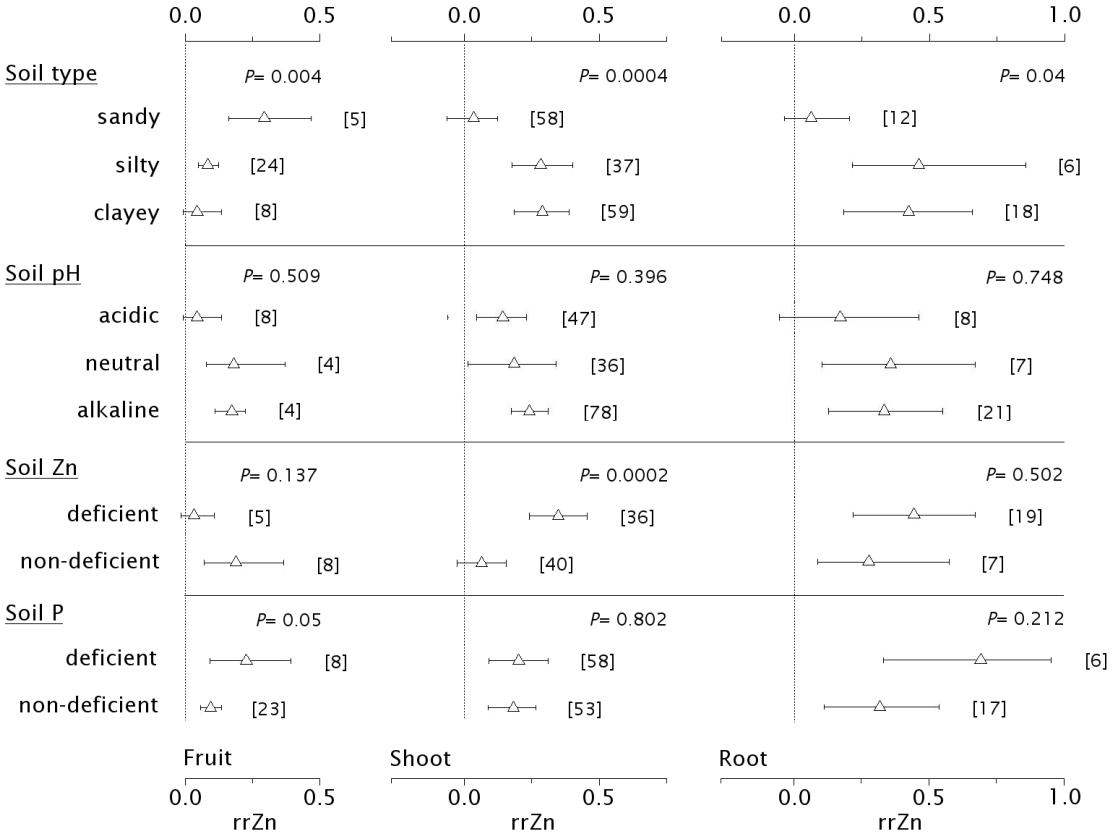

Fig. III.2. Effect of the edaphic factors soil texture, soil pH, soil Zn and soil P concentration on rrZn in datasets *fruit*, *shoot* and *root*. Effects were represented as means and bias corrected CIs. Values in parentheses were numbers of trials included in the analysis. Significance test for between-level differences was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

Soil Zn only significantly influenced rrZn in the *shoot* dataset; plants grown in non-deficient soil Zn concentrations (> 0.5 mg* kg soil^{-1}) showed a reduction in rrZn. This pattern was also consistent in different soil textures and soil pHs (Table III.S4).

The variable soil P had a marginally significant effect in the *fruit* dataset; plants grown in substrate with non-deficient P concentrations (> 9 mg* kg soil^{-1}) yielded lower rrZn as plants grown in P deficient soil.

The impact of the experimental setting

Setting had only a significant effect on rrZn in dataset *fruit*; plants grown under controlled environmental conditions yielded higher effect size values compared to field studies (Fig. III.3). Nevertheless the number of trials for lab studies was low compared to field studies.

Environmental factors as mediators of AMF effects

The overall positive effect of rrZn was not influenced by fertilization but there was a trend in *shoot* for application of P fertilizer to reduce rrZn (Fig. III.3). As a consequence of this trend, we further tested the impact of all remaining independent variables on rrZn in the two subsets of *shoot*: *fertilization- no* and *fertilization- yes* (Fig. III.S2 and III.S3). Plant type and soil texture differed in their effect on rrZn when tested in both fertilization subsets.

Experimental duration did not affect rrZn in either *shoot* or *root*. For *fruit* dataset, data for this variable were insufficient for any analysis.

Furthermore, rooting space had also no significant effect on rrZn, meaning that inadequately small growing space had no detrimental impact on the effect size. As for experimental duration, insufficient data were available for *fruit*.

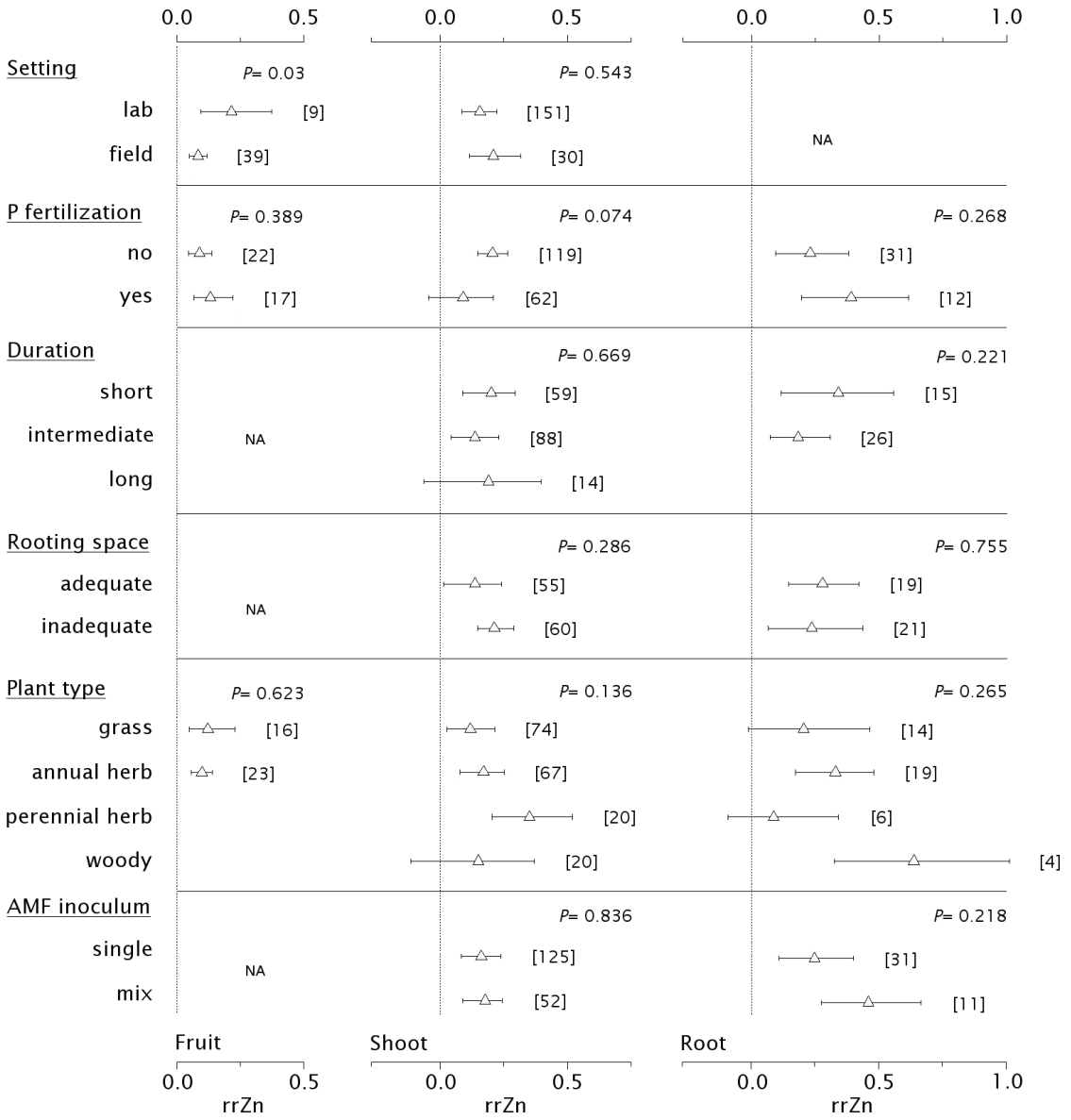

Fig. III.3. Effect of setting and important experimental growing conditions on rrZn in datasets *fruit*, *shoot* and *root*. Effects were represented as means and bias corrected CIs. Values in parentheses were numbers of trials included in the analysis. Significance test for between-level differences was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

Plant type had no significant effect but perennial herbs in *shoot* and woody plants in *root* seemed to have higher rrZn as grasses and annual herbs. For *fruit*, only grasses and annual herbs were used as experimental plants and no significant effect was present. The analysis in the two *fertilization*- subsets revealed that perennial plants seemed to profit more

from P fertilizer application and hence showed higher values for rrZn than other plant types and not fertilized perennial plants (Fig. III.S2), but due to low sample size this result needs to be treated with caution.

Single or mixed species AMF inocula did not differ significantly in their effect on rrZn.

Discussion

Overall AMF effect on Zn uptake in different target tissues

Our analysis provided strong, quantitative evidence that AMF positively influence Zn concentrations in crops irrespective of tissue type (Fig. III.1). This finding supported our first hypothesis and was in agreement with the existing literature (Garg and Kaur 2013; Kothari et al. 1991; Liu et al. 2000). The positive effect tended to decrease from *root* to *fruit*; the AMF-mediated Zn concentration increased in *roots* by 32%, in *shoot* and *fruit* by 18% and 13%, respectively. The symbiosis enhanced the diffusion-limited process of Zn acquisition and provided an improved phytoavailable Zn pool for the associated plants. Thus, AMF might influence *root* Zn nutrition directly while the Zn translocation from *root* to *shoot* and *fruit* was limited by bottlenecks of xylem/ phloem loading (Palmgren et al. 2008; Stomph et al. 2009) or storage and compartmentation (Hacisalihoglu and Kochian 2003).

The high rrZn in *root* might be partially explained by Zn attached to or incorporated in intraradical AM fungal structures of mycorrhizal roots (Olsson et al. 2011); the numerator could be potentially positively biased. Separating root and fungal structures is impossible and thus also disentangling these Zn sources.

Edaphic factors affecting AMF-mediated crop Zn nutrition

Soil texture significantly affected rrZn for all datasets but the pattern obtained for *fruit* differed from *shoot* and *root*; here, sandy soils yielded lowest values for rrZn (Fig. III.2). The soils in our dataset were mainly low in organic matter and thus Zn was not retained by chelation processes. Additionally, in sandy soils, Zn is quite soluble and mobile and thus AMF associated plants gain no benefit as compared to non-mycorrhizal plants. For *fruit*, the opposing pattern might be due to the confounding effect of test plants used in studies with

sandy soils which were exclusively members of the *Poaceae*. Thus, the pattern detected might be induced by changes in Zn translocation during seed development (Palmgren et al. 2008).

Soil pH only had a significant effect on rrZn in *shoot,* but *fruit* and *root* showed a comparable trend (Fig. III.2); rrZn was lowest in acidic soils. With decreasing pH the solubility and phytoavailability of Zn increases (Marschner and Dell 1994). Although AMF are known to alleviate pH stress in general (Clark and Zeto 1996; Rouphael et al. 2010) this benefit can be reduced in acidic soils due to fungistatic effects on mycelium and spores (Abbott and Robson 1985; Siqueira et al. 1984). However, the trials included in soil pH level acidic were dominated by non-deficient soil Zn levels, i.e. most studies with acidic growth substrate had a soil Zn concentration higher than 0.5 mg* kg soil^{-1} and thus did probably not suffer from Zn deficiency.

Non-deficient soil Zn concentrations (> 0.5 mg Zn* kg soil^{-1}) reduced rrZn in *shoot* and *root* (Fig. III.3) (Karagiannidis and Hadjisavva-Zinoviadi 1998; Marschner and Dell 1994; Smith and Read 2008). This reduction was probably caused by increased Zn availability and thus a diminished AMF benefit. The Zn concentrations of maximum 8.3 mg Zn* kg soil^{-1} was too low to cause toxic effects even in susceptible plant species (Alloway 2009). However, whether soil Zn is deficient or not for plants is mainly determined by soil texture and soil pH (Armour et al. 1990; Haq and Miller 1972; Haynes and Swift 1983). In our dataset, the reduction in rrZn for non-deficient soil Zn was also detectable in *soil texture* and *soil pH* subsets (Table III.S4). For the *soil texture* subsets, the mycorrhizal effect in sandy soils was less pronounced for Zn deficient soils than in silty or clayey soils but this was also consistent with overall effect of soil texture and soil pH on rrZn (Fig. III.2).

We found a more pronounced AMF effect for deficient soil P concentrations (0-9 mg P* kg soil^{-1}) in *fruit* and *root* but not *shoot* (Fig. III.2). The AMF-mediated Zn uptake is influenced by the P status of the associated plant being affected by concentration of phytoavailable P concentration of the soil (Clark and Zeto 1996; Lambert et al. 1979). An

increased phytoavailable soil P concentration does not necessarily cause a reduced mycorrhizal effect as demonstrated for *shoot*. Even though the growth substrate was potentially non-deficient in P, AMF enhanced Zn and P supply compared to control plants (Fig. III.S4).

The impact of the experimental setting

In the *shoot* dataset, no differences were detectable for rrZn of lab vs. field studies. In contrast to our hypothesis, the opposite was true for *fruit* (Fig. III.3); lab studies yielded higher effect size values as were measurable in field trials. Due to low sample size, we were not able to further analyze the impact of this difference on the other independent variables as we did for the variable fertilization in dataset *shoot*. For AMF-mediated Zn tissue concentration, more research is needed to verify the usefulness of lab studies and the generalization of results to field situations (Limpens et al. 2012). Carrying out experiments under controlled environmental conditions is a useful approach to examine complex topics such as the effect of AMF on Zn uptake and translocation from belowground tissue to edible plant portions where multiple and interacting factors are involved. However, there is a risk that the simplicity diminishes the degree to which results can be extrapolated to realistic conditions.

Environmental factors as mediators of AMF effects

In our dataset, plant types did not significantly affect rrZn (Fig. III.3). In the literature, woody plants and perennial herbs have been shown to be more responsive to AMF in relation to plant growth and P nutrition than annual grasses (Boerner 1992); and this was congruent with the trend we found (Fig. III.3). However, a potential plant type effect should be small in magnitude and this could explain why we failed to retrieve any significant effect.

Rooting space, a measure of adequate pot size of different plant species and intraspecific competition, did not significantly affect rrZn, either. Root restriction has multiple

side effects, e.g. water and nutrient deficiency (Kharkina et al. 1999) as well as overlapping root and hyphal depletion zones (Hayman 1983). The lack of relevance for rooting space in our dataset might be explained by alleviation of a restricted rooting space effect by AMF (Facelli et al. 1999), or maybe the test plants were simply not affected by a rooting space of less than 1g root biomass per litre as suggested by Poorter et al. (2012).

We expected that the experimental duration levels intermediate and long yielded higher mycorrhizal effects than short experiments. In experiments with longer duration not only is the symbiosis better established but resources are also increasingly depleted (Daft 1991; Schroeder and Janos 2004). However, we only found such a trend for rrP and rrbiomass (Table III.S3). For our dataset, Zn might not become limiting over time. Additionally, the three duration levels were not biased by unequal number of trials for deficient and non-deficient soil Zn.

AMF inoculum was expected to yield higher rrZn for mixed than for single species inocula because a more diverse assemblage of species increases the probability of presence of beneficial or complementary species being more effective against stress factors than single species inocula (Hart and Forsythe 2012; Hart and Reader 2002; Hoeksema et al. 2010; Maherali and Klironomos 2007; Vogelsang et al. 2006). For *root*, there was only a trend that mixed inocula had a positive effect on rrZn but this marginal effect did not propagate to the shoot.

Conclusions

Our synthesis of 104 studies showed a positive impact of AMF on crop tissue Zn concentration. In addition, we found that edaphic factors influencing Zn mobility and bioavailability were more important than environmental factors. Focusing on *shoot* as target tissue, soils with silty and clayey texture, neutral pH and deficient Zn concentration resulted in highest rrZn. The improvement of *fruit* Zn concentration via AMF was most pronounced in sandy and P deficient soils. Irrespective of the tissue, AMF-induced Zn enhancement could be of great interest for perennial and woody crop species, e.g. fruit trees.

Under these specific conditions AMF could be particularly useful in diminishing Zn deficiency in crops and hence livestock and humans. The effect of AMF is not as strong as the respective effect of mineral Zn fertilizers in high output cereal and vegetable production. However, there is a role for these plant symbionts in local, sustainable and organic agriculture where soil quality is additionally improved by soil-protecting techniques, e.g. reduced tillage and constraint application of pesticides and synthetic fertilizers. In these systems, AMF could also improve Zn tissue concentration of modern cultivars for which a decrease in micronutrients was found over the last 160 years (Fan et al., 2008). Although modern cultivars are bred under high fertilizer input conditions, they have not necessarily lost their ability to profit from AMF with respect to growth and nutrition (Chu et al. 2013; Lehmann et al. 2012). Thus, future research should address the AMF-mediated Zn uptake in crops with diverse breeding history with a major focus on edible plant portions under controlled and field conditions. As demonstrated here, plant tissue (*root*, *shoot* and *fruit*) showed variable responses to edaphic and environmental factors and thus it is important to test effects directly in the tissues of interest instead of extrapolating on the basis of other plant tissues.

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Appendix C. Supplementary material

CHAPTER 5

Summary

Soil health is the basis for food production and determines the quality of the food and the timeframe for which this quality can be maintained by the soil. The integration of soil microorganisms in management concepts for sustainable, organic agriculture is increasingly important for soil health and thus food production. Improper management practices can cause diminishing quality of soil. Avoiding disturbance factors like tillage or high fertilizer input, sustainable agriculture can attain positive growth promoting effects without loss of yield (Davies et al 2012; Pretty and Hine 2001). There are factors that can be optimized and adapted to demands and preconditions of farmers and consumers, e.g. techniques for soil ploughing and fertilization or nutritional value of food. However, important aspects like the interaction of crop plants and soil microorganisms are less transparently observable and improvable; especially the symbiosis of AMF and mycorrhizal crop plants is highly complex and influenced by both biotic and abiotic components.

The aim of the present dissertation was to focus on the three topics identified in the introduction.

1) Effect of breeding history on AMF-mediated growth promotion
2) Effect of breeding history on root architecture and hence soil stability
3) Role of AMF for Zn nutrition of crops.

Thus, the question could be addressed to what extent AMF-mediated services are of use for modern, sustainable agriculture. The main focus was the evaluation of the interaction of AMF and crop plants to verify the impact of breeding conditions on the symbiosis. Possible

negative effects could be inadvertently caused by breeding and selection for high yield. The consequences could be a reduced ability of modern crop plants to interact with AMF and to profit from their services. This severe problem was addressed in chapters 2 and 3. In chapter 2, we used an approach for quantitative data synthesis (meta-analysis) to challenge the assumption that new genotypes (release year after 1950) are less mycorrhizal responsive than old cultivars (release year between 1900 and 1950) and landraces or wild ancestors (Hetrick et al. 1992, 1993). In chapter 3, we tested experimentally whether breeding for high yield caused negative effects in root architecture in German barley breeding lines, and thus on soil stability and quality. In chapter 4, we tested the potential role of AMF for Zn nutrition of crops under various biotic and abiotic conditions.

Chapter 2

Here, we showed that new cultivars can benefit from AMF in regard to plant growth. New cultivars had a higher mycorrhizal responsiveness then landraces and wild ancestors. This positive trend was only detectable for the comparison of the release year groups (new, old, ancestor) but not for the analysis of the release year dates. In the latter analysis only new and old cultivars were included because landraces and uncultivated ancestors have of course no release year date.

The majority of integrated studies did not apply additional P-fertilizer neither at the beginning nor during the time of the experiment. Therefore, it is likely that cultivars bred under high fertilizer input conditions and thus with high nutrient demands during growth and yield production suffered from P-limitation. Considering such conditions, these crop plant genotypes should profit from AMF-mediated nutrient supply and thus show increased growth. In contrast, landraces and wild ancestors are adapted to nutrient limitation, by physiological and anatomical traits (Chapin et al. 1986; Koide et al. 1988). The selected and desired trait for increased yield was introduced to modern germplasms at the price of high nutrient demands.

In this combination of P-limitation and high yielding cultivars, AMF are capable of improving plant growth. However, these findings are only related to shoot data. Information about edible tissue like fruit and seed were not available to permit statistical tests. Thus, more research is needed to evaluate the impact of AMF on edible biomass of crop plants with differing breeding history.

Chapter 3

Plant breeding reduced length of primary roots, total root length density and total seminal root length (Bertholdsson and Kolodinska-Brantestam 2009; Sanguineti et al. 2006; Zhu et al. 2003). According to these findings, we were also able to detect reduction in very fine root length (root diameter < 0.2mm) in one of two barley breeding lines; there was a continuous reduction detectable for cultivars released between 1984 and 1991. For a second breeding line, such a negative trend present for the years 1914 to 1961 was interrupted by the introduction of the cultivar 'Franka'; the available data on this breeding line did not give the information if increased fine root length was a specifically selected trait or if this change in root architecture appeared by chance.

Roots with a diameter smaller then 1mm are important for soil exploration and increase of root surface area and thus nutrient acquisition. A reduction of this root architecture trait would reduce the nutrient uptake efficiency of affected cultivars and additionally would diminish their yield under suboptimal growth conditions (Föhse et al. 1991; Manske et al. 2000). However, such a negative trend can be interrupted and desired root traits can be re-introduced in germplasms as revealed for cultivar 'Franka'.

So far, we were not able to show that the reduced very fine root length could cause ripple-on effects on soil stability. This might be due to either the poor mycorrhizal status of barley or the well aggregated soil used as growth substrate. For a re-evaluation of this topic, a

mycorrhiza responsive crop plant, like onion or lettuce, should be used; although these plant species have only limited available data for pedigrees and release years.

Chapter 4

The AMF-mediated supply of immobile nutrients like P and Zn to their host plants is well researched (e.g. Bürkert and Robson 1994; Marschner and Dell 1994). However, we showed for the first time that AMF are capable of increasing Zn concentration in root, shoot and fruit tissue under various growing conditions; they are most effective in clayey soil with neutral pH and under deficient Zn and P soil concentrations.

Although plants have their own Zn uptake pathways, AMF can still enhance the Zn nutrition status. Zn uptake is diffusion-limited due to its low mobility in the soil solution. Thus, AMF can increase the surface area of roots, expand the explored soil volume and hence the possible available soil Zn pool. There is evidence that AMF have Zn transporters; for *Glomus intraradices*, a Zn transporter was identified (GintZnT1; Gonzalez-Guerrero et al. 2005). Its putative function is the Zn transport through hyphae towards the plant and subsequently the supply into the apoplastic space between fungi and associated plant (Cavagnaro 2008). Whether or not AMF influence the Zn translocation inside the plant is unknown. Further research is needed to verify the impact of AMF on the xylem-loading processes for Zn transportation from root to shoot and shoot to fruit, respectively (Broadley and White 2007; Palmgren et al. 2008). These data would be vital for the assessment of the true potential of AMF-mediated Zn supply.

However, due to the present data, we can suggest that AMF are beneficial for Zn nutrition of root, shoot and fruit or seed tissue, especially in soils with low input management practices as in sustainable, organic agriculture.

Synthesis

AMF have potentially multiple roles in agriculture which they can perform even today when interacting with modern high-yielding crop varieties. They improve nutrient uptake not only for P but for essential micronutrients (important for a healthy diet) as well; here shown for Zn (see chapter 4). Due to their ability to supply associated plants with nutrients, AMF can promote plant growth under nutrient limited conditions, especially in genotypes with high nutrient demand (see chapter 2)

The inadvertently reduced root architecture, caused by plant breeding for high yield, was also detectable in German breeding lines (see chapter 3). However, we could show in one of the concerned breeding lines that such a negative and problematic trend can be interrupted by potentially selective breeding. Furthermore, we were not able to reveal any ripple-on effects of reduced root architecture on soil aggregate stability.

As a consequence, breeding of high yielding crop plants did not necessarily cause negative effects for the interaction of crop plants and AMF; on the contrary, if the interaction is studied under nutrient-limited conditions, the beneficial services of AMF are also detectable for modern cultivars. The symbiosis of plants and fungi and its advantages present since the advent of land plants can be an important component for modern, sustainable agriculture. AMF are an integral component of soil and plant interactions and as such they need to be understood and used actively.

Future perspective

The data obtained from this dissertation should be further deepened and extended. One major goal would be the focus on the negative effect of the breeding history on Zn and Fe concentration. Fan et al. (2008) detected a negative trend for wheat yield Zn and Fe concentration over the last 100 years of wheat breeding. Thus, it would be recommended to tested whether or not this negative trend could be diminished or even eliminated by the

application of AMF. Besides wheat, additional mycorrhizal crop plant species and breeding lines should be incorporated, e.g. onion and flax. Furthermore, in such an experiment the impact of the breeding history on root architecture and soil quality could be re-evaluated.

As an expansion for chapter 4, an additional meta-analysis should be conducted with the aim to test for the general effect of AMF on crop plant micronutrient concentration for Cu, Mn and Fe. These micronutrients are important for plant growth and thus productivity of crops. The impact of any AMF-mediated effect on crop plant nutrition and hence productivity would further highlight the important role of AMF in modern, sustainable agriculture.

CHAPTER 6

Zusammenfassung

Das Einbeziehen von Bodenmikroorganismen in der nachhaltigen Landwirtschaft hat enorme Bedeutung für die Bodengesundheit und Nahrungsproduktion. Die Gesundheit des Bodens bildet die Grundlage der Nahrungsproduktion und bestimmt die Qualität der zu erzeugenden Nahrung und die Zeitspanne, in der diese Qualität vom Boden gewährleistet werden kann. Falsche Handhabung kann die Qualität mindern und die Nutzbarkeit des Bodens nachhaltig reduzieren oder gar gänzlich aufheben, z.B. durch schädliche Bodenmanagementpraktiken. Diesem und ähnlichen schädlichen Faktoren wurde in der nachhaltigen, organischen Landwirtschaft bereits Einhalt geboten und positive Effekte konnten erzielt werden ohne Eintreten von Ertragseinbußen (Davies et al 2012; Pretty and Hine 2001). Neben Faktoren die zeitnah optimiert und an die Ansprüche und gegebenen Bedingungen angepasst werden können, wie z.B. Techniken zur Bodenauflockerungen, Düngung und Fruchtfolge, ist die Interaktion von Nutzpflanzen und Bodenmikroorganismen weniger offensichtlich zu analysieren und zu verbessern. Insbesondere das Zusammenspiel von AMF und mykorrhizierbaren Nutzpflanzen ist hoch komplex und viele biotische und abiotische Wechselbeziehungen wirken direkt und indirekt ein.

Das Ziel dieser Dissertation war es, die drei Themen, die in der Einleitung herausgearbeitet wurden, zu bearbeiten:

1) Einfluss der Zuchtbedingungen auf das durch AMF verbesserte Pflanzenwachstum
2) Einfluss der Zuchtbedingungen auf Wurzelarchitektur und folglich Bodenstabilität
3) Rolle der AMF für Zn Ernährung von Nutzpflanzen.

Dabei habe ich mich mit der Frage beschäftigt, in wie weit durch AMF vermittelte Dienstleistungen in der modernen Landwirtschaft eine Rolle spielen können. Der Schwerpunkt lag auf der Evaluierung der Interaktionsfähigkeit von AMF und Nutzpflanzen, um den Einfluss von Zuchtbedingungen besser einschätzen zu können. Mögliche negative Effekte könnten sich durch den Fokus auf Ertragssteigerung unabsichtlich in modernen Zuchtlinien manifestiert haben und zu einer verminderten Interaktionsfähigkeit von Pflanze und AMF geführt haben. Dieses schwerwiegende Problem adressierten wir in Kapitel 2 und 3. In Kapitel 2 wurde mithilfe einer quantitativen Analyse von Primärdaten (Meta-Analyse) untersucht, ob neue Kultivare (Zulassungsjahr nach 1950) eine geringere Reaktionsfähigkeit gegenüber AMF haben als alte Sorten (Zulassungsjahr zwischen 1900 und 1950) oder Landsorten bzw. unkultivierte Genotypen, die hauptsächlich durch natürliche Selektion entstanden sind (Hetrick et al. 1992, 1993). In Kapitel 3 sollte experimentell getestet werden, ob negative Effekte auf Wurzelarchitektur bedingt durch die auf Ertrag fokussierte Pflanzenzucht auch in deutschen Gerstezuchtlinien nachweisbar sind und sich womöglich auf die Bodenstabilität und damit -qualität auswirken. In Kapitel 4 sollte generell mithilfe einer weiteren Meta-Analyse getestet werden, in wie weit AMF auch zur Verbesserung der Mikronährstoffkonzentration, mit Fokus auf Zink, in verschiedenen Nutzpflanzenarten und unter verschiedenen biotischen und abiotischen Bedingungen beitragen können; und ob sie somit als eine umweltschonende Option für eine verbesserte Zinkversorgung von Nutzpflanzen in Frage kommen.

Kapitel 2

Hier haben wir gezeigt, dass neue Kultivare von AMF in Bezug auf Biomassezuwachs profitieren können. Neue und alte Kultivare haben eine höhere Reaktionsfähigkeit gegenüber AMF als Landsorten bzw. unkultivierte Sorten. Dieser positive Trend konnte nur für den Vergleich der Zulassungsjahr-Gruppen neu und alt mit Landsorten gefunden werden. Wenn

der Einfluss der Zulassungsjahreszahlen direkt getestet wurde, konnte kein Effekt festgestellt werden, da hier nur neue und alte Kultivare in die Analyse einbezogen werden konnten; Landsorten und unkultivierte Sorten haben naturgemäß kein Zulassungsjahr.

Die Mehrheit der verwendeten Studien gab weder zu Beginn noch im Verlauf der Experimente Phosphordünger zum Wachstumssubstrat. Es ist also wahrscheinlich, dass besonders Kultivare, die unter hoher Phosphordüngung gezüchtet wurden, unter Phosphorlimitierung standen. Unter diesen Bedingungen ist eine positive Reaktion auf AMF in Bezug auf Biomassezuwachs wahrscheinlich. Währenddessen sind Landsorten lokal an bestehende Nährstofflimitierungen adaptiert (Chapin et al. 1986; Koide et al. 1988). Diese „angezüchtete" Fähigkeit mehr Ertrag zu erbringen, wurde in neuen Kultivaren zum Preis eines gesteigerten Nährstoffbedarfs erreicht. In dieser Konstellation von Phosphorlimitierung und neuem nährstoffintensiven Kultivaren können AMF eine Verbesserung des Pflanzenwachstums bewirken. Jedoch beziehen sich diese Ergebnisse nur auf Sprossgewebedaten. Informationen zu essbarem Pflanzengewebe wie Früchten oder Samen waren nicht in statistisch ausreichender Menge vorhanden, um den Einfluss von AMF in neuen, alten Kultivaren und Landsorten zu testen. Weitere Versuche sind nötig, um den Effekt der Zuchthistorie auf die Fruchtbiomasse zu evaluieren.

Kapitel 3

Pflanzenzucht hat negative Auswirkungen auf die Wurzelarchitektur (Bertholdsson and Kolodinska-Brantestam 2009; Sanguineti et al. 2006; Zhu et al. 2003). Diesen negativen Effekt konnten wir in einem von zwei deutschen Gerstezuchtlinien nachweisen. Dabei lag unser Fokus auf der Gesamtlänge jener Wurzeln, deren Durchmesser kleiner gleich 0.2mm war. Eine stetige Reduzierung dieser sehr feinen Wurzeln von Kultivaren von 1894 bis 1991 war in einer Zuchtlinie feststellbar. Für die zweite Zuchtlinie wurde dieser negative Trend (von 1914 bis 1961) mit der Züchtung des 1980 zugelassenen Kultivars „Franka"

unterbrochen. Ob es sich um ein gezielt gezüchtetes Merkmal handelt, konnte aus den vorhandenen Daten nicht entnommen werden.

Wurzeln mit einem Durchmesser unter 1mm sind bedeutsam für die Bodenerschließung und die Oberflächenvergrößerung und damit wichtig für die Aufnahme von Nährstoffen. Die Reduzierung dieses Wurzelarchitekturmerkmales würde die Nährstoffaufnahmeeffizienz betroffener Kultivare mindern und ihren Ertrag unter suboptimalen Bedingungen schmälern (Föhse et al. 1991; Manske et al. 2000). Aber es ist nachweisbar möglich solch einen negativen Trend zu unterbrechen und gewünschte Wurzelmerkmale in eine Zuchtlinie wieder einzuführen, wie an Kultivar „Franka“ sichtbar. Nichtsdestotrotz scheint sich der negative Effekt von Zucht auf feine und sehr feine Wurzeln nicht zwangsweise auf die Bodenstabilität zu übertragen, zumindest nicht für unser Testsystem. Dies mag zum einen durch die geringe Mykorrhizierbarkeit von Gerste und zum anderen durch die hohe Bodenstabilität des Testsubstrats begründet sein. Für eine erneute Analyse dieses Themas sollte eine Mykorrhiza-abhängige Nutzpflanze verwendet werden, z.B. Zwiebel oder Salat. Obwohl für diese Pflanzenarten die Verfügbarkeit von Stammbäumen und Zulassungsjahreszahlen sehr begrenzt ist.

Kapitel 4

AMF sind nachweislich besonders effektive in der Bereitstellung von immobilen Nährstoffen wie Phosphor und Zink (z.B. Bürkert and Robson 1994; Marschner and Dell 1994). Hier konnten wir zeigen, dass AMF die Zinkkonzentration unter verschiedensten Wachstumsbedingungen erhöhen können, sowohl in Wurzel-, Spross- als auch Fruchtgewebe. Besonders effektive ist die Zinkversorgung durch AMF in lehmigen Böden mit neutralem pH-Wert und einer mangelhaften Zink- und Phosphorkonzentration.

Auch wenn Pflanzen über eigene Aufnahme- und Transportsysteme für Nährstoffe wie Zink besitzen, können AMF unterstützend wirken. Da Zink wegen seiner geringen Mobilität

in der Bodenlösung Diffusions-limitiert ist, können AMF mit ihren Hyphen die Aufnahmeoberfläche und den Einzugsbereich erhöhen und somit den verfügbaren Boden-Zinkpool erweitern. AMF besitzen nachweislich Zinktransporter; für *Glomus intraradices* wurde ein solcher Transporter identifiziert (GintZnT1; Gonzalez-Guerrero et al. 2005). Dessen mutmaßliche Funktion ist der Zinktransport durch die AMF Hyphen und die Übertragung in den apoplastischen Raum zwischen Pilz und assoziierter Pflanze (Cavagnaro 2008).

Ob AMF einen direkten Einfluss auf die Zinktranslokation innerhalb der Pflanze haben ist nicht bekannt. Dabei wäre es von entscheidendem Interesse, ob AMF den Xylem-Beladungsprozess für den Zinktransport von Wurzel zu Spross und/ oder Spross zu Frucht direkt oder indirekt manipulieren können (Broadley and White 2007; Palmgren et al. 2008). Nur mit diesem Wissen ließe sich ihr wahres Potential für die Zinkversorgung von Nutzpflanzen beurteilen.

Aber aufgrund der momentanen verfügbaren Datenlage können wir schlussfolgern, dass AMF eine wertvolle Ressource zur Anreicherung von Zn in Wurzel-, Spross-, Frucht- und Samengewebe von Nutzpflanzen darstellen, besonders wenn wenig gedüngt wird, wie es in nachhaltiger, organsicher Landwirtschaft praktiziert wird.

Synthese

AMF haben viele potentielle Rollen in der Landwirtschaft, die sie auch heute ausüben können, selbst wenn sie mit modernen Hochleistungssorten konfrontiert werden. Sie verbessern die Nährstoffaufnahme nicht nur für Phosphor sondern auch für essentielle Mikronährstoffe, die bedeutsam sind für eine gesunde Ernährung; hier nachgewiesen für Zn (siehe Kapitel 4). Aufgrund ihrer Rolle als Nährstofflieferant können sie unter entsprechenden nährstofflimitierenden Bedingungen die Biomasse von assoziierten Pflanzen erhöhen, wobei nährstoffintensive Kultivare potentiell mehr von AMF profitieren können (siehe Kapitel 2).

Die, durch die gezielte Zucht für mehr Ertrag und daraus resultierenden, wenn auch unbeabsichtigten, Konsequenzen für die Wurzelarchitektur lassen sich auch in deutschen Gerstelinien nachweisen (siehe Kapitel 3). Jedoch konnte an einer weiteren Zuchtlinie gezeigt werden, dass sich ein solcher problematischer Trend durch gezielte Zucht aufheben lässt. Wir konnten auch nicht nachweisen, dass die negativen Effekte der Pflanzenzucht auf Wurzelarchitektur Konsequenzen für die Bodenqualität haben.

Demzufolge hat die auf Ertrag fokussierte Nutzpflanzenzucht nicht zwangsweise negative Effekte auf die Interaktion von AMF und Nutzpflanzen, besonders nicht wenn sie unter nährstofflimitierenden Bedingungen betrachtet wird. Die Vorteile dieser Symbiose von Pflanze und Pilz, die seit Beginn der Landpflanzen selbst besteht, kann auch heute noch unter den richtigen Rahmenbedingungen in der modernen, nachhaltigen Landwirtschaft genutzt werden. AMF sind ein integraler Bestandteil von Boden und Pflanzen und sollten auch als solcher verstanden und aktiv genutzt werden.

Zukunftsperspektiven

Die gewonnen Informationen dieser Dissertation sollten nun erweitert werden. Dabei sollte ein Hauptschwerpunkt der negative Einfluss der Zuchthistorie auf die Mirkonährstoffkonzentration für Zink und Eisen (Fan et al. 2008) sein. In einem Experiment müsste untersucht werden, ob der festgestellte negative Trend für die Nährstoffkonzentration in Weizensamen durch den gezielten Einsatz von AMF abgemildert oder gar aufgehoben werden kann. Dabei sollte neben Weizen weitere nachweislich mykorrhizierte Nutzpflanzen getestet werden. Des Weiteren könnte in solch einem Experiment der Effekt der Zuchthistorie auf die Wurzelarchitektur und damit die Bodenqualität re-evaluiert werden. Da die erste Studie dieser Art im Rahmen meiner Dissertation mit einer eher ungeeigneten Versuchspflanze durchgeführt wurde.

Ebenfalls sollte als Erweiterung für Kapitel 4 eine zusätzliche Meta-Analyse durchgeführt werden, die zum Ziel hat, den Effekt von AMF auf die Mikronährstoffkonzentration von Nutzpflanzen für Kupfer, Mangan und Eisen zu verifizieren. Diese Mikronährstoffe sind allgemein bedeutsam für das Pflanzenwachstum und damit für die Produktivität von Nutzpflanzen im Speziellen. Deshalb ist eine Beurteilung eines potentiellen AMF induzierten Effekts auf die Nährstoffversorgung von Nutzpflanzen entscheidend für die zukünftige Rolle der AMF in der modernen, nachhaltigen Landwirtschaft.

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Contribution to the publications

I. Lehmann A, Barto EK, Powell JR, Rillig MC (2012) Mycorrhizal responsiveness trends in annual crop plants and their wild relatives - a meta-analysis on studies from 1981 to 2010. Plant and Soil 355: 231-250

Own contribution

AL performed all the analysis and wrote the manuscript. EKB and JRP mentored the analysis. JRP wrote the applied R script. All authors reviewed the manuscript.

II. Lehmann A, Rillig MC. 201X. Are there temporal trends in root architecture and soil aggregation for Hordeum vulgare breeding lines? Applied Soil Ecology 65: 31– 34

Own contribution

AL designed the experiment, performed all the analyses and wrote the manuscript. All authors reviewed the manuscript.

III. Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2013) Arbuscular mycorrhizal influence on Zinc nutrition in crop plants - a meta-analysis. *In preparation for submission*

Own contribution

AL performed all the analyses and wrote the manuscript. SDV and EFL mentored the analyses. All authors reviewed the manuscript.

APPENDIX A

Table I.S1 References, plant genera and number of different plant genotypes used as experimental organisms as well as availability of biomass parameters (root, shoot or total dry weight. "+" indicates that all three parameters were reported) and host P efficiency data used in the meta-analysis

Reference	Plant	Biomass	P efficiency	No. of genotypes
Al-Karaki and Clark (1998)	Wheat	+	+	2
Al-Karaki and Al-Raddad (1997)	Wheat	+	+	2
Al-Karaki (1998)	Wheat	+	+	2
Azcón and Ocampo (1981)	Wheat	+	+	10
Baon et al. (1993)	Barley	+	+	4
Behl et al. (2003)	Wheat	root	-	5
Boyetchko and Tewari (1994)	Barley	+	-	7
Bryla and Koide (1998)	Tomato	shoot	-	1
Bryla and Koide (1990)	Tomato	shoot	-	8
Bryla and Koide (1990)	Tomato	shoot	-	8
Chen et al. (2004)	Barley	+	+	2
Gao et al. (2007)	Rice	+	-	5
Grandison and Cooper (1986)	Alfalfa	shoot	-	1
Hetrick et al. (1995)	Wheat	total	-	54
Hetrick et al. (1996)	Wheat	total	-	10
Hetrick et al. (1992a)	Wheat	total	-	47
Hetrick et al. (1992b)	Wheat and Barley	total	-	37
Ibijbijen et al. (1996)	Bean	total	-	4
Jackson et al. (2002)	Lettuce	+	+	2
Jakobson et al. (2005)	Barley	total	+	1
Kaeppler et al. (2000)	Maize	shoot	-	18
Kahlil et al. (1994)	Maize and Soybean	+	+	6
Kapulnik and Kushnir (1991)	Wheat	total	-	27
Koide et al. (1988)	Oats	shoot	+	2
Liu et al. (2000)	Maize	+	-	2
Mendoza and Borie (1998)	Barley	+	+	2
Mickelson and Kaeppler (2005)	Maize	shoot	-	15
Poulton et al. (2001)	Tomato	leave	-	2
Rajapakse et al. (1989)	Pea	shoot	-	2
Raju et al. (1990)	Sorghum	total	+	2
Rouphael et al. (2010)	Cucumber	+	+	1
Sensoy et al. (2007)	Pepper	+	+	2
Simpson and Daft (1993)	Groundnut	+	-	1
Vierheilig and Ocampo (1991a)	Wheat	+	+	6
Vierheilig and Ocampo (1991b)	Wheat	+	-	6
Xavier and Germida (1998)	Wheat	shoot	+	3
Yücel et al. (2009)	Wheat	+	-	36
Zhu et al. (2003)	Barley	+	+	2
Zhu et al. (2001)	Wheat	+	+	6

Online Resource 1

Evaluation of trustworthiness of mycorrhizal responsiveness indices.

We tested two different indices to calculate mycorrhizal responsiveness:

1) The response ratio: lnMNC = ln(M/NC); a simple ratio of mycorrhizal and non-mycorrhizal plant biomass. Ratios were widely used throughout the literature.
2) Absolute mycorrhizal responsiveness: R = M-NC. This is a modified version of the model presented by Janos (2007) for a specific P level.

For our (non-parametric regression) analysis, we needed an index that was linearly related to non-mycorrhizal plant biomass (NC), to get a trustworthy rendition of the mycorrhiza effect.
A non-linear relationship of an index and NC would be characterized by extreme values of NC (outliers) biasing the slope and thus the interpretation of the mycorrhiza effect in the given population.

To test the indices for their trustworthiness, we used the regression method proposed by Sawers et al. (2010) and Galvan et al. (2011).

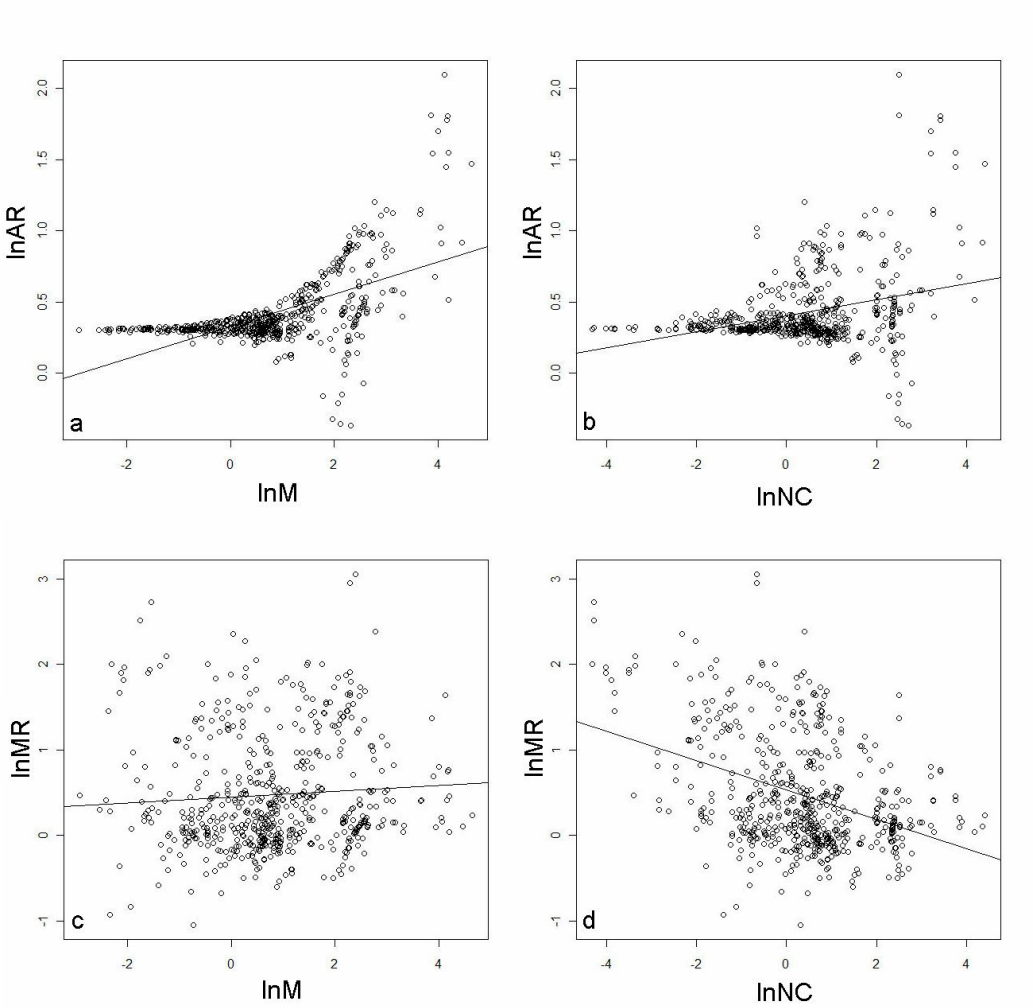

Fig. I.S2. Regression of indices (for measuring mycorrhizal effects) against non-mycorrhizal plant biomass (NC) and mycorrhizal plant biomass (M) for both indices mycorrhizal responsiveness (MR) and absolute responsiveness (AR). (a) Natural logarithm of the response ratio ln (M/NC) against lnNC. (b) Natural logarithm of the absolute responsiveness (lnR) against lnNC. (c) Natural logarithm of the response ratio ln (M/NC) against lnM. (d) Natural logarithm of the absolute responsiveness (lnR) against lnM. The graphical parameters are given in Table Online Resource 2.

Table I.S2 Measurements for evaluation of trustworthiness of mycorrhizal effect indices. Correlation calculated with Kendall's Tau, P< 0.05 (*), P < 0.01 (**) and P < 0.001 (***).

x	Correlation(x, lnNC)	Correlation(x, lnM)	Common variation	Specific variation
lnAR	0.09**	0.38***	0.09	0.91
lnMR	-0.23***	0.07*	0.16	0.84

The two indices lnR and lnMNC were less strongly correlated to NC due to high variability in the dataset. For lnR, the correlation was the lowest and followed a logistic model, thus this index would over-estimate the mycorrhiza effect, especially in plants showing a high dependence upon mycorrhiza.

Dependence is defined as "the inability of a plant to grow without mycorrhizas below a particular level of soil phosphorus" (Janos 2007).

Both indices suggested a high variability in specific variation in the population of this dataset, i.e. variation in plant growth response to AMF of mycorrhizal or non-mycorrhizal plants alone.

The response ratio lnMNC was more trustworthy for our dataset than lnR. In a study on AMF responsiveness in onion (Galvan et al. 2011), absolute responsiveness reflected the mycorrhizal effect best and showed a linear relationship with NC. Onions are highly mycorrhizal responsive plants. For less responsive plants this index might be inappropriate. Therefore, it is not surprising that absolute R is strongly linked to mycorrhizal biomass (Fig. OR2) and there non-linearity was most clearly evident.

The response ratio lnMNC is more obviously influenced by NC and thus showed for this variable the highest correlation and the best linear fit. So, we decided to use the response ratio lnMNC as our effect size.

Table I.S2 Effect of different moderator variables on the effect sizes mycorrhizal P acquisition efficiency (mPAE), mycorrhizal P utilization efficiency (mPUE) and percent root length colonized by AMF (%AM). The significance of relationship is represented by 95% confidence interval (CI) calculated with the "error" bootstrap (Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Subset	Moderator	Level	Mean	Trials	CI
C.d.	Pre-germination			354	[-7.41; 2.46]
C.d.	Pre-germination	No	44.25	131	
C.d.	Pre-germination	Yes	35.95	223	
Gl intra	YORgroup			42	[-9.80; 11.37]
Gl intra	YORgroup	Ancestor	32.25	8	
Gl intra	YORgroup	Old	NA	0	
Gl intra	YORgroup	New	23.70	34	
Gl mosseae	YORgroup			47	[-10.21; 2.30]
Gl mosseae	YORgroup	Ancestor	52.51	10	
Gl mosseae	YORgroup	Old	44.70	4	
Gl mosseae	YORgroup	New	53.03	33	
C.d.	density			398	[-12.20; -9.13]***

NA indicates that no data was available for calculation for level mean via "metagen".

C.d. is the abbreviation for "complete dataset", i.e. calculation of moderator effect on %AM was performed on the complete dataset and not on a subset.

Table I.S3 Pearson's Chi-squared analysis for independency test of moderators. Chi-squared values are presented above the diagonal and P-values below. A *P*-value < 0.05 indicates a non-independent relationship between tested moderators.

	Plant	Density	Duration	Pre-germination	AMFspec	Treatment P	Treatment P conc	Soil pH	YORgroup	YOR	Year
Plant		4890.93	5073.84	375.92	910.71	334.0	2583.02	7477.86	314.45	3388.97	9565.28
Density	< 0.0001		5185.55	726.63	916.53	222.49	1692.76	5425	250.38	2961.74	9174.61
Duration	< 0.0001	< 0.0001		399.32	564.63	303.22	1850.14	7325.3	308.58	2282.83	6970.34
Pre-germination	< 0.0001	< 0.0001	< 0.0001		139.29	11.03	80.82	573.12	32.82	249.21	787.36
AMFspec	< 0.0001	< 0.0001	< 0.0001	< 0.0001		56.42	149.76	896.39	53.47	523.75	1334.79
Treatment P	< 0.0001	< 0.0001	< 0.0001	0.0009	< 0.0001		1163	369.42	60.21	174.02	563.85
Treatment Pconc	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0049	< 0.0001		1995.62	76.72	1325.19	3230.9
Soil pH	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		336.73	3909.91	8786.16
YORgroup	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0005	< 0.0001	< 0.0001	< 0.0001		1116	368.48
YOR	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		4916.31
Year	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

Table I.S4 Relationship of mycorrhizal responsiveness (lnMNC) and the moderator variable plant type for the subsets "Preger YES" and "Preger NO". The significance of relationship is represented by 95% confidence interval (CI) calculated with the "error" bootstrap (Noortgate and Onghena 2005). Significance of moderator effect was calculated with a two-tailed test and is presented in the table with asterisks (P= 0.05 (*), P = 0.01 (**) and P = 0.001 (***)).

Subset	Moderator level	Mean	Trials	CI
Preger YES				**[0.2594; 0.4464]*** **
	cereals	**0.1684**	**263**	
	legumes	**1.0212**	**42**	
	vegetables	**0.426**	**28**	
Preger NO				**[-0.5389; -02721]*** **
	cereals	**0.8148**	**151**	
	legumes	**0.236**	**22**	
	vegetables	**0.3089**	**8**	

Online Resource 2
"Error" bootstrap code for R.

```
## function for implementing bootstrap methods in meta-analysis ##
## based on Van den Noortgate and Onghena 2005 Behavior Research Methods 37, 11-22 ##
## coded by Jeff Powell (jeffpowell2@gmail.com) ##

## data from Van den Noortgate and Onghena 2005
study<-factor(1:10)
grade<-c(6,5,3,3,2,4,8,1,3,5)
n1<-n2<-c(90,40,36,20,22,10,10,10,39,50)
N<-n1+n2
d<-c(-0.583,0.535,0.779,1.052,0.563,0.308,0.081,0.598,-0.178,-0.234)
err<-(N/(n1*n2))+((d^2)/(2*N))

#mima(yi=d,vi=err,mods=grade)

library(metafor)

z<-rma.uni(yi=d,vi=err) #init w/o mods
zz<-rma.uni(yi=d,vi=err,mods=grade) #init w/ mods


# error here is estimated as described in Van den Noortgate and Onghena 2005, which may be different from how you want to estimate error

boot.rma<-function(init.mod,type=c('effect size','error'),n=list(),boot.reps=10,control=list(maxit=100)){
	if(type!='effect size'&type!='error') stop('indicate correct type of analysis')
	boot.out<-
matrix(NA,nrow=boot.reps,ncol=ncol(init.mod[['X']]),dimnames=list(NULL,colnames(init.mod[['X']])))
	n1<-n[[1]]
	n2<-n[[2]]
	N<-n1+n2
	boot.samp<-function(){
		y<-init.mod
		if(type=='effect size'){ #effect size bootstrap (parametric)
			u.boot<-rnorm(length(resid(y)),0,sqrt(y[['tau2']]))  # step 1 (u.boot = u*)
			del.boot<-fitted(y)+u.boot  # step 2 (del.boot = delta*)
			err.boot<-(N/(n1*n2))+((del.boot^2)/(2*N))  # step 3 (err.boot = vi*) --> is this how
error is estimated?
			e.boot<-rnorm(length(del.boot),0,sqrt(err.boot))  # step 3 (e.boot = e*)
			d.boot<-del.boot+e.boot  # step 4 (d.boot = d*)
			}
		if(type=='error'){ #error bootstrap (nonparametric)
			u<-(y[['tau2']]/(y[['tau2']]+y$vi))*(y$yi-fitted(y))  # step 1 using equation 8 (u = level
2 residuals from y)
			#r<-(y$yi-u)/sqrt(y$vi)  # step 3 using equation 8 (r = level 1 residuals from y) <-- this
is wrong, don't use this (see message at end)
			r<-(y$yi-fitted(y)-u)/sqrt(y$vi)  # step 3 using equation 3 (r = level 1 residuals from y)
			u.infl<-function(p) (y[['tau2']]-var(p*u))^2  # function for reflation of residuals (from
paragraph containing equation 8)
			u<-u*optimize(u.infl,c(0,10))$minimum  # reflation of residuals
			u.boot<-sample(u,replace=T)  # step 1 (u.boot = u*)
			del.boot<-fitted(y)+u.boot  # step 2 using equation 5 (del.boot = delta*)
			r.infl<-function(p) (1-var(p*r))^2  # function for reflation of residuals (from paragraph
containing equation 8)
			r<-r*optimize(r.infl,c(0,10))$minimum  # reflation of residuals
			r.boot<-sample(r,replace=T)  # step 3 (r.boot = r*)
			err.boot<-(N/(n1*n2))+((del.boot^2)/(2*N))  # step 3 (err.boot = vi*) --> is this how
error is estimated?
			e.boot<-r.boot*sqrt(err.boot)  # step 3 from equations 2+3 (e.boot = e*)
```

```
                d.boot<-del.boot+e.boot  # step 4 (d.boot = d*)
                }
        res<-list(d.boot,err.boot)
        names(res)<-c('d.boot','err.boot')
        res
        }
    for(i in 1:boot.reps){
        go<-T
        yy<-NULL
        while(go){  # for dealing with convergence errors during estimation on bootstrap replicates, resamples bootstrap replicate on error
                bootsamp<-boot.samp()
                if(ncol(init.mod[['X']])==1) try(yy<-rma.uni(yi=bootsamp[['d.boot']],vi=bootsamp[['err.boot']],control=control),silent=T) else try(yy<-rma.uni(yi=bootsamp[['d.boot']],vi=bootsamp[['err.boot']],mods=init.mod[['X']][,2:ncol(init.mod[['X']])],control=control),silent=T)
                if(!is.null(yy)) ifelse(any(attr(yy,'class')=='try-error'),go<-T,go<-F)
                }
        boot.out[i,]<-yy[['b']][,1]
        }
    boot.out
    }
```

APPENDIX B

Table II.S1. Dry aggregates size distribution (DASD, in %) of two barley breeding lines. Data are means of five replicates ± standard error.

Line	Cultivar [a]		DASD									
			>2mm [b]		2-1mm		1mm-212µm		212-53µm		<53µm	
A	Groninger	(1894)	14.78 (2.13)		24.56 (2.09)		53.43 (2.84)		7.04 (1.43)		0.19 (0.05)	
	Herfordia	(1950)	16.29 (1.04)		24.99 (0.16)		52.44 (1.17)		6.09 (0.63)		0.18 (0.04)	
	Birgit	(1976)	15.21 (2.23)		23.94 (1.90)		52.96 (3.08)		7.68 (1.01)		0.21 (0.02)	
	Monika	(1991)	14.28 (1.56)		22.66 (1.04)		55.17 (1.67)		7.60 (0.87)		0.30 (0.07)	
B	Kalk	(1914)	12.70 (0.98)		22.17 (1.59)		55.17 (0.90)		9.63 (1.72)		0.33 (0.05)	
	Dea	(1953)	12.83 (0.60)		21.38 (0.26)		57.20 (0.44)		8.27 (0.43)		0.32 (0.04)	
	Senta	(1963)	13.37 (1.06)		22.95 (0.77)		56.24 (1.71)		7.20 (0.31)		0.25 (0.03)	
	Franka	(1980)	16.60 (1.77)		23.94 (1.17)		52.42 (2.02)		6.81 (0.96)		0.23 (0.03)	
	Carola	(1998)	41.06 (0.71)		22.38 (0.90)		54.68 (0.71)		8.63 (0.87)		0.25 (0.02)	
	Regression [c]		***R²***	***P***	***R²***	***P***	***R²***	***P***	***R²***	***P***	***R²***	***P***
A			0.01	0.92	0.04	0.44	0.007	0.74	0.02	0.62	0.09	0.22
B			0.11	0.11	0.02	0.47	0.04	0.35	0.07	0.22	0.16	0.05

[a] Cultivar name, values in brackets are release year dates.

[b] Analysed soil size class fraction.

[c] R^2 and *P*-value are derived from simple linear regression.

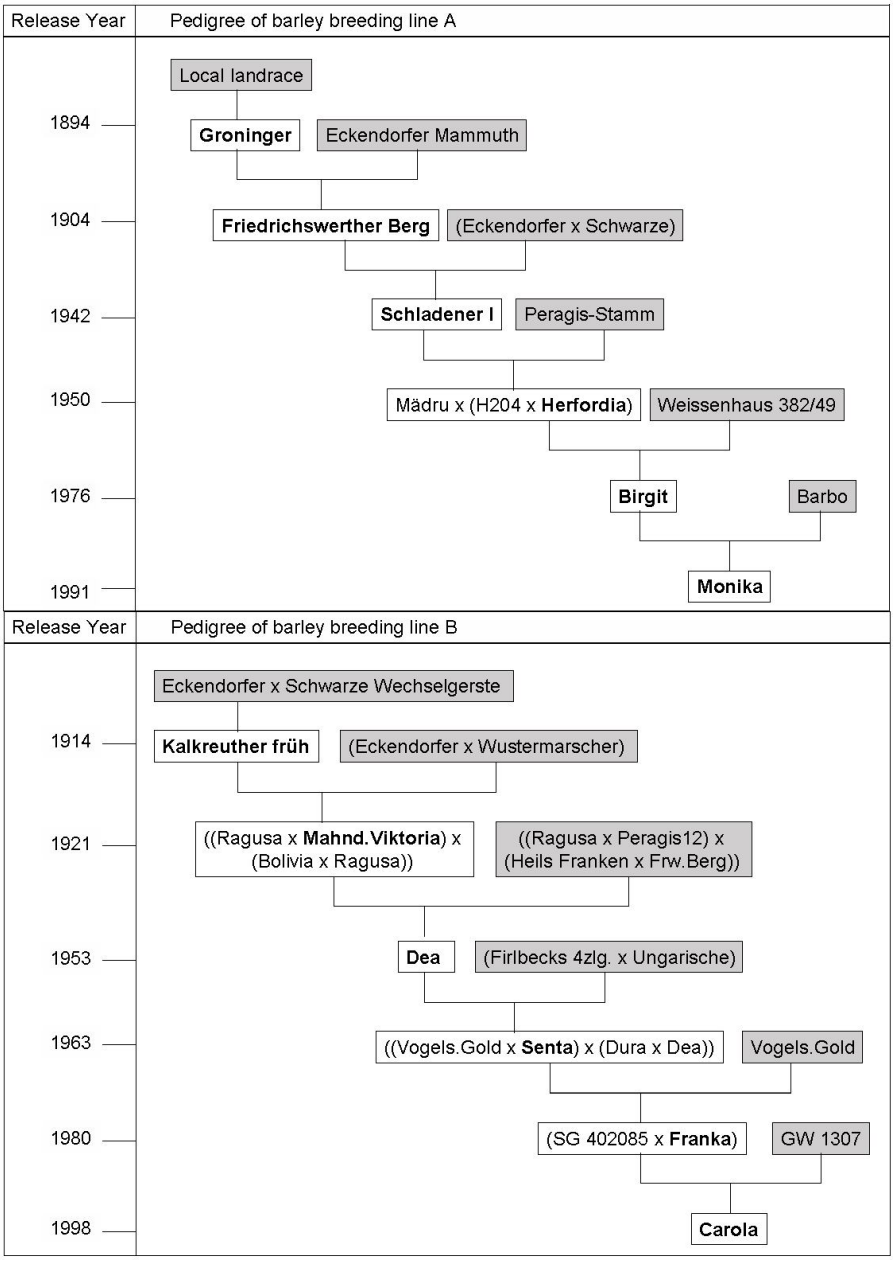

Fig. II.S1. Schemes of the two German barley breeding line pedigrees used in this study. Cultivars written in bold are those cultivars used in the experiment. The release year dates are related to the cultivars written in bold. The cultivars in grey boxes represent the parental genotypes not being used in this study.

Information on parental lineages is available in the "barley lineage catalogue" of the Bayrische Landesanstalt für Landwirtschaft. [http://www.lfl.bayern.de/ipz/gerste/09740/ ; 10.05.2012; 11:07]

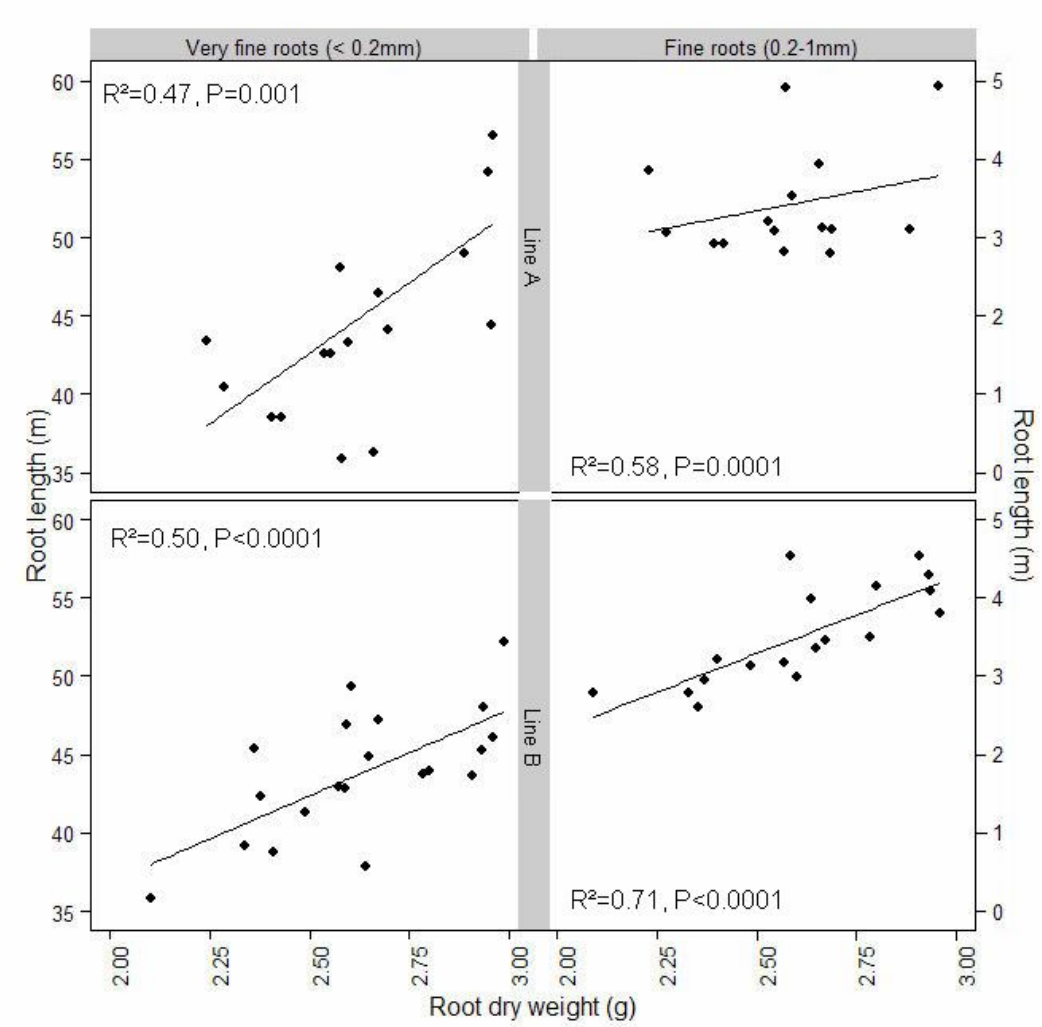

Fig. II.S2. Interaction of root length and root dry weight of two barley breeding lines with focus on very fine and fine roots (diameter <0.2 and 0.2-1mm, respectively). R^2 and *P*-value derived from simple linear regression.

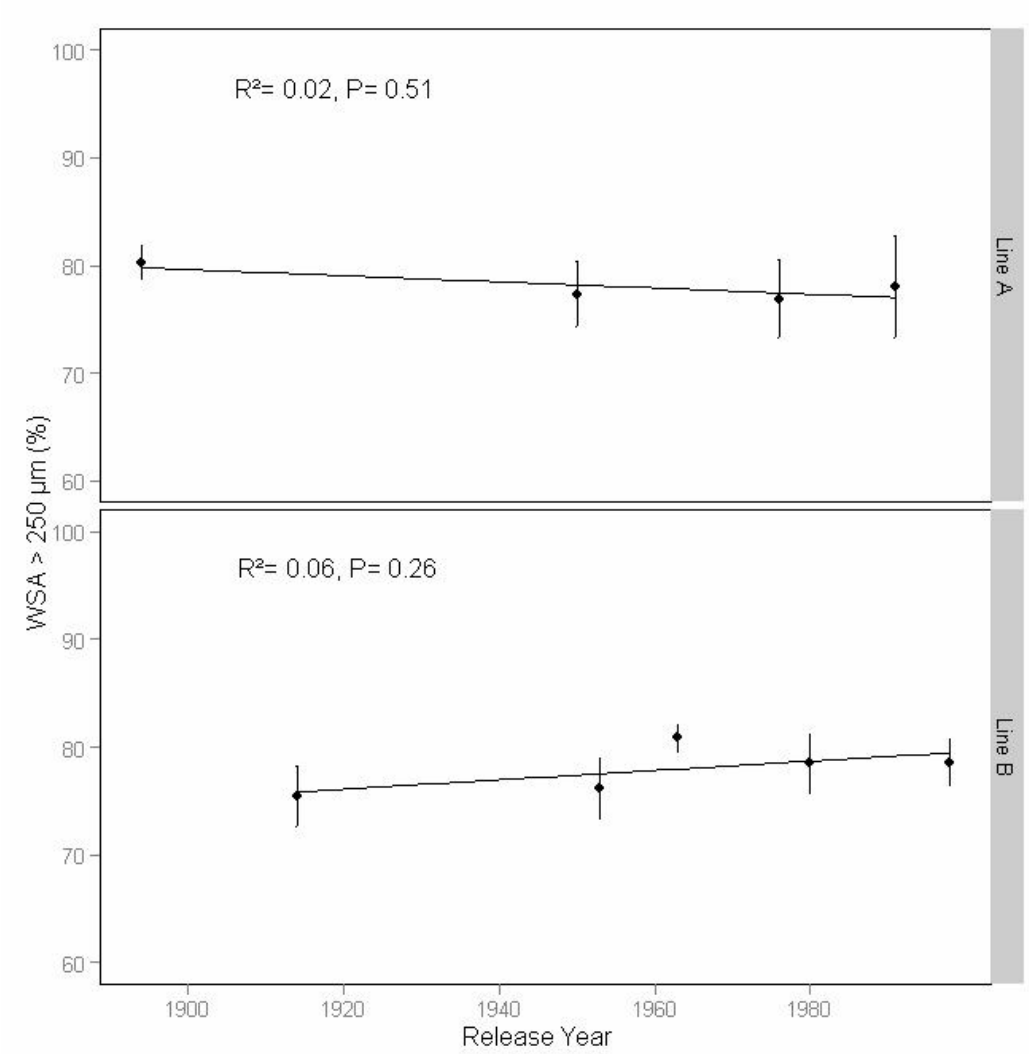

Fig. II.S3. Percentage of water-stable aggregates for soil samples of two barley breeding lines. Bars indicate standard error. R^2 and *P*-value derived from simple linear regression.

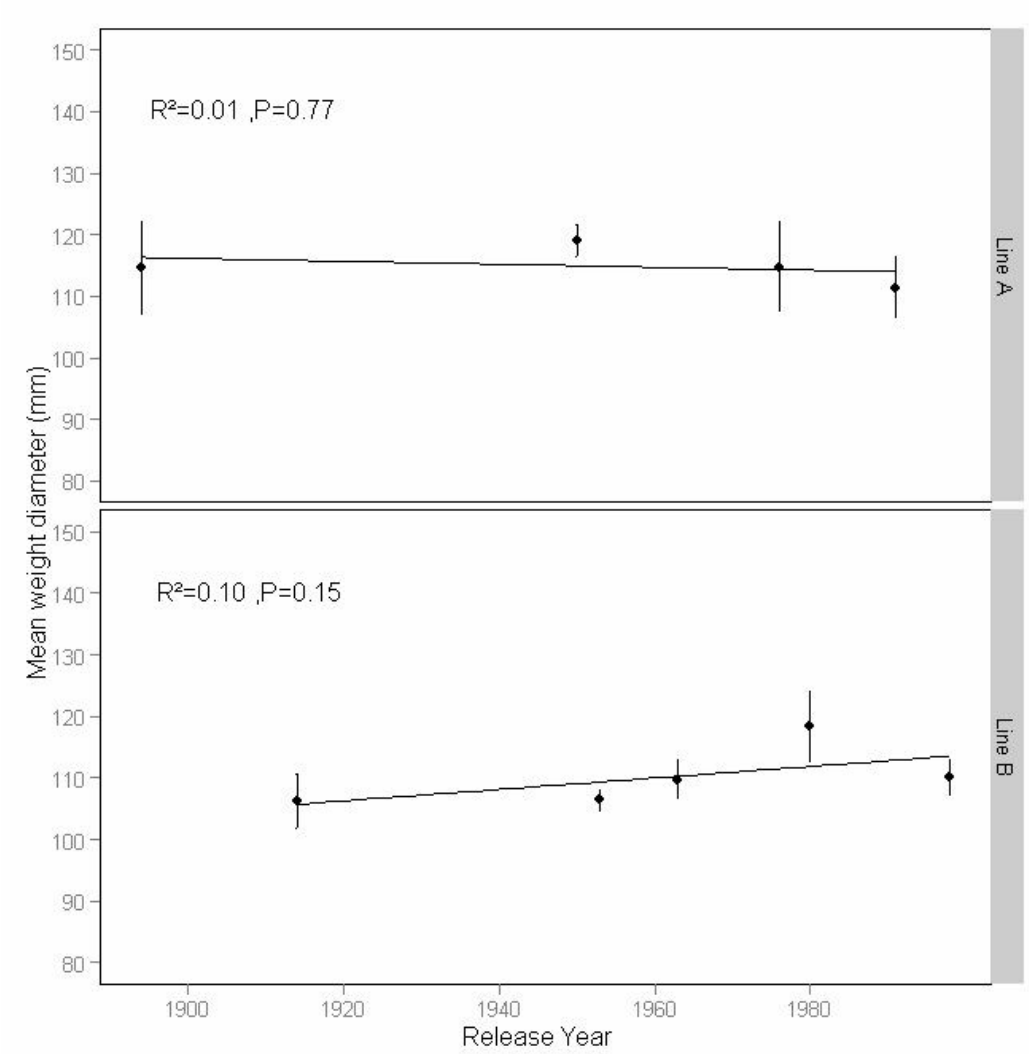

Fig. II.S4. Mean weight diameter (sum of all aggregate size fractions) for two barley breeding lines. Bars indicate standard error. R^2 and *P*-value derived from simple linear regression.

APPENDIX C

I. Publication Bias

We tested our datasets for publication bias by plotting the effect size rrZn against the sample size (replicates) and variance (within-study variance; Egger et al., 1997).

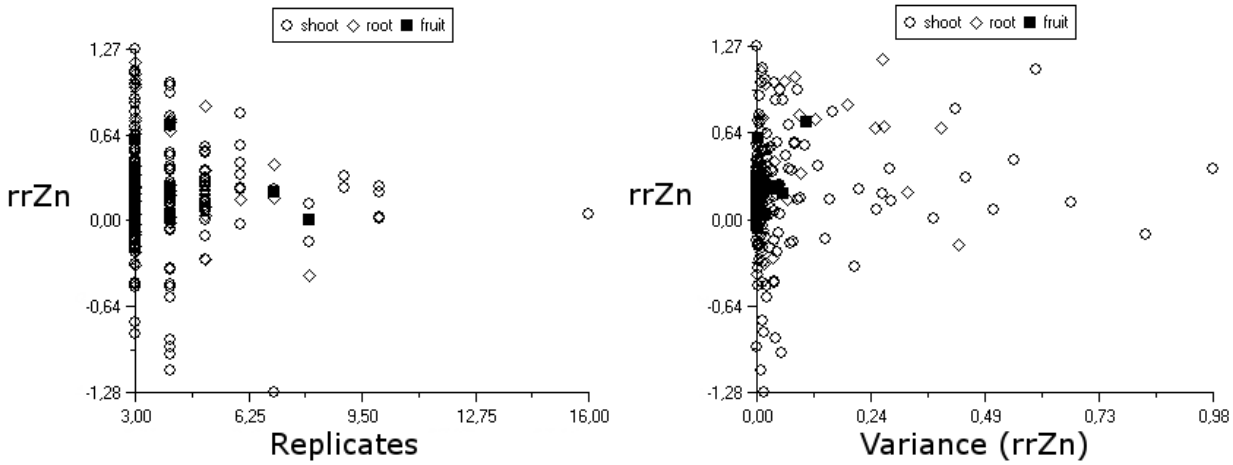

Fig. III.S1. Scatterplots of effect size against sample size (replicates) and sample variance for rrZn, respectively, for *fruit*, *shoot* and *root* dataset.

There were no patterns suggesting the existence of a publication bias (Fig. III.S1), as would be evident by funnel asymmetry (Nagakawa and Santos, 2012)

II. Random-effects meta-analysis

i. Effect of additional nutrient solution application (not the fertilizer treatment)

The independent variable soil Zn and soil P had two levels: deficient and non-deficient. For level deficient of both variables, studies were included in the dataset that applied nutrient solutions once at the beginning of the experiment or weekly throughout the experiment (soil Zn: Kothari and Singh (1996), one application 2 mg $ZnSO_4$* kg $soil^{-1}$; Cavagnaro et al. (2008) used 30 mL of minus-P Long Ashton solution per week; soil P: Ortas et al. (2002) and Ortas and Akpinar (2011) used quarter-strength Hewitt's nutrient solution throughout the experiment). We tested whether the exclusion of these studies altered the results compared to the whole dataset.

Table III.S1 Comparison of results for effect of soil Zn and soil P on rrZn with all studies included or exclusion of studies with additional fertilizer application. Effect size mean, lower (lb) and upper (ub) confidence interval border, number of trials and *P*-value are presented.

Dataset: *shoot*

		All studies included					Studies with additional nutrient solution application excluded				
Moderator	**Level**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value** [a]	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value** [a]
SoilZn	deficient	0.3493	0.2435	0.4576	36		0.3098	0.2027	0.4243	32	
	non-deficient	0.0839	-0.0203	0.1569	40	0.0004	0.0847	-0.0238	0.1538	40	0.0022
Soil P	deficient	0.2033	0.0963	0.3137	58		0.1792	0.0686	0.2832	56	
	non-deficient	0.1835	0.0897	0.2655	53	0.7976	0.1762	0.0796	0.2611	53	0.9706

Dataset: *root*

		All studies included					Studies with additional nutrient solution application excluded				
Moderator	**Level**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value** [a]	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value** [a]
Soil Zn	deficient	0.4445	0.2148	0.666	19		0.429	0.1855	0.6733	17	
	non-deficient	0.2759	0.089	0.5524	7	0.4932	0.2763	0.0789	0.5448	7	0.4500

[a] The *P*-value referred to the between-level differences, i.e. if the *P*-value ≤ 0.05 than confidence intervals of independent variable levels were not overlapping each other and thus the independent variable significantly influenced the effect size.

Although the four concerning studies potentially had no longer growth substrate with deficient Zn or P soil concentrations, we could not detect any significant changes in results when excluding these studies (Table III.S1). Therefore, we decided to not exclude the four studies.

ii. Effect of P fertilization on impact of independent variables on rrZn

There was a trend for P fertilizer application to reduce rrZn in the *shoot* dataset (Fig. III.3). Therefore, we repeated the analysis in two *shoot* subsets, namely *fertilization- no* and *fertilization-yes*.

In general, we found that (i) the effect size values for *fertilization-yes* were more negative and overlapped with zero, (ii) the levels of the independent variables showed similar patterns in both subsets being (iii) almost always not significant (Fig. III.S2 and III.S3). There were two exceptions: plant type and soil texture. For plant type, perennial plants profited more from P fertilizer application in terms of rrZn as other plant types and not fertilized perennial plants (Fig. III.S2). For soil texture, the diminishing effect of sandy soil on rrZn was more pronounced in fertilized soil. However, the sample size for both independent variables was rather small and thus these results needed to be interpreted with caution.

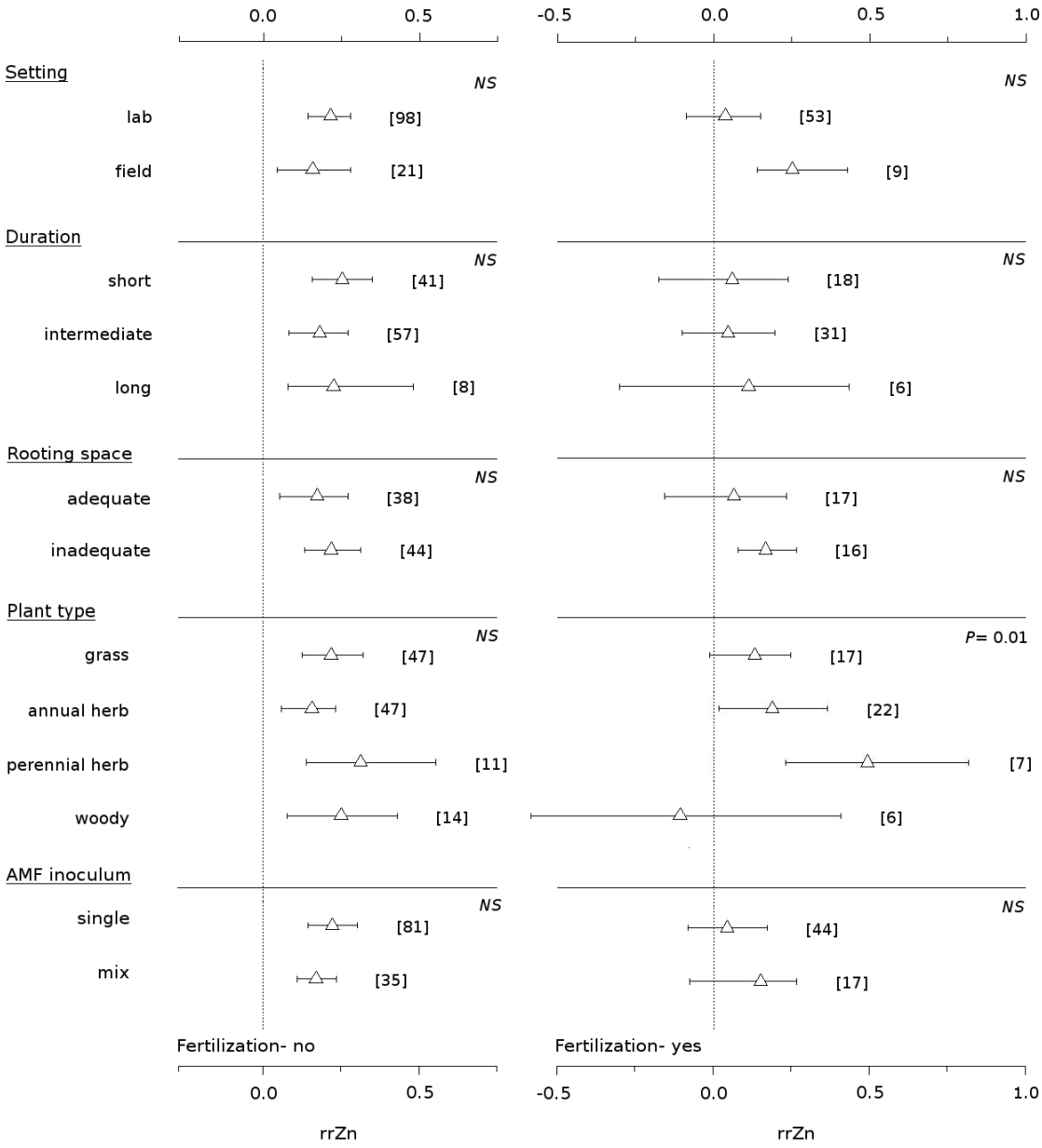

Fig. III.S2. Effect of independent variables on rrZn in subset Fertilization- no and Fertilization- yes of *shoot* dataset. This analysis was performed to test for the impact of P fertilizer application on the relationship of rrZn and independent variables. Effects are represented as means and bias corrected CIs. Values in parentheses are numbers of trials included in the analysis. Significance test was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

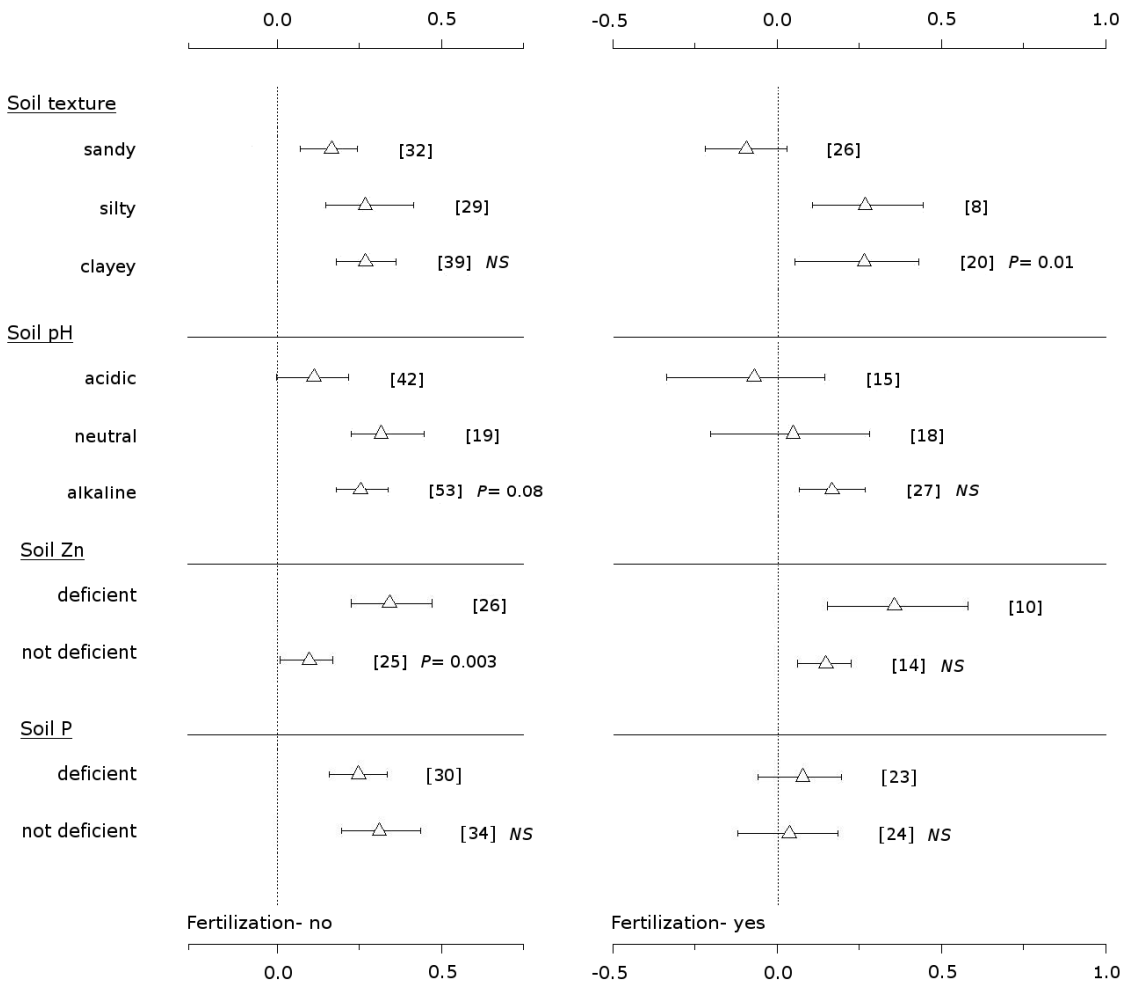

Fig. III.S3. Effect of independent variables on rrZn in subset *fertilization- no* and *fertilization- yes* of the *shoot* dataset. This analysis was performed to test for the impact of P fertilizer application on the relationship between rrZn and independent variables. Effects are represented as means and bias corrected CIs. Values in parentheses are numbers of trials included in the analysis. Significance test was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

iii. rrP and rrbiomass

The effects sizes rrP and rrbiomass were log response ratios of (rr) of mycorrhizal and non-mycorrhizal nutrient concentration:

$$rrP = \ln\left(\frac{P_M}{P_C}\right) \text{ and}$$

$$rrbiomass = \ln\left(\frac{biomass_M}{biomass_C}\right), \text{ respectively,}$$

where P_M and $biomass_M$ represented the tissue nutrient concentration (mg * kg DWT^{-1}) of mycorrhizal plants and P_C and $biomass_C$ that of control plants.

Following we present results for *fruit*, *shoot* and *root* datasets. For *fruit* and *root*, numbers of studies and trials were limited.

Table III.S2 Effect of independent variables on rrP and rrbiomass in the *fruit* dataset. Effect size mean, lower (lb) and upper (ub) confidence interval border, number of trials and *P*-value are presented. NA stands for not applicable.

Dataset: *fruit*

		rrP					rrbiomass				
Moderator	**Level**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**
Overall		0.0586	-0.0217	0.1103	38		0.1572	0.0748	0.2258	11	
Setting	lab	-0.0255	-0.3445	0.1455	8	0.1572	0.1892	0.1384	0.2579	9	0.1248
	field	0.0786	0.0329	0.1223	30		-0.1249	-0.3511	0.0572	2	
Fertilization	no	0.0916	0.0383	0.1385	22	0.2947	0.1791	0.1252	0.2085	6	0.7158
	yes	0.0165	-0.1508	0.1146	16		0.1362	-0.0326	0.2611	5	
Duration	short	0.0883	0.0834	0.0919	2	0.7264	0.1176	0.1044	0.1466	2	0.5366
	Intermediate	0.0572	-0.0273	0.1129	36		0.176	0.0453	0.2573	8	
Rooting space	adequate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	inadequate	NA	NA	NA	NA		NA	NA	NA	NA	
Plant type	grass	-0.0017	-0.1695	0.093	15	0.1282	0.1825	0.1218	0.2763	6	0.5524
	annual herb	0.0972	0.037	0.1492	23		0.1062	-0.1276	0.2052	5	
AMF inoculum	single	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	mix	NA	NA	NA	NA		NA	NA	NA	NA	
Soil texture	sandy	-0.1902	-0.6588	0.0911	4	0.0442	0.1888	0.1077	0.3259	4	0.2778
	silty	0.0761	0.0263	0.1176	24		0.1876	0.1426	0.2173	3	
	clayey	0.1093	0.0303	0.2208	8		-0.1272	-0.3511	0.0572	2	
Soil pH	acidic	0.1207	0.0407	0.2252	4	0.2144	0.2172	-0.3511	0.3489	3	0.4932
	neutral	-0.0714	-0.6469	0.3487	4		0.1538	0.122	0.1973	7	
	alkaline	0.0678	0.0276	0.102	30						

[a] The *P*-value referred to the between-level differences, i.e. if the *P*-value ≤0.05 then confidence intervals of independent variable levels were not overlapping each other and thus the independent variable significantly influenced the effect size.

Table III.S3 Effect of independent variables on rrP and rrbiomass in the *shoot* dataset. Effect size mean, lower (lb) and upper (ub) confidence interval border, number of trials and *P*-value are presented. *P*- value ≤ 0.05 are significant.

Dataset: *shoot*

		rrP					rrbiomass				
Moderator	**Level**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**
Overall		0.3049	0.2534	0.3629	163		0.3171	0.2471	0.402	156	
Setting	lab	0.3126	0.2515	0.3766	141	0.412	0.3233	0.2472	0.4108	147	0.505
	field	0.2496	0.1641	0.3429	22		0.2204	0.0963	0.4444	9	
Fertilization	no	0.2695	0.2065	0.3312	106	0.1086	0.3142	0.2328	0.4305	103	0.7682
	yes	0.368	0.2658	0.471	57		0.3411	0.2208	0.4969	53	
Duration	short	0.2305	0.1358	0.3271	57	0.1656	0.2588	0.1619	0.3524	57	0.155
	Intermediate	0.319	0.2446	0.4002	81		0.3371	0.2491	0.4546	82	
	long	0.42	0.2628	0.6208	13		0.5476	0.1596	1.2328	12	
Rooting space	adequate	0.2784	0.1739	0.3797	46	0.949	0.2553	0.1827	0.3496	45	0.781
	inadequate	0.274	0.1832	0.3542	52		0.2731	0.1956	0.3611	59	
Plant type	grass	0.2292	0.1403	0.3202	67	0.0006	0.2575	0.1853	0.3362	66	0.019
	annual herb	0.284	0.2049	0.3701	62		0.3512	0.2352	0.4906	58	
	prennial herb	0.2803	0.1703	0.3978	15		0.1299	0.0015	0.2772	14	
	woody	0.5861	0.4611	0.7245	19		0.6846	0.3293	1.3090	18	
AMF inoculum	single	0.316	0.2501	0.3781	117	0.653	0.3857	0.2972	0.5032	117	0.0158
	mix	0.2876	0.1852	0.401	42		0.1558	0.0768	0.2831	35	
Soil texture	sandy	0.2829	0.1753	0.3837	54	0.1856	0.2249	0.1351	0.3213	54	0.0268
	silty	0.4097	0.3176	0.5212	37		0.5386	0.3262	0.8741	32	
	clayey	0.3536	0.268	0.4463	49		0.4099	0.2823	0.5776	45	
Soil pH	acidic	0.177	0.0511	0.3024	54	0.0008	0.2239	0.1246	0.3538	52	0.2102
	neutral	0.482	0.3952	0.5799	34		0.3814	0.2659	0.5129	35	
	alkaline	0.3188	0.2624	0.3794	69		0.3881	0.2613	0.5549	62	

[a] The *P*-value referred to the between-level differences, i.e. if the *P*-value ≤0.05 then confidence intervals of independent variable levels were not overlapping each other and thus the independent variable significantly influenced the effect size.

Table III.S4 Effect of independent variables on rrP and rrbiomass in the *root* dataset. Effect size mean, lower (lb) and upper (ub) confidence interval border, number of trials and *P*-value are presented. *P*- value ≤ 0.05 are significant. NA stands for not applicable.

Dataset: *root*

		rrP					rrbiomass				
Moderator	**Level**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**	**Mean**	**lbCI**	**ubCI**	**Trials**	***P*-value [a]**
Overall		0.2868	0.2143	0.3683	38		0.3625	0.1993	0.5845	38	
Setting	lab	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	field	NA	NA	NA	NA		NA	NA	NA	NA	
Fertilization	no	0.2525	0.1768	0.3206	26	0.2332	0.3031	0.1053	0.6202	26	0.4056
	yes	0.3648	0.2157	0.5627	12		0.4891	0.2595	0.7995	12	
Duration	short	0.2906	0.2114	0.3881	13	0.0074	0.3461	0.1591	0.6386	13	0.0028
	Intermediate	0.2415	0.1467	0.3381	23		0.1854	0.0551	0.3239	23	
	long	1.1815	1.1787	1.2040	2		1.8883	1.1879	2.5823	2	
Rooting space	adequate	0.2609	0.1816	0.3594	18	0.7404	0.2129	0.0633	0.4303	18	0.6928
	inadequate	0.2861	0.1874	0.3782	17		0.2632	0.1195	0.4351	18	
Plant type	grass	0.2659	0.1592	0.4183	11	0.006	0.2078	0.0595	0.3529	13	0.0002
	annual herb	0.2575	0.1743	0.3371	18		0.3267	0.1465	0.5204	18	
	prennial herb	0.1754	0.0272	0.2772	5		-0.0398	-0.1095	0.0996	4	
	woody	0.8471	0.5331	1.1797	4		1.9295	1.1879	2.5823	3	
AMF inoculum	single	0.3151	0.2273	0.4196	26	0.5854	0.3889	0.2013	0.6506	28	0.6526
	mix	0.2643	0.1554	0.4228	11		0.275	0.0224	0.6971	10	
Soil texture	sandy	0.2057	0.0871	0.3598	9	0.0952	0.0799	-0.0817	0.2794	10	0.0144
	silty	0.4853	0.326	0.8835	6		0.914	0.3544	1.7430	6	
	clayey	0.31	0.214	0.4218	18		0.4355	0.2284	0.7059	18	
Soil pH	acidic	0.1741	0.067	0.3269	11	0.1588	0.2239	-0.0596	0.7358	9	0.59
	neutral	0.272	0.1103	0.3964	5		0.2786	0.0786	0.4905	7	
	alkaline	0.3648	0.2709	0.501	20		0.4733	0.2285	0.8295	21	

[a] The *P*-value referred to the between-level differences, i.e. if the *P*-value ≤0.05 then confidence intervals of independent variable levels were not overlapping each other and thus the independent variable significantly influenced the effect size.

iv. Effect of soil type and soil pH on soil Zn for rrZn

We tested the influence of soil texture and soil pH on soil Zn by analyzing the impact of soil Zn on rrZn in 6 subsets: *sandy*, *silty*, *clayey*, *acidic*, *neutral* and *alkaline*.

We found the same pattern for soil Zn in all subsets; non-deficient soil Zn concentrations reduced the mycorrhizal effect irrespective of soil type or soil pH level (Table III.S5).

Therefore, we concluded that studies categorized as soil Zn deficient indeed had very low Zn soil concentrations that promoted AMF Zn-uptake.

Table III.S5 Effect of soil Zn on rrZn in soil texture and soil pH subsets in *shoot* dataset. Effect size mean, lower and upper confidence interval border, number of trials and *P*-value are presented.

Subset		Soil Zn level	Mean	lbCI	ubCI	Trials	*P*-value [a]
Soil texture	*sandy*	deficient	0.0753	-0.3712	0.4275	7	0.2712
		non-deficient	-0.1258	-0.2665	0.0027	26	
	silty	deficient	0.3222	0.1409	0.535	14	0.0772
		non-deficient	0.0873	-0.0435	0.1875	9	
	clayey	deficient	0.2947	0.1742	0.4164	22	0.0656
		non-deficient	0.0621	-0.2362	0.2215	16	
Soil pH	*acidic*	deficient	0.2687	0.0999	0.516	8	0.152
		non-deficient	-0.0168	-0.3303	0.1801	16	
	neutral	deficient	0.313	0.1676	0.5012	4	0.481
		non-deficient	-0.0927	-0.7351	0.2453	4	
	alkaline	deficient	0.3544	0.2401	0.4749	28	0.0002
		non-deficient	0.0493	-0.0508	0.1324	23	

[a] The *P*-value referred to the between-level differences, i.e. if the *P*-value ≤ 0.05 than confidence intervals of independent variable levels were not overlapping each other and thus the independent variable significantly influenced the effect size.

v. Zn- P- Biomass interaction

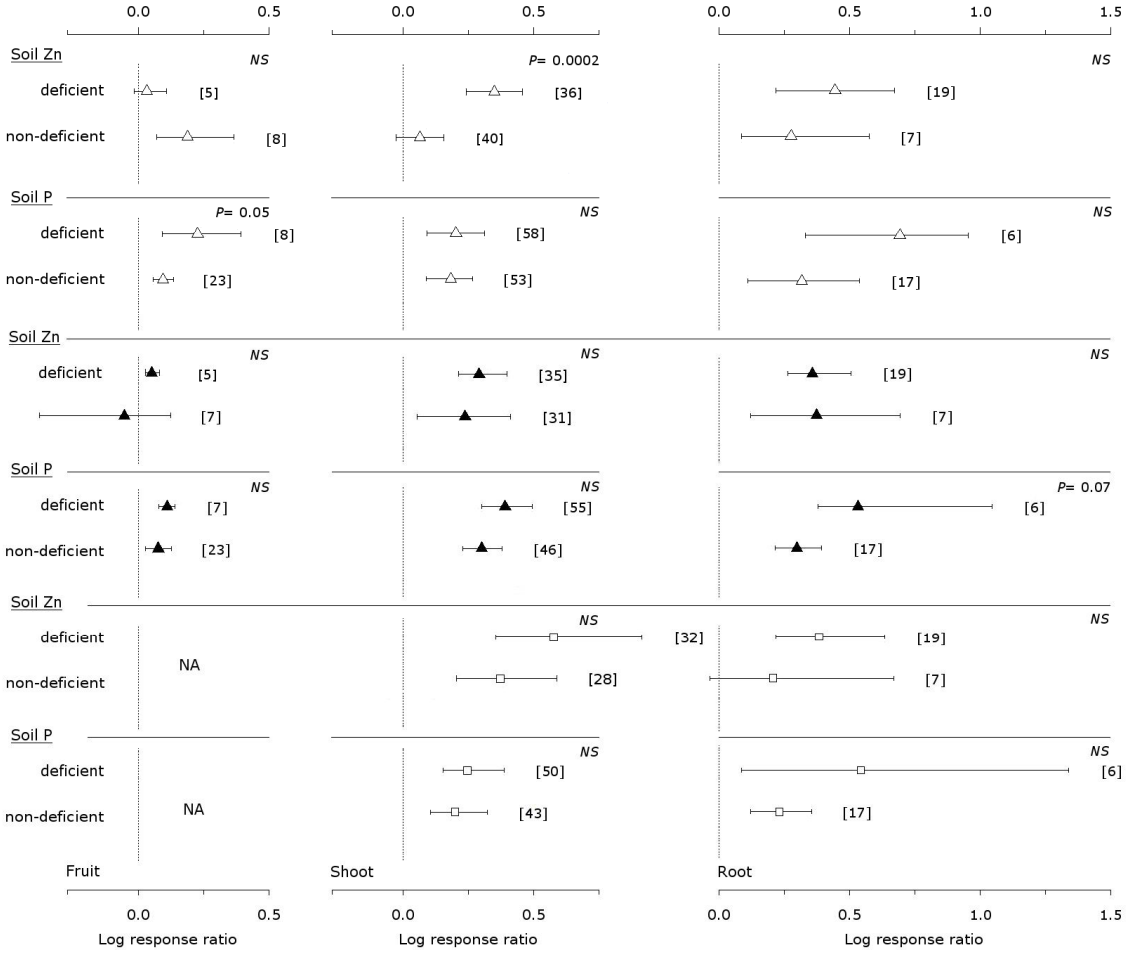

Fig. III.S4. Effect of soil Zn (DTPA- extractable Zn in mg Zn* kg soil-1) and soil P (Olsen P-extractable P in mg P* kg soil-1) on rrZn (white triangles), rrP (black triangles) and rrbiomass (white squares) for datasets *fruit*, *shoot* and *root*. Effects are represented as means and bias corrected CIs. Values in parentheses are numbers of trials included in the analysis. Significance test was based on a permutation test (random effects design) and *P*-values ≤ 0.05 were significant.

III. Sensitivity Analysis

The robustness of the summary effect size estimates had to be verified for any disproportional impact of single studies. Therefore, a sensitivity analysis (Copas and Shi, 2000) was performed to identify studies with an exceptionally high or low effect in the *shoot*, *root* and *fruit* datasets and *fertilization* subsets (Fig. III.S5 to III.S15).

However, we only applied this procedure on independent variables significantly affecting rrZn.

The sensitivity analysis was done in Metawin by sequentially excluding one study at a time from the dataset. After excluding a study, a new random effects meta-analysis was performed and the effect size estimate and the biasCIs were compared with those of the complete dataset. Effect size estimates and biasCIs for each level of the categorical independent variables were investigated.

If the biasCIs did not include the effect size estimate of the complete dataset, then this specific study had a disproportional impact. Consequently, the meta-analysis of the complete dataset had to be repeated without this specific study.

i. Dataset: *fruit*

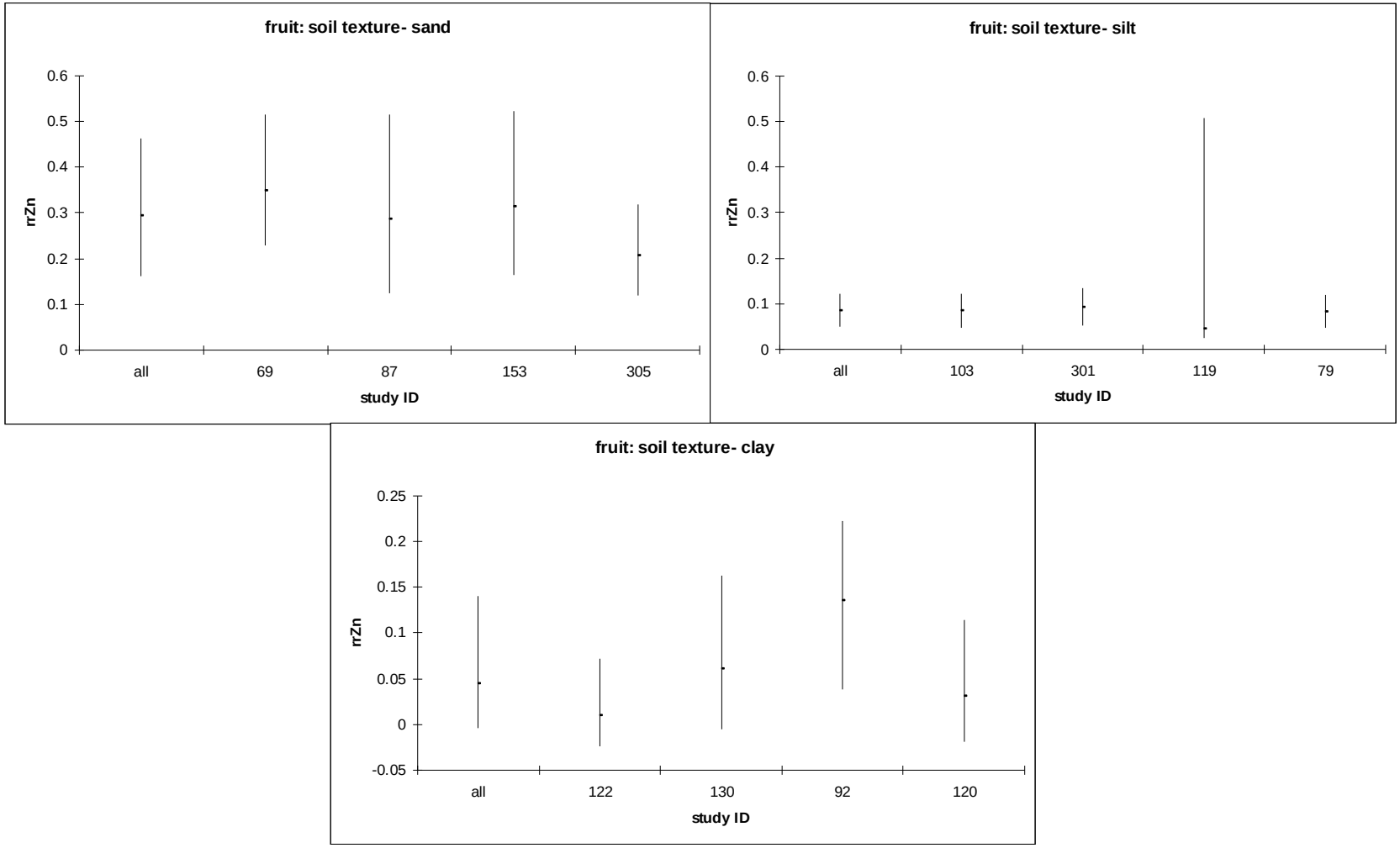

Fig. III.S5. Sensitivity analysis of experimental moderator soil texture on rrZn in *fruit* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

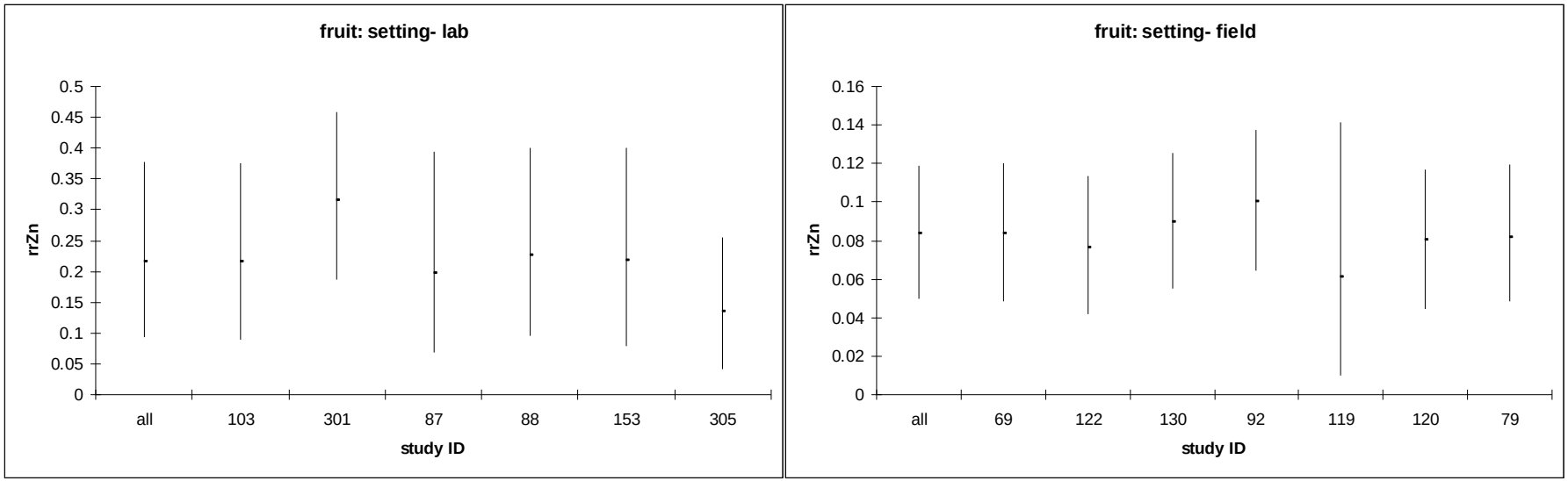

Fig. III.S6. Sensitivity analysis of experimental moderator setting on rrZn in *fruit* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

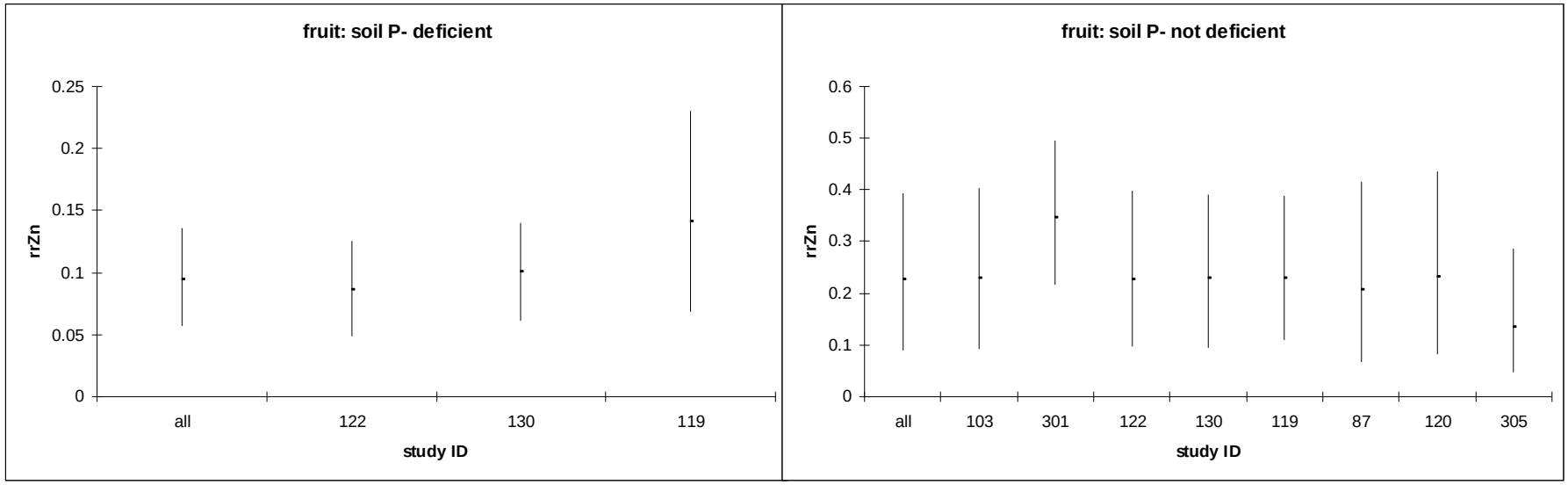

Fig. III.S7. Sensitivity analysis of experimental moderator soil P on rrZn in *fruit* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

ii. Dataset: *shoot*

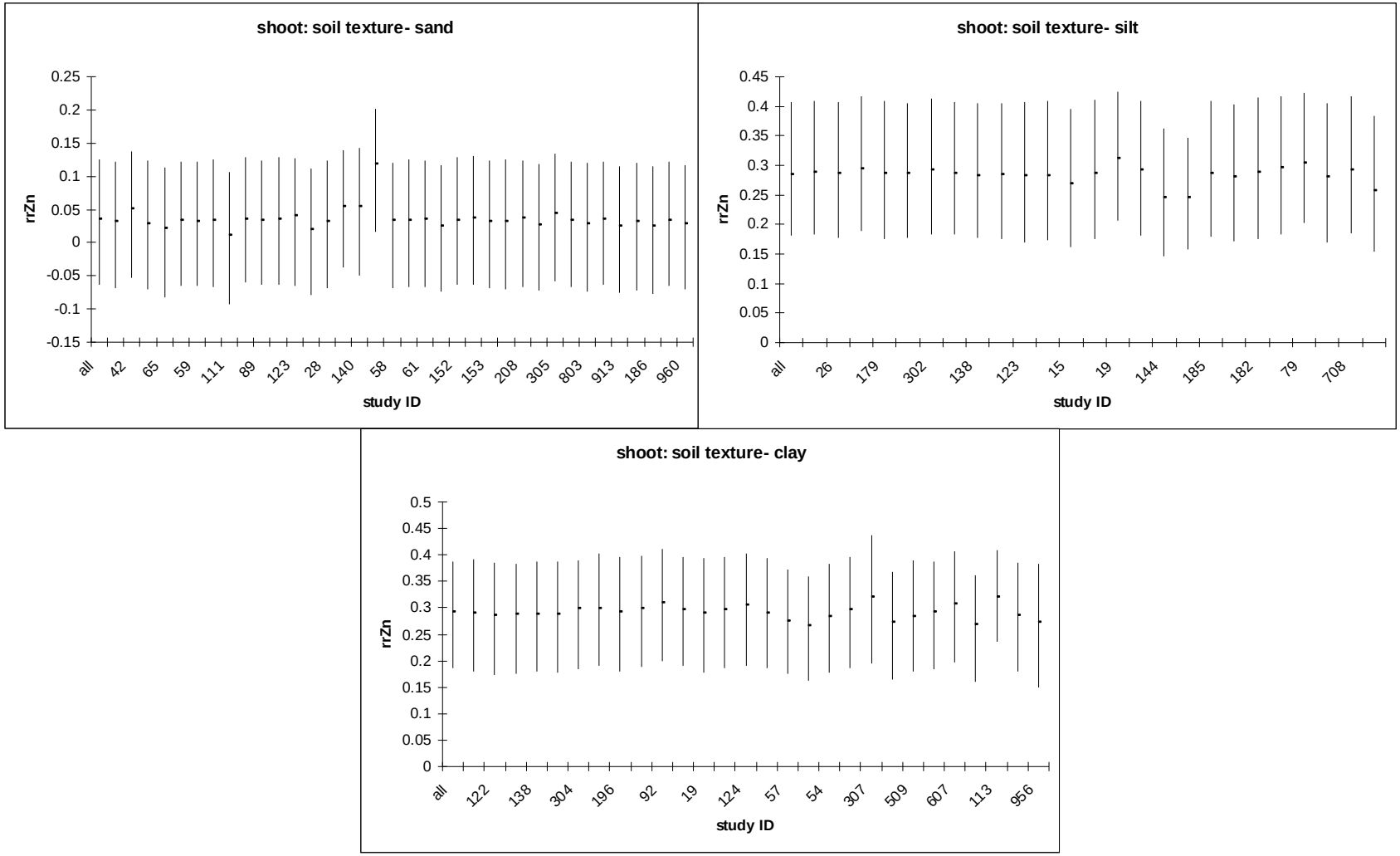

Fig. III.S8. Sensitivity analysis of experimental moderator soil texture on rrZn in *shoot* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

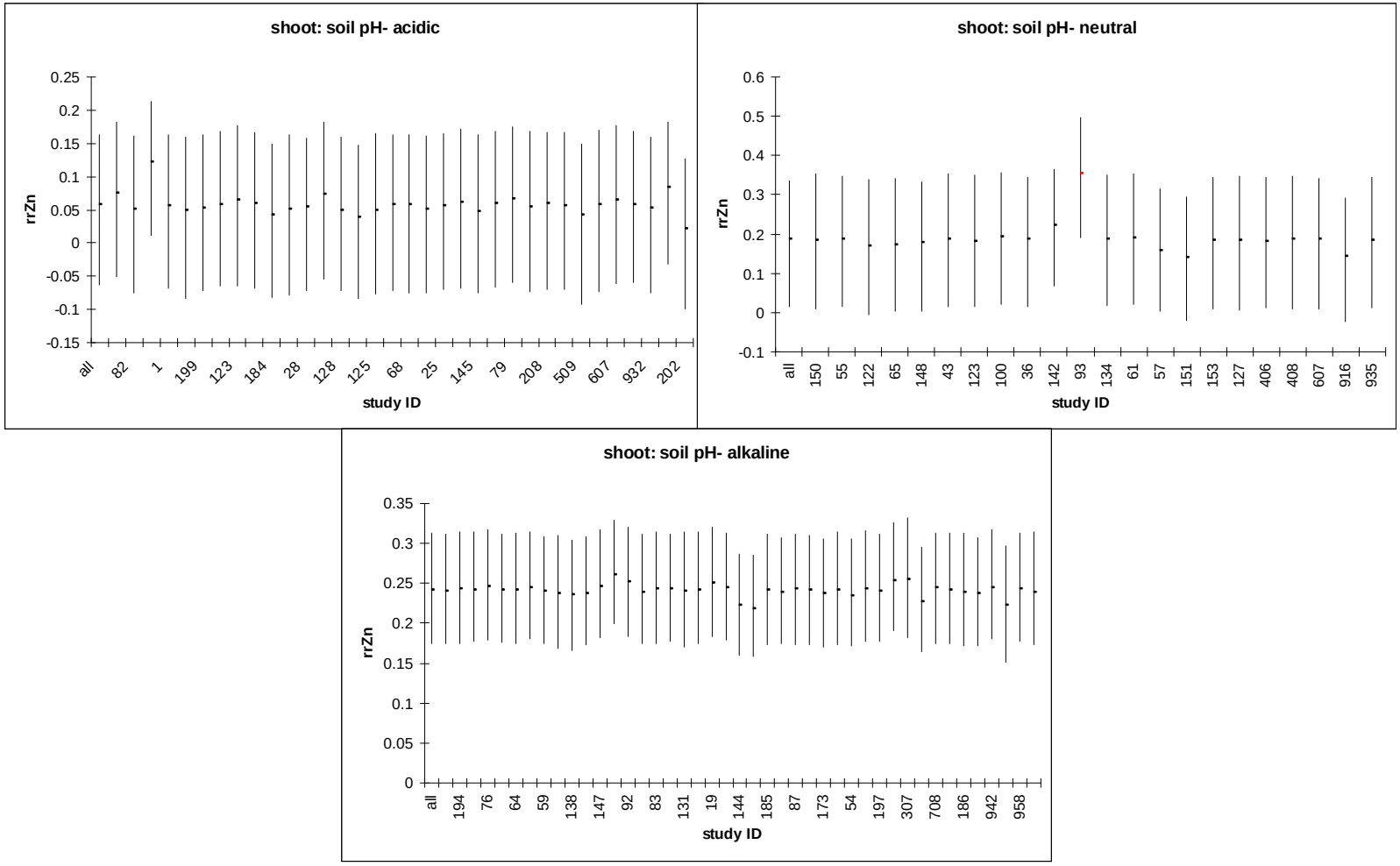

Fig. III.S9. Sensitivity analysis of experimental moderator soil pH on rrZn in *shoot* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. Red squares represent studies with a disproportional impact on effect size.

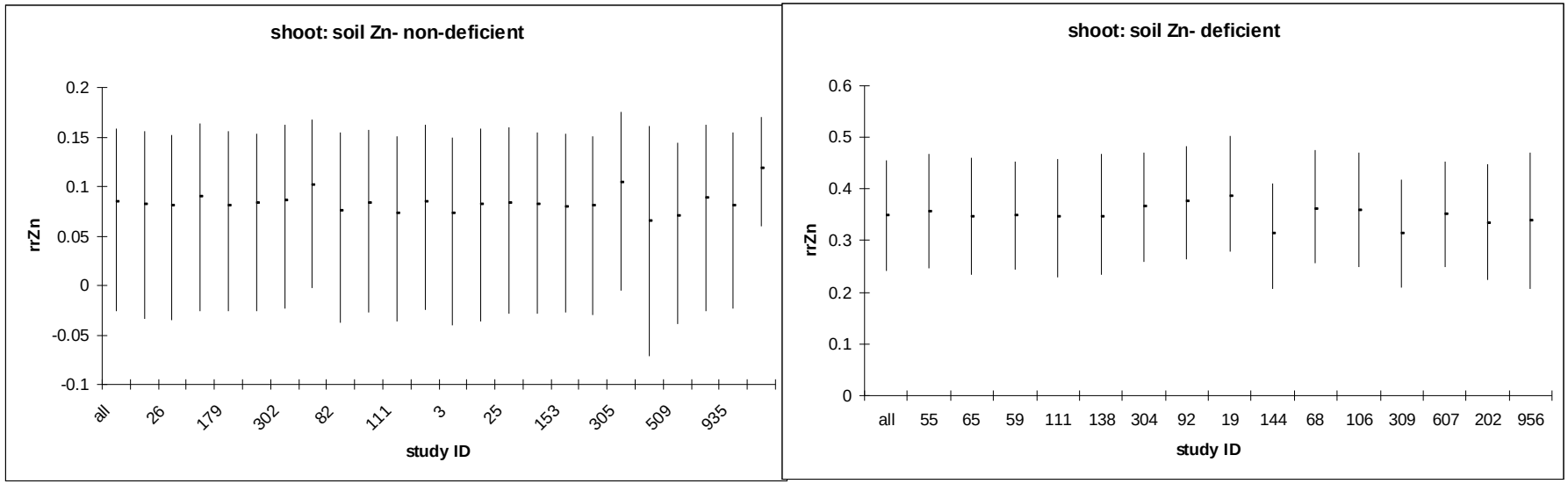

Fig. III.S10. Sensitivity analysis of experimental moderator soil Zn on rrZn in *shoot* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

iii. Dataset: *root*

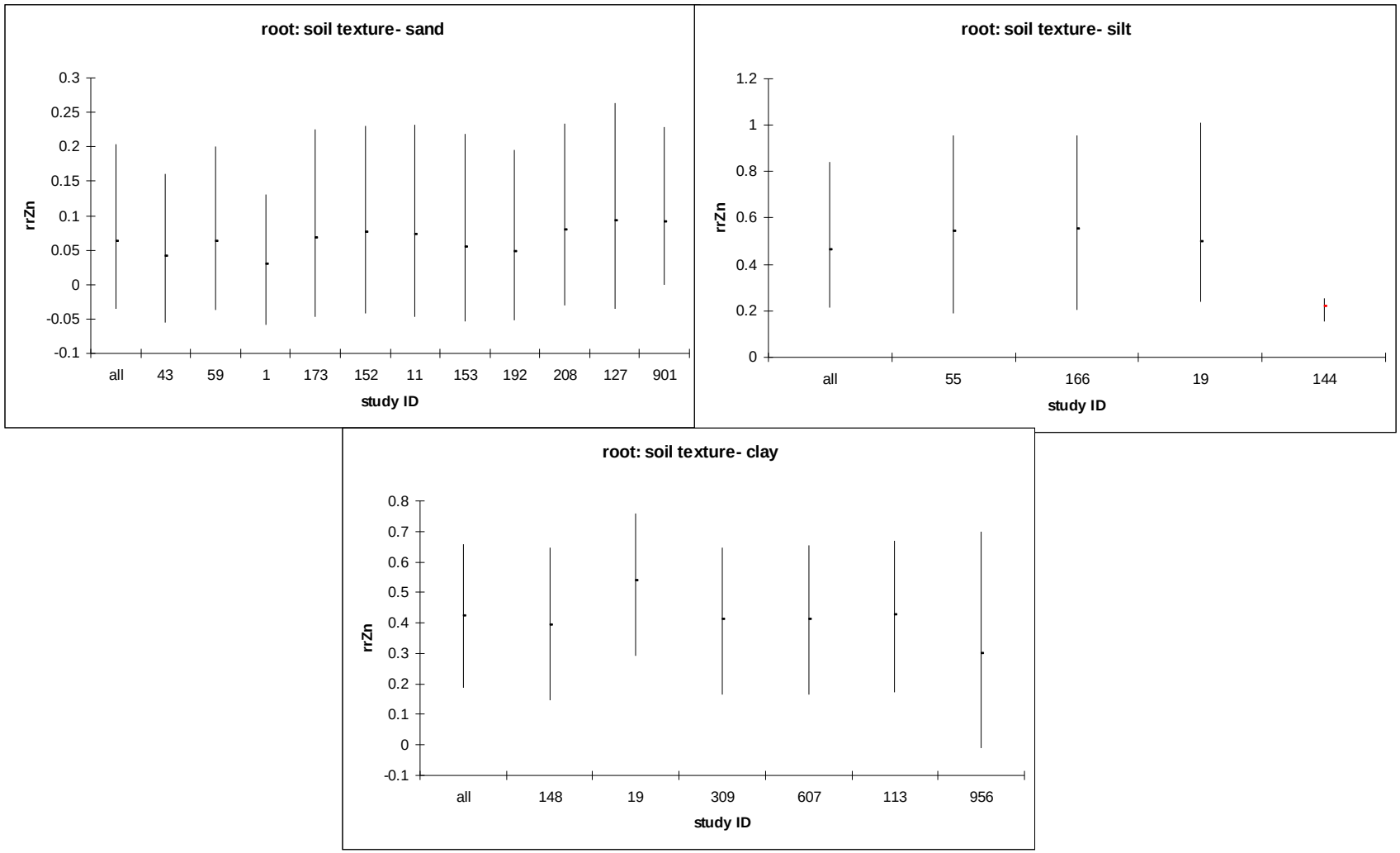

Fig. III.S11. Sensitivity analysis of experimental moderator soil texture on rrZn in *root* dataset. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. Red dots represent studies with a disproportional impact on effect size.

iv. Fertilization subsets

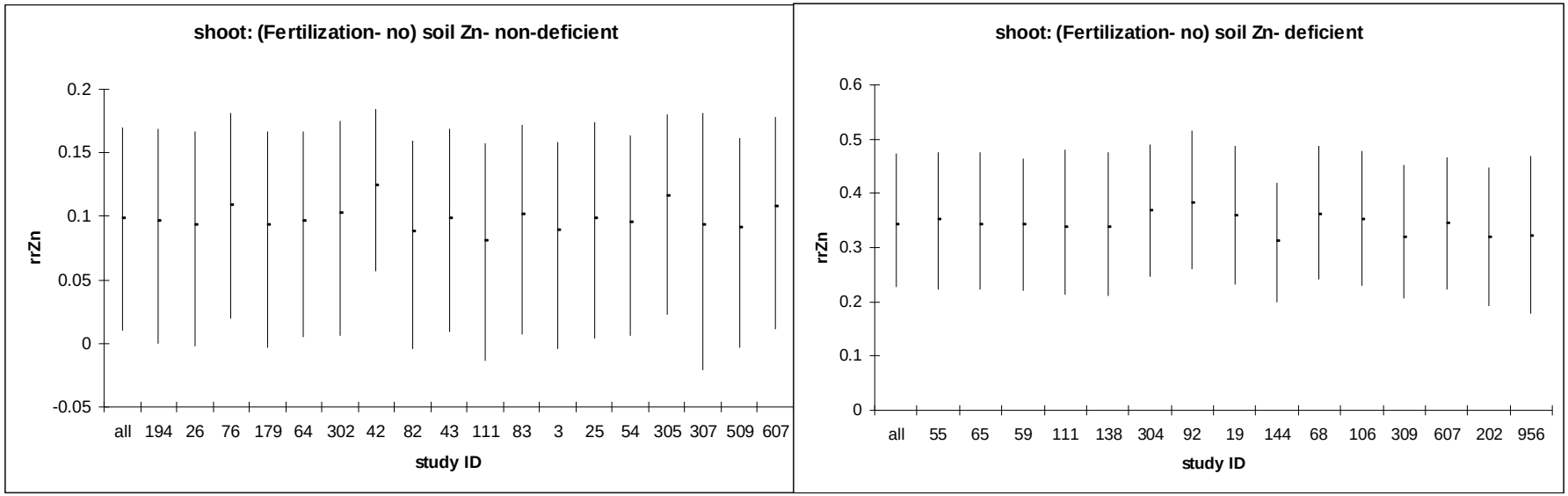

Fig. III.S12. Sensitivity analysis of experimental moderator soil Zn on rrZn in *Fertilization- no* subset of dataset *shoot*. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

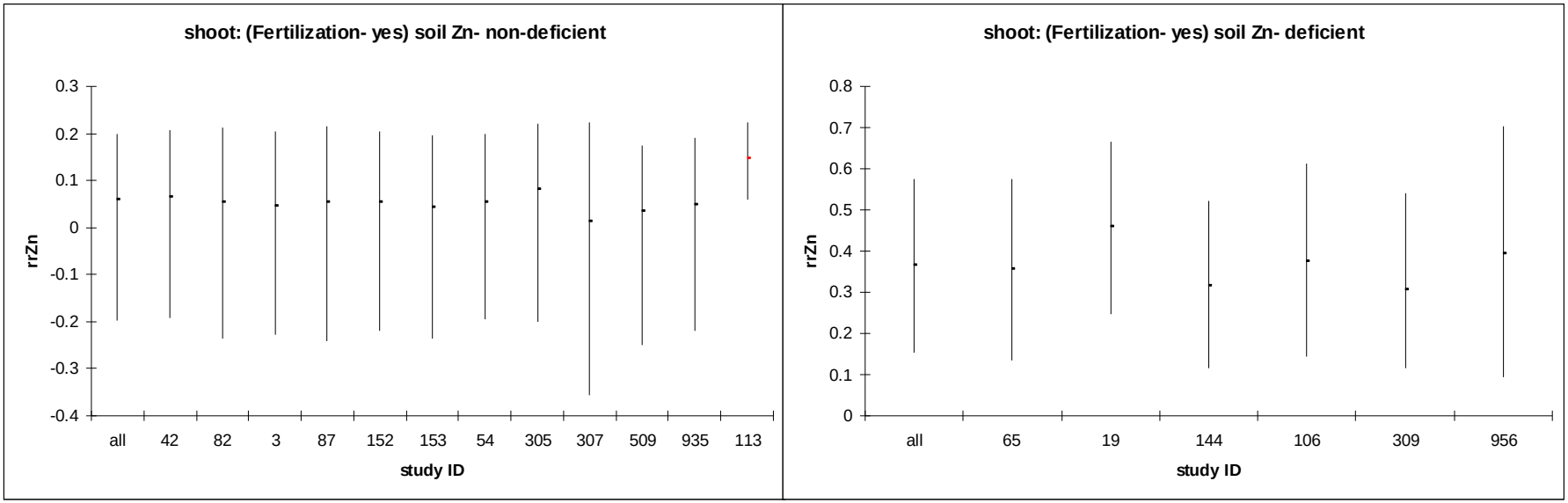

Fig. III.S13. Sensitivity analysis of experimental moderator soil Zn on rrZn in *Fertilization- yes* subset of dataset *shoot*. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.

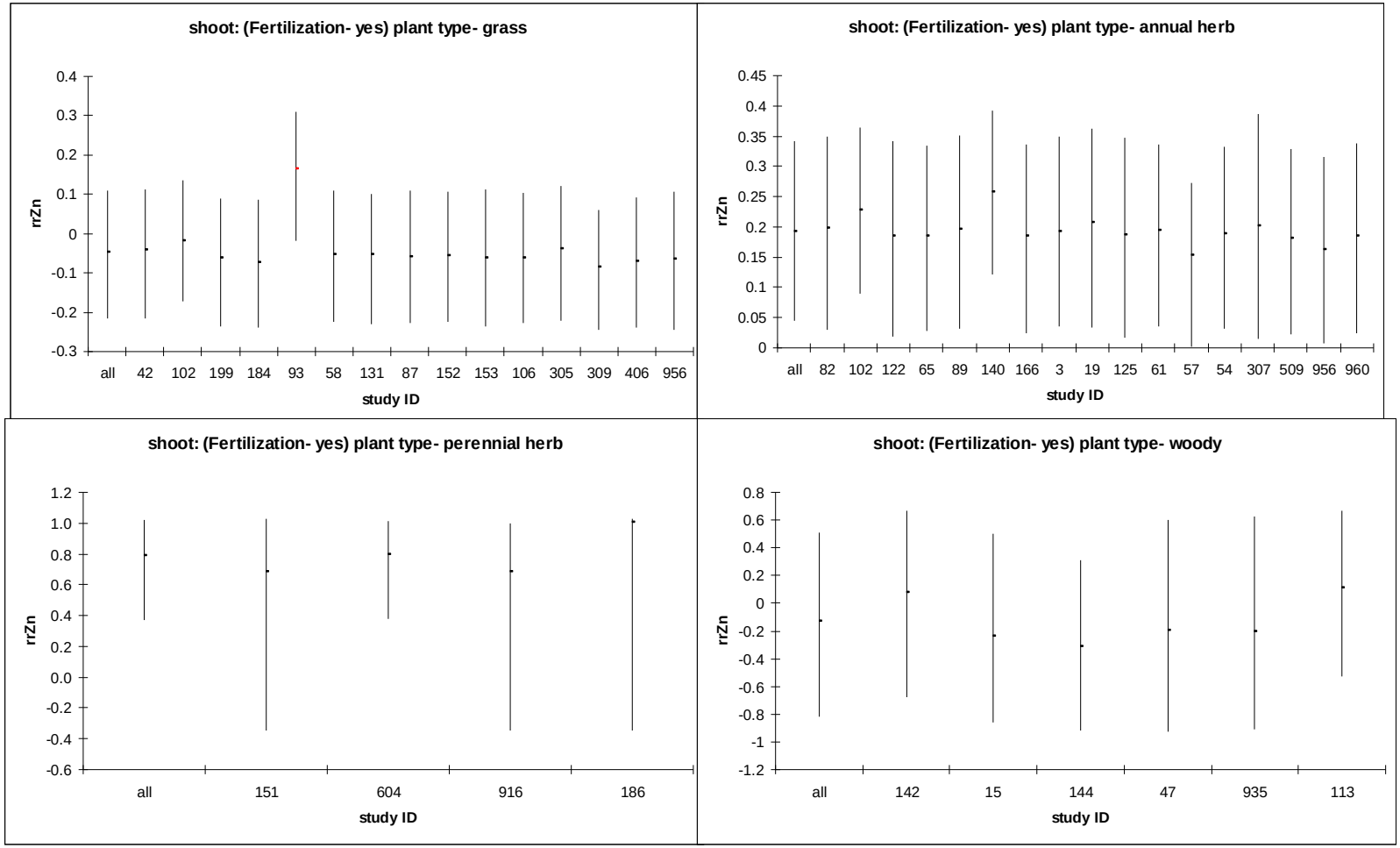

Fig. III.S14. Sensitivity analysis of experimental moderator plant type on rrZn in *Fertilization- yes* subset of dataset *shoot*. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. Red dots represent studies with a disproportional impact on effect size.

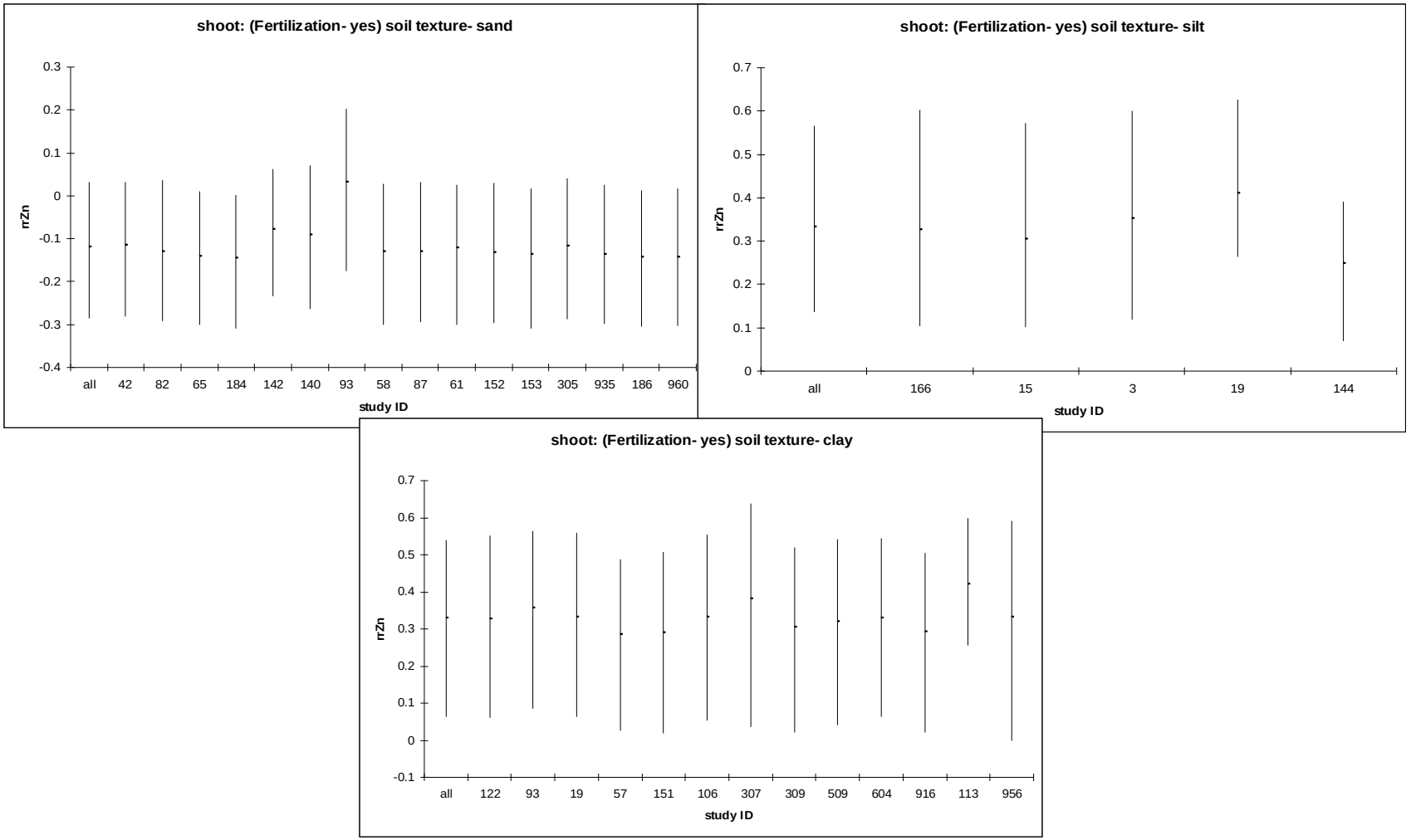

Fig. III.S15. Sensitivity analysis of experimental moderator soil texture on rrZn in *Fertilization- yes* subset of dataset *shoot*. Means and biasCI were presented for all (overall effect with no study excluded) and sequentially exclusion of one study. The values on the x-axes represented study ID of excluded study. No study with disproportional impact was detectable.