Plant, Cell & Environment

Plant, Cell and Environment (2016) **39**, 136–146 doi: 10.1111/pce.12600

Original Article

Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils

Luise Köhl[1,2], Catherine E. Lukasiewicz[2] & Marcel G. A. van der Heijden[1,2,3]

[1]Plant–Soil interactions, Institute for Sustainability Sciences, Agroscope, CH-8046 Zurich, Switzerland, [2]Plant–Microbe Interactions, Institute of Environmental Biology, Faculty of Science, Utrecht University, 3508 TB Utrecht, The Netherlands and [3]Institute of Evolutionary Biology and Environmental Studies, University of Zurich, CH-8057 Zurich, Switzerland

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are promoted as biofertilizers for sustainable agriculture. So far, most researchers have investigated the effects of AMF on plant growth under highly controlled conditions with sterilized soil, soil substrates or soils with low available P or low inoculum potential. However, it is still poorly documented whether inoculated AMF can successfully establish in field soils with native AMF communities and enhance plant growth. We inoculated grassland microcosms planted with a grass–clover mixture (*Lolium multiflorum* and *Trifolium pratense*) with the arbuscular mycorrhizal fungus *Rhizoglomus irregulare*. The microcosms were filled with eight different unsterilized field soils that varied greatly in soil type and chemical characteristics and indigenous AMF communities. We tested whether inoculation with AMF enhanced plant biomass and *R. irregulare* abundance using a species specific qPCR. Inoculation increased the abundance of *R. irregulare* in all soils, irrespective of soil P availability, the initial abundance of *R. irregulare* or the abundance of native AM fungal communities. AMF inoculation had no effect on the grass but significantly enhanced clover yield in five out of eight field soils. The results demonstrate that AMF inoculation can be successful, even when soil P availability is high and native AMF communities are abundant.

Key-words: arbuscular mycorrhizal fungi; field soil; inoculation; *Lolium*; qPCR; *Rhizoglomus irregulare*; *Trifolium*.

INTRODUCTION

There is an increased interest to utilize beneficial soil biota as a tool to enhance plant nutrition and plant productivity (Barrios 2007). The presence of beneficial soil biota can be stimulated by altering agricultural practices such as crop rotation or tillage intensity that favour particular groups of microorganisms (Altieri 1999, Köhl *et al.* 2014). In addition, beneficial soil biota can also be deliberately introduced into agroecosystems through inoculation or seed coating in order to add a desired function or enhance an already existing one (Berg 2009, Vessey 2003).

Within the soil microbial community, arbuscular mycorrhizal fungi (AMF) are well known for their ability to enhance plant nutrient uptake, improve plant growth and influence ecosystem functioning (Smith & Read 2008). Up to 50% of the soil microbial biomass consists of AMF (Olsson *et al.* 1999). AMF form a symbiosis with over 80% of the land plants including many important crops (Smith & Read 2008). AMF can provide a range of soil nutrients to plants in exchange for carbohydrates. In addition, AMF can also contribute to soil aggregate formation (Leifheit *et al.* 2014), protect their hosts against abiotic (Galli *et al.* 1994, Bothe 2012) and biotic stresses (Azcón-Aguilar & Barea 1996), influence nutrient cycling (Bender & van der Heijden 2015, Cavagnaro *et al.* 2006) and soil respiration (Wamberg *et al.* 2003, Langley *et al.* 2005), and reduce the production of the greenhouse gas N_2O (Bender *et al.* 2014).

Arbuscular mycorrhizal fungi are native to all terrestrial ecosystems and can be found in almost every soil (Abbott & Robson 1982, Öpik *et al.* 2006, Jansa *et al.* 2009). Several studies report reduced AMF diversity upon land use intensification (Helgason *et al.* 1998, Verbruggen *et al.* 2010). The reduction of mycorrhizal abundance and species diversity is due to factors related to intensive agricultural management such as high fertilization, intensive tillage, fallow and crop sequence with non-host crops (Jansa *et al.* 2006, Koide & Peoples 2012, Säle *et al.* 2015). It has been shown in microcosms that this loss of fungal diversity in soil can disrupt a range of soil ecosystem services (Maherali & Klironomos 2007, van der Heijden *et al.* 1998, Wagg *et al.* 2014). Moreover, some studies indicate that intensive agriculture selects for inferior mutualists (Johnson 1993, Scullion *et al.* 1998).

Soil inoculation with beneficial AMF has been proposed to overcome this limitation, and contribute to more efficient nutrient use. Inoculation with beneficial AMF is increasingly considered for species-poor and often sterile soils in nurseries (Azcón-Aguilar & Barea 1997) and in tropical crop production where soils are low in plant available phosphorus and AMF abundance (Ceballos *et al.* 2013, Sieverding 1991). The hesitant application of AMF in commercial agriculture in the temperate zone might be due to high application costs, the perception that AMF are not very beneficial when

Correspondence: M. G. A. van der Heijden. Fax: +41 58 468 72 01; e-mail: marcel.vanderheijden@agroscope.admin.ch

P-availability is high, and that AMF may even lead to plant growth depression in some crops (Ryan & Graham 2002). Despite these concerns, meta-analyses have revealed that biomass production and P-uptake can indeed be increased by inoculation of soil with AMF (McGonigle 1988, Lekberg & Koide 2005, Hoeksema *et al.* 2010).

One of the crucial biotic soil factors determining the success of the fungal inoculant is the indigenous mycorrhizal community. If the strain is compatible with a particular soil, it still needs to outcompete the indigenous AMF community, and AMF already established in the field may be competitively superior (priority effect) compared with the introduced ones (Verbruggen *et al.* 2013). Furthermore, it is thought that ecosystems can only support AMF populations to a certain quantity (carrying capacity) preventing further establishment if this carrying capacity has already been reached. Thus, it seems questionable, if inoculation can be successful in fields with high fungal abundance. Despite numerous inoculation studies, only a few attempts using molecular tools have been made to assess, if a foreign strain can successfully colonize host plants and persist in field soil despite of other AMF being present (Farmer *et al.* 2007, Ceccarelli *et al.* 2010, Pellegrino *et al.* 2012, Sýkorová *et al.* 2012). Moreover, all these studies focused on one particular field, and it has not yet sufficiently been tested whether a particular inoculant can establish in a wide range of soils. It is also still unclear, whether the same fungal isolate as it often occurs in commercial inoculum can successfully established in a broad range of field sites. Such a broad applicability is one pre-condition for commercial AMF inocula.

In this study, we introduced *Rhizoglomus irregulare* to a range of agriculturally managed field soils. *R. irregulare* (formerly named *Rhizophagus irregularis/Glomus irregulare/Glomus intraradices*, Sieverding *et al.* 2014) is a widespread AMF present in almost any ecosystem investigated (Öpik *et al.* 2006), and is especially abundant in agricultural soils (Jansa *et al.* 2003, Oehl *et al.* 2010). Earlier studies with this isolate have shown that it has a positive impact on the growth and nutrition of a range of plant species, when added to sterilized soil (Scheublin *et al.* 2007, van der Heijden *et al.* 2015, Wagg *et al.* 2011a). Here, we specifically test whether (1) the introduced AMF can establish in a wide range of field soils; (2) the AMF is able to establish and compete with different resident AMF communities; and (3) whether AMF inoculation enhances plant productivity and nutrient uptake. In order to test this, we inoculated or mock inoculated the AMF *R. irregulare* into microcosms planted with a grass–clover mixture. The microcosms were filled with unsterilized field soil originating from eight agriculturally managed fields that differed strongly in soil type and chemical characteristics.

MATERIALS AND METHODS

Field soil

Eight different soils from tilled fields distributed across Switzerland were used as experimental soil. We specifically chose field sites that differed strongly in soil type and chemical characteristics (Table 1). These different soils also varied in cropping history and agricultural management, like fertilization. All soils are representative for the temperate zone. Soils were taken from the tilled layer before fertilization in the spring. As a control, we selected a field soil with a very low mycorrhizal inoculum potential that had been stored long term at the research station Agroscope (Fig. 1(c)). Soils were sieved to 5 mm for homogenization and to remove larger fragments and stones.

Soil physical and chemical properties (Table 1) were analysed by lbu (Thun, Switzerland). The initial total N content in the dried soils was quantified with an elemental

Table 1. Soil type, origin, physical and chemical properties of the soils used in the greenhouse inoculation experiment

Soil	Soil type[a]	Location	pH	Clay	Loam	Humus	AAE10-Ex P[b]	CO2-Ex P[c]	Total N	NO_3-N	NH_4-N	CEC[d]
				%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	meq/100 g
A	Fluvisol	Paradislihof, Rietheim	7.8	16.0	31.0	1.6	52.8	5.3	1295.0	20.2	0.19	9.3
B	Cambic Stagnosol	Laubbergerhof, Rietheim	7.3	21.0	31.0	3.8	109.3	3.0	2540.0	77.4	0.24	23.4
C	Regosol	Hardhof, Tegerfelden	8.0	21.0	41.0	2.1	210.1	18.8	1660.0	24.8	0.22	13.5
D	Histosol	Riedmatt, Rümlang	8.0	21.0	51.0	10.5	63.9	2.9	6610.0	32.3	0.23	46.9
E	Gleysol	Gordola, Ticino	6.2	16.0	41.0	2.3	56.2	1.6	1300.0	7.8	3.85	10.9
F	Cambisol	Agroscope, Zürich	6.6	16.0	31.0	2.5	62.1	3.8	2030.0	19.3	0.56	14.2
G	Eutric Stagnosol	Agroscope, Zürich	7.6	26.0	41.0	5.5	118.2	3.7	4400.0	53.0	0.31	35.4
H	Control soil	Agroscope, Zürich	5.6	11.0	31.0	1.0	7.8	0.3	1160.0	29.2	25.71	45.9

[a]According to IUSS Working Group WRB (2006);
[b]ammonium acetate EDTA extraction; cannot be interpreted when pH > 6.8;
[c]extraction with CO_2-saturated water;
[d]cation exchange capacity.

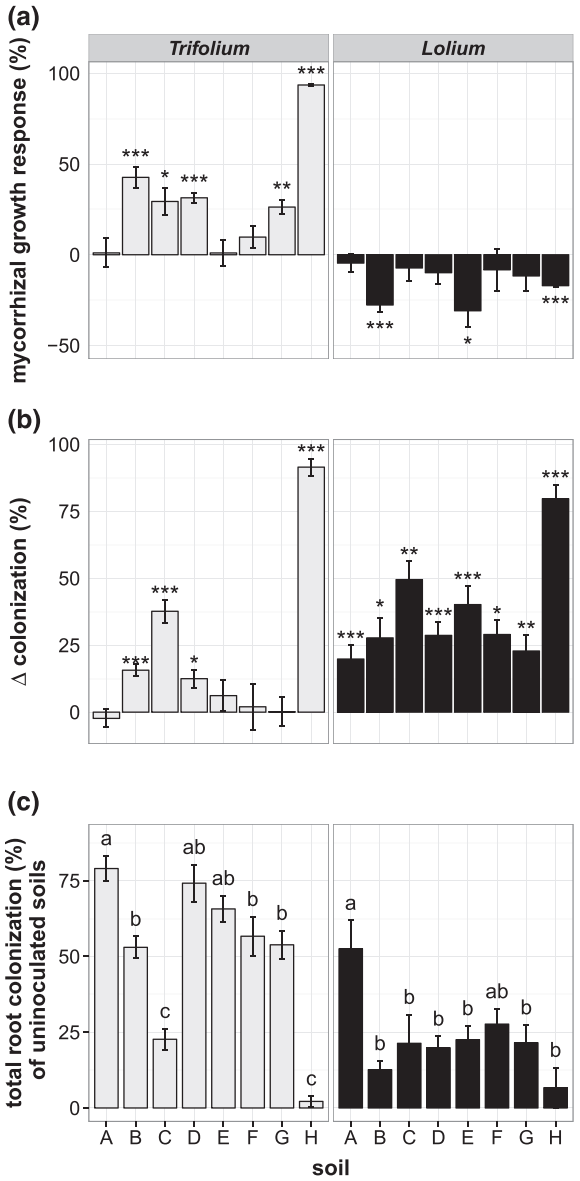

Figure 1. (a) Mycorrhizal growth response of *Lolium* and *Trifolium* plants (%), (b) change in total root colonization (%) due to inoculation with *R. irregulare* and (c) total root colonization (%) of uninoculated treatments in microcosms with eight different unsterile field soils (A-H). Bars depict means ± standard error of the mean of six replicates. Asterisks indicate that the effect size is significantly different from zero ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***) according to a one-sample *t*-test. Means with the same letter do not differ significantly (Tukey test, $p < 0.05$).

analyser Euro EA 3000 (HEKAtech, Wegberg, Germany). Available soil ammonium and nitrate concentrations were determined according to the Swiss reference methods of the research station Agroscope (Forschungsanstalt Agroscope Reckenholz-Tänikon ART & Forschungsanstalt Agroscope Changins-Wädenswil ACW 1996) using the Berthelot reaction (Krom 1980) and the cadmium reduction method (van Staden 1982) followed by a Griess assay (Griess 1879), respectively. The absorption of the resulting coloured complexes was quantified with the continuous flow analyser SAN++ analyser (Skalar Analytical B.V., Breda, the Netherlands).

AMF inoculum

Soils were either inoculated with the AM fungus *R. irregulare* (Błaszk., Wubet, Renker & Buscot) Sieverd., G. A. Silva and Oehl (formerly known as *R. irregularis*/*G. irregulare*/*G. intraradices* (Sieverding *et al.* 2014)), isolate BEG21 (accession number DQ377990, SAF22, Swiss Collection of Arbuscular Mycorrhizal Fungi, Agroscope, Zurich, Switzerland, www.agroscope.ch/saf) or received a non-mycorrhizal control inoculum. *R. irregulare* is a common AMF with a worldwide distribution (Öpik *et al.* 2006), and is very abundant in a wide range of ecosystems, including many agricultural fields in Switzerland (Sýkorová *et al.* 2007, Oehl *et al.* 2010). *R. intraradices* is reported to be a good root colonizer (Pellegrino *et al.* 2011) and very resistant to intensive agricultural management practices (Oehl *et al.* 2010). Furthermore, this AM fungus is commonly used in commercially available biofertilizers (Faye *et al.* 2013). The isolate was propagated in the greenhouse on *Plantago lanceolata* in an autoclaved substrate made of 15% grassland soil and 85% quartz sand. After 8 months of growth, the pots were left to dry out, and aboveground biomass was discarded. The roots were then cut into small pieces and mixed thoroughly with the rest of the substrate to serve as the soil inoculum. A non-mycorrhizal control was prepared analogously to the AMF inoculum, but without AMF added. The *R. irregulare* inoculum contained roots that were at least 72% colonized by AMF and 75 spores per gramme of inoculum. No AMF spores or root colonization was observed in the control inoculum.

Set-up of the AMF inoculation trial in the greenhouse

The experiment was set up as a full factorial block design and consisted of two factors: 'soil type' (eight different field soils, A-H, where H served as control soil with low abundance of AMF, Table 1) and 'mycorrhizal inoculation' [microcosms inoculated with AM fungi (I) or inoculated with a non-mycorrhizal control (C)]. Each of the 16 treatments was replicated six times resulting in 96 microcosms.

Grassland microcosms were established in PVC tubes with a diameter of 15.2 cm (surface corresponding to 1.8e-06 ha) and a height of 40 cm. For better drainage, 1040 g of an autoclaved gravel mixture was added to the bottom of the tubes. A total of 5.125 L sieved field soil was added to each microcosm, and 5.1% v/v soil inoculum (275 ml in total) was mixed with the upper 450 ml of soil. Each microcosm was covered with 175 ml of the corresponding soil on the top to prevent cross contamination.

The microcosms were planted with a model grassland community consisting of *Trifolium pratense* L. 'Formica' (red clover) and *Lolium multiflorum* Lam. 'Oryx' (Italian ryegrass), plant genotypes often planted in Swiss pastures

(Boller*et al.* 2002, Frick *et al.* 2008). A grass–clover mixture was chosen as it is widespread in both agricultural and natural grassland ecosystems where these species commonly coexist (Nyfeler 2009). Moreover, the two plant species belong to different functional groups (a legume and a grass) and show different mycorrhizal growth responses (MGR) (*Trifolium* is a highly responsive species (Köhl *et al.* 2014; van der Heijden *et al*. 2015) and *Lolium* is an unresponsive species (Wagg *et al.* 2011a)). Before planting, seeds (propagated by Agroscope, Zurich, Switzerland) were surface sterilized with 5% household bleach for 5 min, 70% ethanol for 10 min and then rinsed thoroughly with sterilized water. Plants were germinated on 1.5% sterile water agar. Twelve individuals of each plant species were planted into the microcosms according to a predefined design. During the first 2 weeks non-surviving seedlings were replaced. All microcosms were kept in the greenhouse (see supporting information for growth conditions). In Switzerland, temporary grass-clover ley is often sown in summer and fertilized for the first time in spring, when the pasture has established. As our experiment lasted only 13 weeks, we choose not to fertilize and give the plant community and mycorrhizal fungi time to establish.

All microcosms received 5 ml of an AMF-free filtered washing of the two different inocula. This was performed to equalize differences in the non-mycorrhizal microbial communities between the two soil inocula (Ames *et al.* 1987). The microbial wash was prepared by suspending 100 g of each inoculum together in 1 L deionized water. The suspension was filtered through several sieves (25–250 μm) and finally through filter paper (N°598, ∅210 mm, Schleicher and Schuell, Dassel, Germany).

Harvest and analysis

After 8 weeks, shoots were cut 6 cm aboveground to simulate hay making or grazing, which is typical for most grasslands in Switzerland. After 13 weeks, the microcosms were harvested and shoot dry weight, root dry weight, shoot N and P content, and AMF root colonization levels for each plant species were determined (see supporting information for details).

Quantification of R. irregulare in the roots by qPCR

A subsample of the root sample, containing roots of both species, was selected randomly, frozen and lyophilized. DNA was extracted, and quantitative PCR was conducted using primers and a hydrolysis probe, which are specific for the nuclear large ribosomal subunit (nLSU) of *R. irregulare*, following Thonar *et al.* (2012) (see supporting information for details).

Statistical analysis

Statistical analyses were conducted using the software R 2.14.1 (R Development Core Team 2011).

The effect of soil type and AMF inoculation on plant responses (biomass, shoot nutrient content, mycorrhizal structures in the roots) was analysed separately with mixed-effect models (Pinheiro & Bates 2000) using the function lme in the library nlme. Soil type, inoculation treatment and their interaction were used as fixed effect, whereas block functioned as random effect. When homoscedasticity was not guaranteed, the varIdent() function was used to allow each treatment to have different variances. Plant biomass was combined for two harvests. As plant mortality had a significant effect on *Lolium* biomass, the plant biomass was standardized by the number of survived individuals for both *Lolium* and *Trifolium*. nLSU copy numbers were log10 transformed before all analyses. The control soil H accounted for a significant amount of variance in the biomasses, as well as the root colonization. As this soil served as control (no fresh field soil, low inoculum potential), a separate analysis was performed without this soil to estimate the effects of field soils and AMF inoculation.

To assess the effect of the AMF inoculation on plant growth, growth responses were calculated as effect size of the inoculation treatment relative to the non-inoculated control for each soil type. The effect size of the mycorrhizal inoculation on the root colonization was evaluated as difference between the total colonization of the inoculated (I) and the mean of the uninoculated soil (C_{mean}) (Lekberg & Koide 2005) for each soil separately (Equation 1).

$$\Delta AMF = I - C_{\text{mean}} \tag{1}$$

I mycorrhizal root colonization (%) of plants growing in microcosms with mycorrhizal inoculum

C_{mean} mean mycorrhizal root colonization (%) of plants growing in microcosms that were not uninoculated (average of six replicates for each soil type)

The same was performed for the total root length colonized by AMF and *R. irregulare* LSU copy number determined by qPCR.

The MGR (Veiga *et al.* 2011) was used to express the effects of AMF inoculation on biomass production for each soil type. To calculate the MGR, two equations are required, one for plants which perform better with AMF ($I > C_{\text{mean}}$) and one for plants growing better without AMF ($I < C_{\text{mean}}$) (Equation 2 and 3).

$$\text{if } I > C_{\text{mean}}, \text{ then } MGR = \left(1 - \left(\frac{C_{\text{mean}}}{I}\right)\right) \times 100\% \tag{2}$$

$$\text{if } I > C_{\text{mean}}, \text{ then } MGR = \left(-1 + \left(\frac{I}{C_{\text{mean}}}\right)\right) \times 100\% \tag{3}$$

I biomass of plants growing in microcosms with mycorrhizal inoculum

C_{mean} mean biomass of plants growing in microcosms that were not uninoculated (average of six replicates for each treatment)

The effect of mycorrhizal inoculation on plant P and N content in the experiment was evaluated analogously to the MGR of the biomass. Means and standard errors of the mean of the raw data can be found in the supporting information (Table S1–S3).

The calculated effect sizes were assessed with a one sample *t*-test to determine, if the difference between the inoculated an uninoculated soil was different from zero. Differences between mycorrhizal and non-mycorrhizal treatments in N:P ratios of aboveground nutrient concentrations were assessed with a two sample *t*-test for each soil.

Correlations between two variables were assessed using Pearson's correlation.

All figures and tables presented show estimates of the means with their standard error. Two DNA samples for qPCR were below the detection limit of 9.6e + 09 copies/μl. Due to the given detection limit, we cannot state that these samples were free of *R. irregulare* DNA; thus, we assigned them a value of 1% of the detection limit. One sample (soil A-I) was not quantifiable because of PCR-inhibitors and was excluded from the analysis. One microcosm (soil B-C) was discarded from biomass and qPCR analysis as only 4 out of 12 *Lolium* plants survived in the course of the experiment despite repeated replanting.

RESULTS

Mycorrhizal colonization

Total root colonization of *Lolium* was enhanced by *R. irregulare* inoculation in all soil types while root colonization of *Trifolium* was enhanced in four out of eight soil types (Fig. 1 (b), Table 2). Effects of inoculation on *Trifolium* root colonization depended on soil type as indicated by a significant 'soil type' x 'mycorrhizal inoculation' interaction ($F_{6,\ 63} = 4.06$, $p = 0.0017$). The effect of inoculation on root colonization of *Lolium* was superior over the effect of soil identity (Table 2). Such a hierarchy could not be detected for *Trifolium* root colonization.

The change in total *Trifolium* root colonization upon inoculation was significant for soil B (total: $t_5 = 7.07$, $p = 0.0009$), C (total: $t_5 = 8.97$, $p = 0.0003$), D (total: $t_5 = 3.78$, $p = 0.013$) and control soil H (total: $t_5 = 28.68$, $p < 0.0001$, supporting information Table S4). The inoculation driven change in total *Lolium* root colonization was significant for all soils (Fig. 1(b)). Total colonization was enhanced by up to 166% in *Trifolium* roots (soil C) and up to 232% in *Lolium* roots (soil C) (soil H: 4217% *Trifolium*, 1194% *Lolium*). In soil H, no arbuscules were detected without inoculation.

R. irregulare was present in all field soils and in soil H before inoculation, as detected with qPCR. nLSU copy number in the uninoculated treatments varied between soils with soil A having the highest amount of *R. irregulare* and the control soil H (followed by soil C) with the lowest (supporting information Table S2). nLSU copy number increased in all soils after inoculation (Fig. 2), but the response depended on soil type ($F_{6,\ 27} = 29.91$, $p < 0.0001$). The highest increase in *R. irregulare* nLSU copy number was observed in the control soil H (1e + 4.26 more nLSU copies), followed by soil C (1e + 2.32). Total root length colonized was positively correlated with *R. irregulare*

Table 2. Results for the mixed effect models using soil type and inoculation treatment as well as their interaction as fixed effect and block as random effect

		Soil			Inoculation			Inoculation:Soil		
Source of variation		d.f.	F		d.f.	F		d.f.	F	
Biomass (g)										
Trifolium		6, 62	5.76	***	1, 62	37.92	***	6, 62	3.17	**
Lolium		6, 62	32.50	***	1, 62	27.03	***	6, 62	4.34	**
Total biomass		6, 62	19.67	***	1, 62	0.89		6, 62	3.23	**
Roots		6, 62	3.99	**	1, 62	2.18		6, 62	0.65	
Nutrient content (mg)										
N[v]		6, 62	61.55	***	1, 62	14.53	***	6, 62	1.76	
P		6, 62	62.00	***	1, 62	0.07		6, 62	0.70	
Nutrient concentration (mg/g)										
N[v]		6, 62	13.21	***	1, 62	8.01	**	6, 62	0.62	
P		6, 62	19.76	***	1, 62	8.39	**	6, 62	2.41	*
AMF colonization (%)										
Arbuscular	*Trifolium*	6, 63	9.52	***	1, 63	9.11	**	6, 63	1.63	
Arbuscular[v]	*Lolium*	6, 63	2.96	*	1, 63	11.96	**	6, 63	1.94	
Total	*Trifolium*	6, 63	16.39	***	1, 63	15.14	***	6, 63	4.06	**
Total	*Lolium*	6, 63	9.26	***	1, 63	113.14	***	6, 63	1.73	
log10 (nLSU)		6, 61	0.21		1, 61	70.71	***	6, 61	2.31	*

AMF, arbuscular mycorrhizal fungi; nLSU, nuclear large ribosomal subunit. All analyses excluded the control soil H (analyses including H see supporting information Table S7). Asterisks indicate significance levels.

*$p < 0.05$;

**$p < 0.01$;

***$p < 0.001$.

[v]Because of heterogeneity in the variance structure the varIdent() function was used.

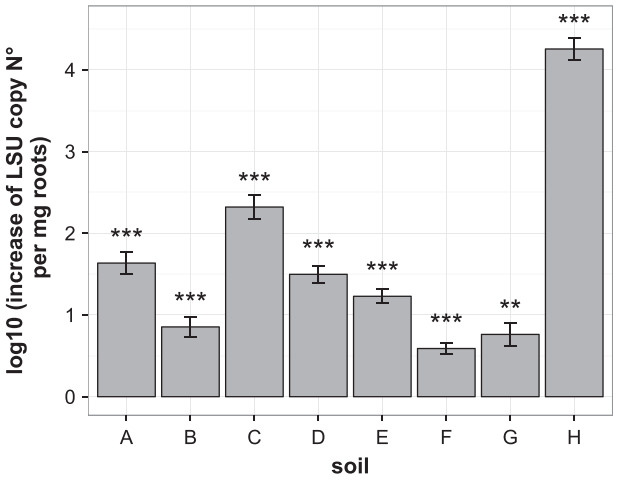

Figure 2. Increase of intraradical *R. irregulare* nuclear large ribosomal subunit (nLSU) copy number (log10) upon inoculation with *R. irregulare* for eight different unsterile field soils (A-H). nLSU copy number was determined by *R. irregulare* specific qPCR. According to qPCR, results *R. irregulare* was naturally present in all soils (10^{12}–10^{15} nLSU copies, supporting information Table S2). Bars depict means ± standard error of the mean of 6 replicates (soil A $N = 5$). Asterisks indicate that the change in LSU copy number due to inoculation is significantly different from zero ($p < 0.01$**, $p < 0.001$***) according to a one-sample *t*-test.

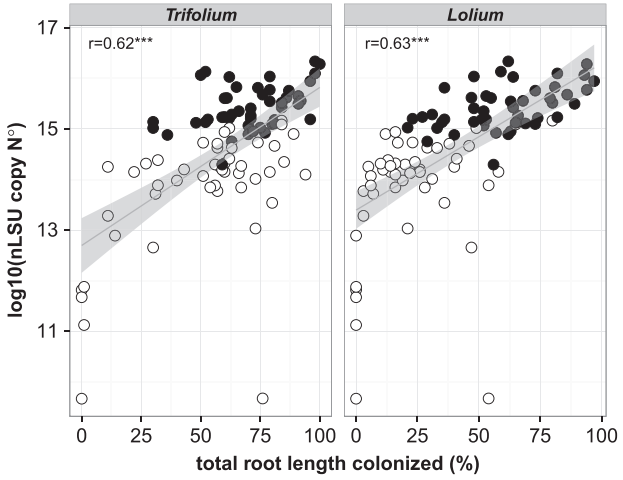

Figure 3. Relation between total root length colonized per plant species and nuclear large ribosomal subunit (nLSU) copy number of *R. irregulare* in the roots ($N = 94$) for inoculated (black) and uninoculated soils (white). All soils were included in the regression analysis (without control soil H: *Lolium* $r = 0.50$***, *Trifolium* $r = 0.31$**). Grey shades visualize the confidence interval. When correlations were performed for each inoculation treatment separately, total root length colonized was still positively correlated with nLSU copy number of *R.irregulare* (*Lolium*: uninoculated $r = 0.31$*, inoculated $r = 0.47$***, *Trifolium*: uninoculated $r = 0.55$***, inoculated $r = 0.41$**). Visualization by soil type is shown in the supporting information (Fig. S2). ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***).

nLSUcopy number (Fig. 3, *Lolium*: $t_{92} = 7.70$, $r = 0.63$, $p < 0.0001$; *Trifolium*: $t_{92} = 7.55$, $r = 0.62$, $p < 0.0001$, supporting information Table S5).

Biomass production

Trifolium

Trifolium biomass generally increased with AMF inoculation, the effect varying with soil type (significant 'soil type' x 'mycorrhizal inoculation' interaction: $F_{6,\ 62} = 3.17$, $p = 0.0089$, Table 2). The growth response to the inoculation (MGR) was significantly influenced by the soil type, regardless of whether the control (H) was included or not (without H: $F_{6,\ 28} = 9.07$, $p < 0.0001$, Table 3).

A significant increase in *Trifolium* biomass following inoculation was observed in five out of eight soils (Fig. 1(a)). A significant growth increase was observed for soil B ($t_5 = 7.64$, $p = 0.0006$), soil C ($t_5 = 4.00$, $p = 0.01$), soil D ($t_5 = 10.98$, $p = 0.0001$), soil G ($t_5 = 6.43$, $p = 0.0013$) and the control soil H ($t_5 = 250.78$, $p < 0.0001$). The control soil (H) yielded the highest biomass increase (1477%). In the other responsive soils, *Trifolium* biomass increased in the range of 33% to 51%, equivalent to an additional yield of 1240 to 2215 kg *Trifolium* shoots per hectare.

Lolium

Lolium biomass was decreased by AMF inoculation in all soils, with the effect size of the inoculation depending on the soil type (significant 'soil type' x 'mycorrhizal inoculation' interaction: $F_{6,\ 62} = 4.34$, $p = 0.001$, Fig. 1(a), Table 2). The growth response to inoculation (MGR) was significantly affected by the soil type, regardless of whether the control (H) was included or not (without H: $F_{6,\ 28} = 6.21$, $p = 0.0003$, Table 3). Three soils showed a statistically significant growth reduction of *Lolium*. These soils were B ($t_5 = -7.23$, $p = 0.0008$), E ($t_5 = -3.39$, $p = 0.02$) and the control H ($t_5 = -28.83$, $p < 0.0001$). Biomass reduction corresponded to 371 to 607 kg per hectare (soils B and E).

Correlation analysis revealed that the change in root colonization by inoculation explained 59% of the change in aboveground *Trifolium* biomass (MGR), when all soils (including soil H) were included in the correlation ($t_{46} = 8.32$, $p < 0.0001$, $r = 0.77$, Table S6). When the control soil H was excluded, the correlation was still significant but only explained 10% of the variation in *Trifolium* biomass ($t_{40} = 2.15$, $p = 0.016$, $r = 0.32$). The increase in nLSU copy number after inoculation did not explain any variation in MGR of *Trifolium* (excluding H: $t_{39} = 0.25$, $p = 0.8$, $r = 0.04$). The decrease in *Lolium* biomass correlated with the increase in arbuscules in *Lolium* roots ($t_{40} = -2.09$, $p = 0.043$, $r = -0.31$). Correlation analysis (using the means per treatment) revealed that the MGR of both species to inoculation could not be explained by soil pH (MGR *Trifolium*: $t_6 = 1.48$, $r = 0.52$, $p = 0.19$; MGR *Lolium*: $t_6 = -1.10$, $r = -0.41$, $p = 0.31$) or soil phosphorus concentration (MGR *Trifolium*: $t_6 = 1.21$, $r = 0.44$, $p = 0.27$, MGR *Lolium*: $t_6 = -0.44$, $r = -0.18$, $p = 0.68$). *Lolium* growth response was partly explained by initial ammonium content in the soil ($t_6 = 3.47$, $r = 0.82$, $p = 0.013$), but this correlation has to be interpreted with caution as only means were used ($N = 8$).

Table 3. Assessment of the effect sizes with mixed effect models using soil type as fixed effect and block as random effect

		Including soil H			Excluding soil H		
Source of variation		d.f.	F		d.f.	F	
MGR biomass							
Trifolium		7, 33	33.51	***	6, 28	9.07	***
Lolium		7, 33	4.66	**	6, 28	6.21	***
Total biomass		7, 33	7.35	***	6, 28	9.03	***
Roots		7, 33	1.24		6, 28	1.25	
MGR nutrient content							
N		7, 33	4.60	**	6, 28	5.61	***
P		7, 33	2.11		6, 28	2.63	*
MGR nutrient concentration							
N		7, 33	3.18	*	6, 28	2.29	
P		7, 33	5.60	***	6, 28	6.76	***
Delta AMF colonization							
Arbuscular	*Trifolium*	7, 33	28.37	***	6, 28	4.98	**
Total		7, 33	56.04	***	6, 28	9.75	***
Arbuscular[v]	*Lolium*	7, 33	4.23	**	6, 28	3.30	*
Total		7, 33	11.80	***	6, 27	3.26	*
Delta log10 (nLSU)		7, 32	132.11	***	6, 28	29.91	***

AMF, arbuscular mycorrhizal fungi; MGR, mycorrhizal growth response; nLSU, nuclear large ribosomal subunit. Analyses were conducted with and without the control soil H. Asterisks indicate significance levels.
*$p < 0.05$;
**$p < 0.01$;
***$p < 0.001$.
[v]Because of heterogeneity in the variance structure the varIdent() function was used.

Total biomass

The effect of AMF inoculation on total plant biomass (*Trifolium* and *Lolium*) depended on soil type, as revealed by a significant 'soil type' x 'mycorrhizal inoculation' interaction term ($F_{6,\ 62} = 3.23$, $p = 0.0079$, Table 2). Upon inoculation, total biomass of the microcosms increased significantly in soil G ($t_5 = 2.64$, $p = 0.046$, Table S4) and in the control soil H ($t_5 = 5.33$, $p = 0.0031$), and it decreased in soil E ($t_5 = -2.98$, $p = 0.031$). The biomass increase in soil G and H corresponded to 265 and 916 kg more biomass per hectare compared with uninoculated soils, which had a biomass production of 9969 and 13 042 kg*ha^{-1}.

Plant nutrient content

Plant nitrogen and phosphorus content was influenced by soil type (N: $F_{6,\ 62} = 61.55$, $p < 0.0001$, P: $F_{6,\ 62} = 62.00$, $p < 0.0001$, Table 2). AMF inoculation only affected N content ($F_{1,\ 62} = 14.53$, $p = 0.0003$), while no effect on P content was found ($F_{1,\ 62} = 0.07$, $p = 0.8$). N content was significantly increased by inoculation in soil D ($t_5 = 5.50$, $p = 0.0027$; supporting information Figure S1). All plants, except those growing in soil H, had N:P ratios below 14, indicating that all soils were N limited (Figure S1, Koerselman & Meuleman 1996).

DISCUSSION

Our study showed that (1) *R. irregulare* can successfully establish in a wide range of soil types and compete effectively with other AMF to colonize plant roots; (2) clover yield in field soil can be enhanced by AMF inoculation, even when the soil P availability and initial inoculum potential are very high; and (3) effects of AMF inoculation on plant productivity depend on soil type.

R. Irregulare can be successfully introduced and established in a wide range of soil types

R. irregulare is known to be globally distributed (Öpik *et al.* 2006) and well adapted to intensive agricultural practices (Oehl *et al.* 2004). The presence of this fungus in all our uninoculated soils, as demonstrated by qPCR, confirms its general abundance in agricultural soils in Switzerland. This ubiquitous occurrence indicates that *R. irregulare* is compatible with a wide range of soil conditions varying in pH (5.6 to 8.0), P availability (0.3 to 18.8 mg/kg CO_2-extracted P), sand content (17.5% to 57.0%), and humus content (1.0% to 10.5%). This compatibility with the environment (soil conditions and host plant) is a crucial factor determining successful establishment of an AMF inoculant and an important characteristic for commercial application (Verbruggen *et al.* 2013).

Successful establishment of the *R. irregulare* strain in this study was shown by a significant increase in nLSU abundance after inoculation in all eight soils (Fig. 2). This indicates that the investigated *R. irregulare* can be considered a favourable inoculant for a wide range of soils. Moreover, the tested soils were all non-sterile and contained a native AMF community as evidenced by total root colonization levels between 2%

and 79% in the non-inoculated soils (Fig. 1(c)). The fact that the abundance of *R. irregulare* increased upon inoculations confirms other studies that it can successfully establish when being introduced in an existing AMF community (Alkan *et al.* 2006, Janoušková *et al.* 2013). While these other studies focused on one single-field site or a specific soil substrate, the results from this study are valid for a much broader range of conditions. The ability of *R. intraradices* to quickly colonize the host (Hepper *et al.* 1988, Jansa *et al.* 2008) might have enabled the fungus to colonize unoccupied niches sooner than competing indigenous AMF species. As a result, the addition of *R. irregulare* caused a shift in AMF community structure because in all treatments the nLSU copy number was significantly increased by inoculation, while in several soil types, root colonization was not enhanced.

Plant responses to AMF inoculation

Arbuscular mycorrhizal fungi inoculation significantly enhanced clover biomass in five out of eight soils tested (averaged across all sites inoculation resulted in a growth increase of 41%, Fig. 1 (a)), confirming other studies that clover is responsive to AMF (Drew *et al.* 2003, Köhl *et al.* 2014, van der Heijden *et al.* 2015). This study now also shows that AMF inoculation in a range of field soils can enhance clover biomass even if a resident AMF community is already present. In contrast to *Trifolium*, the biomass of the grass *Lolium* declined upon inoculation in all soils (on average with 14%, Fig. 1(a)), confirming other studies that *Lolium* is an unresponsive species or is even suppressed by AMF (Köhl *et al.* 2014, Tawaraya 2003). Our observation that the grass (*Lolium*) and the legume (*Trifolium*) responded differently to inoculation confirms results from Hoeksema *et al.* (2010) that plant functional group is an important determinant in predicting the plant growth responses to inoculation.

Although, both *Lolium* and *Trifolium* were influenced by AMF inoculation, the total biomass was not influenced by AMF inoculation (on average 3% biomass increase). Similarly, AMF did not improve the nutritional status of the grassland mixture. Instead, AMF reduced the competitive inequality between the two plant species by reducing the growth suppression of the AMF responsive legume by the non-responsive grass (Hall 1978, Hartnett *et al.* 1993, Wagg *et al.* 2011b). The direct effect of mycorrhizal colonization on the biomass of the individual species as observed in monocultures (Veiga *et al.* 2011, Wagg *et al.* 2011b) is thus additionally influenced by competitive interactions in mixture. Although, the average net biomass of the grassland communities did not increase significantly, the composition of the aboveground biomass shifted towards a better forage quality (Dewhurst *et al.* 2009) with more biomass of the nitrogen fixing legume.

Colonization response to inoculation

Inoculation with *R. irregulare* increased root colonization by 39% in clover and by 163% in the grass (Fig. 1(b)). This is in agreement with meta-analyses of various inoculation trials (Lekberg & Koide 2005, McGonigle 1988) that showed that inoculation usually enhances root colonization by AMF. This also indicates that the carrying capacity for successful establishment was not reached in the investigated field soils, despite high P availability and high AMF abundance at some of the investigated sites.

Interestingly, qPCR revealed that *R. irregulare* abundance in the roots was successfully increased by inoculation in all soils regardless of the initial inoculum potential (colonization in uninoculated treatments). However, this did not necessarily lead to an enhancement in clover biomass. Root colonization was a much better predictor of plant production than *R. irregulare* abundance (nLSU copy number) in this study (Table S6), although root colonization correlated well with nLSU copy number (Fig. 3). Discrepancies between DNA quantification and staining as measures of fungal biomass have been reported before (Gamper *et al.* 2008, Jansa *et al.* 2008, Pivato *et al.* 2007). We assume in case of *R. irregulare* that intraradically produced spores contribute much more to the pool of DNA than intraradical hyphae (Gamper *et al.* 2008). Furthermore, vital hyphae can be devoid of nuclei leading to a considerable heterogeneity in nuclear distribution (Gamper *et al.* 2008). It is important to stress that the qPCR assay in this study only focused on *R. irregulare*, while root colonization encompasses all fungal structures, regardless of fungal species identity. With this in consideration, it is not surprising that root colonization is a better predictor of plant growth response in this study. Thus, a change in *R. irregulare* abundance does not necessarily lead to an altered overall root colonization, but does indicate a substitution of AMF species within the mycorrhizal community (Gazey *et al.* 2004, Janoušková *et al.* 2013). An altered mycorrhizal community will consequently affect the plant response, as the identity of the fungi colonizing a plant root is important because AM fungi vary in their ability to provide nutrients to plants (Ravnskov & Jakobsen 1995; Smith *et al.* 2000) and plant species respond differently to different AM fungal species (Newsham *et al.* 1995; Streitwolf-Engel *et al.* 2001; Scheublin *et al.* 2007).

Biotic and abiotic soil factors are determining the inoculum response

Generally, it has been observed that the importance of AMF for plants is inversely related to P availability (Marschner & Dell 1994, Stribley *et al.* 1980, Treseder 2004). Our study did not confirm this. Inoculation success (in terms of biomass stimulation and establishment success of *R. irregulare*) depended on the field soil selected. As expected, the control soil H with the lowest plant available P and low initial AMF abundance showed the strongest biomass reaction to additional AMF. However, even though plant available P was highest in soil C, clover nevertheless showed a positive biomass response in this soil upon AMF inoculation (Figure 1). Furthermore, clover did not respond to inoculation in soil E, a soil with a moderate P availability, which would be favourable for inoculation. As soil nutrient status could not predict mycorrhizal inoculation success in this study, we assume that other factors such as initial AMF abundance and AMF community composition influence establishment success.

For successful application, it is necessary to develop a mechanistic model that can predict under which conditions and for which crops application is feasible and commercially attractive. In this sense, it is important to mention that the amount of inoculum we added to the pots was large (corresponding to 1.4×10^5 L/ha). Adding such a large amount is expensive and possibly unrealistic for successful commercial application, despite of substantial progress which has been made in inoculum production over the last years (de Santana *et al*. 2014, IJdo *et al*. 2011, Jolicoeur *et al.* 1999). Other inoculation techniques that use smaller amounts of inoculum (e.g. seed coating or pre-inoculation of seedlings) are likely to be more promising (Vosátka *et al.* 2012).

CONCLUSIONS

Unlike former observations that AMF are not beneficial in agricultural fields (Ryan & Graham 2002, Ryan & Kirkegaard 2012), our results demonstrate that AMF inoculation in field soils can enhance growth of clover irrespective of initial soil P availability and AMF abundance. We have also shown that our tested AMF strain can successfully establish in a wide range of soils with highly variable chemical characteristics suggesting that it has a broad niche and is able to compete successfully with indigenous AMF.

ACKNOWLEDGMENTS

This work has been sponsored by grant 125428 and 143097 of the Swiss National Science Foundation (SNSF). We thank Beat Boller for the provision of seeds, Hans Jörg Bachmann, Hansruedi Bosshard, Brigitte Weiss and Erika Hinnen for help with plant nutrient analysis and Franco Widmer and Jan Jansa for molecular advice. Special thanks to Urs Zihlmann and Fritz Oehl for helping to choose the field sites. We further thank all farmers who provided their field soil, Kyle Hartmann for English-language editing and the editor and two anonymous reviewers for valuable comments on the manuscript. We have no conflict of interest to declare.

REFERENCES

Abbott L. & Robson A. (1982) Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils. *Australian Journal of Agricultural Research* **33**, 1049–1059.

Alkan N., Gadkar V., Yarden O. & Kapulnik Y. (2006) Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, *Glomus mosseae* and *G. intraradices*, by real-time PCR. *Applied and Environmental Microbiology* **72**, 4192–4199.

Altieri M.A. (1999) The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems and Environment* **74**, 19–31.

Ames R.N., Mihara K.L. & Bethlenfalvay G.J. (1987) The establishment of microorganisms in vesicular–arbuscular mycorrhizal and control treatments. *Biology and Fertility of Soils* **3**, 217–223.

Azcón-Aguilar C. & Barea J.M. (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* **6**, 457–464.

Azcón-Aguilar C. & Barea J.M. (1997) Applying mycorrhiza biotechnology to horticulture: significance and potentials. *Scientia Horticulturae* **68**, 1–24.

Barrios E. (2007) Soil biota, ecosystem services and land productivity. *Ecological Economics* **64**, 269–285.

Bender S.F., Plantenga F., Neftel A., Jocher M., Oberholzer H.-R., Köhl L., … van der Heijden M.G.A. (2014) Symbiotic relationships between soil fungi and plants reduce N_2O emissions from soil. *ISME Journal* **8**, 1336–1345.

Bender S.F. & van der Heijden M.G.A. (2015) Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology* **52**, 228–239.

Berg G. (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* **84**, 11–18.

Boller B., Schubiger F. & Tanner P. (2002) Oryx und Rangifer, neue Sorten von Italienischem Raigras. *AGRARForschung* **9**, 260–265.

Bothe H. (2012) Arbuscular mycorrhiza and salt tolerance of plants. *Symbiosis* **58**, 7–16.

Cavagnaro T., Jackson L., Six J., Ferris H., Goyal S., Asami D., … Scow K. (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant and Soil* **282**, 209–225.

Ceballos I., Ruiz M., Fernández C., Peña R., Rodríguez A. & Sanders I.R. (2013) The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. *PLoS ONE* **8**, e70633.

Ceccarelli N., Curadi M., Martelloni L., Sbrana C., Picciarelli P. & Giovannetti M. (2010) Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. *Plant and Soil* **335**, 311–323.

Dewhurst R.J., Delaby L., Moloney A., Boland T. & Lewis E. (2009) Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research* **48**, 167–187.

Drew E.A., Murray R.S., Smith S.E. & Jakobsen I. (2003) Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. *Plant and Soil* **251**, 105–114.

Farmer M.J., Li X., Feng G., Zhao B., Chatagnier O., Gianinazzi S., … van Tuinen D. (2007) Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China. *Applied Soil Ecology* **35**, 599–609.

Faye A., Dalpe Y., Ndung'u Magiori K., Jefwa J., Ndoye I., Diouf M., … Lesueur D. (2013) Evaluation of commercial arbuscular mycorrhizal inoculants. *Canadian Journal of Plant Science* **93**, 1201–1208.

Forschungsanstalt Agroscope Reckenholz-Tänikon ART & Forschungsanstalt Agroscope Changins-Wädenswil ACW (1996) *Schweizerische Referenzmethoden der Forschungsanstalten Agroscope. Band 1 Boden- und Substratuntersuchungen zur Düngeberatung (Acker-, Futter-, Obst-, Wein- und Gartenbau). Änderungen 1997–2012* (vol. Band 1). Forschungsanstalten ART, ACW, Zürich.

Frick R., Jeangros B., Demenga M., Suter D. & Hirschi H. (2008) Red clover variety tests. *Revue suisse d'agriculture* **40**, 245–248.

Galli U., Schüepp H. & Brunold C. (1994) Heavy metal binding by mycorrhizal fungi. *Physiologia Plantarum* **92**, 364–368.

Gamper H.A., Young J.P.W., Jones D.L. & Hodge A. (2008) Real-time PCR and microscopy: are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? *Fungal Genetics and Biology* **45**, 581–596.

Gazey C., Abbott L.K. & Robson A.D. (2004) Indigenous and introduced arbuscular mycorrhizal fungi contribute to plant growth in two agricultural soils from south-western Australia. *Mycorrhiza* **14**, 355–362.

Griess P. (1879) Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt "Ueber einige Azoverbindungen". *Berichte der Deutschen Chemischen Gesellschaft* **12**, 426–428.

Hall I.R. (1978) Effects of endomycorrhizas on the competitive ability of white clover. *New Zealand Journal of Agricultural Research* **21**, 509–515.

Hartnett D.C., Hetrick B.A.D., Wilson G.W.T. & Gibson D.J. (1993) Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *Journal of Ecology* **81**, 787–795.

van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., … Sanders I.R. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**, 69–72.

van der Heijden, M.G.A., de Bruin, S., Luckerhoff, L., van Logtestijn, R.S.P. & Schlaeppi, K. (2015) A widespread plant–fungal–bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME Journal* in press. doi: 10.1038/ismej.2015.120.

Helgason T., Daniell T.J., Husband R., Fitter A.H. & Young J.P.W. (1998) Ploughing up the wood-wide web? *Nature* **394**, 431–431.

Hepper C.M., Azcón-Aguilar C., Rosendahl S. & Sen R. (1988) Competition between three species of *Glomus* used as spatially separated introduced and indigenous mycorrhizal inocula for leek (*Allium porrum* L.). *New Phytologist* **110**, 207–215.

Hoeksema J.D., Chaudhary V.B., Gehring C.A., Johnson N.C., Karst J., Koide R. T., … Umbanhowar J. (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**, 394–407.

IJdo M., Cranenbrouck S. & Declerck S. (2011) Methods for large-scale production of AM fungi: past, present, and future. *Mycorrhiza* **21**, 1–16.

IUSS Working Group WRB (2006) *World reference base for soil resources 2006*. World Soil Resources Reports No. 103., FAO, Rome.

Janoušková M., Krak K., Wagg C., Štorchová H., Caklová P. & Vosátka M. (2013) Effects of inocula additions in presence of a pre-established arbuscular mycorrhizal fungal community. *Applied and Environmental Microbiology* **79**, 6507–6515.

Jansa J., Mozafar A., Kuhn G., Anken T., Ruh R., Sanders I.R., … Frossard E. (2003) Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* **13**, 1164–1176.

Jansa J., Oberholzer H.R. & Egli S. (2009) Environmental determinants of the arbuscular mycorrhizal fungal infectivity of Swiss agricultural soils. *European Journal of Soil Biology* **45**, 400–408.

Jansa J., Smith F.A. & Smith S.E. (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist* **177**, 779–789.

Jansa J., Wiemken A. & Frossard E. (2006) The effects of agricultural practices on arbuscular mycorrhizal fungi. *Geological Society, London, Special Publications* **266**, 89–115.

Johnson N.C. (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* **3**, 749–757.

Jolicoeur M., Williams R.D., Chavarie C., Fortin J.A. & Archambault J. (1999) Production of *Glomus intraradices* propagules, an arbuscular mycorrhizal fungus, in an airlift bioreactor. *Biotechnology and Bioengineering* **63**, 224–232.

Koerselman W. & Meuleman A.F.M. (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* **33**, 1441–1450.

Köhl L., Oehl F. & van der Heijden M.G.A. (2014) Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. *Ecological Applications* **24**, 1842–1853.

Koide R.T. & Peoples M.S. (2012) On the nature of temporary yield loss in maize following canola. *Plant and Soil* **360**, 259–269.

Krom M.D. (1980) Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. *Analyst* **105**, 305–316.

Langley J.A., Johnson N. & Koch G. (2005) Mycorrhizal status influences the rate but not the temperature sensitivity of soil respiration. *Plant and Soil* **277**, 335–344.

Leifheit E.F., Veresoglou S.D., Lehmann A., Morris E.K. & Rillig M.C. (2014) Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation—a meta-analysis. *Plant and Soil* **374**, 523–537.

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

Maherali H. & Klironomos J.N. (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* **316**, 1746–1748.

Marschner H. & Dell B. (1994) Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* **159**, 89–102.

McGonigle T.P. (1988) A numerical analysis of published field trials with vesicular–arbuscular mycorrhizal fungi. *Functional Ecology* **2**, 473–478.

Newsham K.K., Fitter A.H. & Watkinson A.R. (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution* **10**, 407–411.

Nyfeler D. (2009) *Productivity and nitrogen utilisation in productive agricultural grassland: effects of species combinations, species proportions and nitrogen fertilisation*. Doctoral thesis, ETH.

Oehl F., Laczko E., Bogenrieder A., Stahr K., Bösch R., van der Heijden M., … Sieverding E. (2010) Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry* **42**, 724–738.

Oehl F., Sieverding E., Mäder P., Dubois D., Ineichen K., Boller T., … Wiemken A. (2004) Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia* **138**, 574–583.

Olsson P.A., Thingstrup I., Jakobsen I. & Baath E. (1999) Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. *Soil Biology and Biochemistry* **31**, 1879–1887.

Öpik M., Moora M., Liira J. & Zobel M. (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* **94**, 778–790.

Pellegrino E., Bedini S., Avio L., Bonari E. & Giovannetti M. (2011) Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. *Soil Biology and Biochemistry* **43**, 367–376.

Pellegrino E., Turrini A., Gamper H.A., Cafà G., Bonari E., Young J.P.W. & Giovannetti M. (2012) Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. *New Phytologist* **194**, 810–822.

Pinheiro J.C. & Bates D.M. (2000) *Mixed-effects Models in S and S-PLUS*. Springer-Verlag, New York.

Pivato B., Mazurier S., Lemanceau P., Siblot S., Berta G., Mougel C., … Van Tuinen D. (2007) *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**, 197–210.

Ravnskov S. & Jakobsen I. (1995) Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. *New Phytologist* **129**, 611–618.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ryan M.H. & Graham J.H. (2002) Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant and Soil* **244**, 263–271.

Ryan M.H. & Kirkegaard J.A. (2012) The agronomic relevance of arbuscular mycorrhizas in the fertility of Australian extensive cropping systems. *Agriculture, Ecosystems & Environment* **163**, 37–53.

Säle V., Aguilera P., Laczko E., Mäder P., Berner A., Zihlmann U., … Oehl F. (2015) Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* **84**, 38–52.

de Santana A.S., Cavalcante U.M.T., de Barreto Sampaio E.V. & Maia L.C. (2014) Production, storage and costs of inoculum of arbuscular mycorrhizal fungi (AMF). *Brazilian Journal of Botany* **37**, 159–165.

Scheublin T.R., van Logtestijn R.S.P. & van der Heijden M.G.A. (2007) Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *Journal of Ecology* **95**, 631–638.

Scullion J., Eason W.R. & Scott E.P. (1998) The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass-arable rotations. *Plant and Soil* **204**, 243–254.

Sieverding E. (1991) *Vesicular-arbuscular mycorrhiza management in tropical agrosystems* (vol. 224). Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany.

Sieverding E., da Silva G.A., Berndt R. & Oehl F. (2014) *Rhizoglomus*, a new genus of the *Glomeraceae*. *Mycotaxon* **129**, 373–386.

Smith F.A., Jakobsen I. & Smith S.E. (2000) Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytologist* **147**, 357–366.

Smith S.E. & Read D. (2008) *Mycorrhizal Symbiosis* (3rd edn), Academic Press, Elsevier Ltd., Oxford.

van Staden J.F. (1982) Automated simultaneous determination of nitrate and nitrite by pre-valve reduction of nitrate in a flow-injection system. *Analytica Chimica Acta* **138**, 403–408.

Streitwolf-Engel R., Heijden M.G.A.v.d., Wiemken A. & Sanders I.R. (2001) The ecological significance of arbuscular mycorrhizal fungal effects on clonal reproduction in plants. *Ecology* **82**, 2846–2859.

Stribley D.P., Tinker P.B. & Rayner J.H. (1980) Relation of internal phosphorus concentration and plant weight in plants infected by vesicular–arbuscular mycorrhizas. *New Phytologist* **86**, 261–266.

Sýkorová Z., Börstler B., Zvolenská S., Fehrer J., Gryndler M., Vosátka M., … Redecker D. (2012) Long-term tracing of *Rhizophagus irregularis* isolate BEG140 inoculated on *Phalaris arundinacea* in a coal mine spoil bank, using mitochondrial large subunit rDNA markers. *Mycorrhiza* **22**, 69–80.

Sýkorová Z., Wiemken A. & Redecker D. (2007) Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology* **73**, 5426–5434.

Tawaraya K. (2003) Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition* **49**, 655–668.

Thonar C., Erb A. & Jansa J. (2012) Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* **12**, 219–232.

Treseder K.K. (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist* **164**, 347–355.

Veiga R.S.L., Jansa J., Frossard E. & van der Heijden M.G.A. (2011) Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? *PLoS ONE* **6**, e27825.

Verbruggen E., Röling W.F.M., Gamper H.A., Kowalchuk G.A., Verhoef H.A. & van der Heijden M.G.A. (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist* **186**, 968–979.

Verbruggen E., van der Heijden M.G.A., Rillig M.C. & Kiers E.T. (2013) Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist* **197**, 1104–1109.

Vessey J.K. (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255**, 571–586.

Vosátka M., Látr A., Gianinazzi S. & Albrechtová J. (2012) Development of arbuscular mycorrhizal biotechnology and industry: current achievements and bottlenecks. *Symbiosis* **58**, 29–37.

Wagg C., Bender S.F., Widmer F. & van der Heijden M.G.A. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences* **111**, 5266–5270.

Wagg C., Jansa J., Schmid B. & van der Heijden M.G.A. (2011a) Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecology Letters* **14**, 1001–1009.

Wagg C., Jansa J., Stadler M., Schmid B. & van der Heijden M. (2011b) Mycorrhizal fungal identity and diversity relaxes plant–plant competition. *Ecology* **92**, 1303–1313.

Wamberg C., Christensen S., Jakobsen I., Müller A.K. & Sørensen S.J. (2003) The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). *Soil Biology and Biochemistry* **35**, 1349–1357.

Received 15 April 2015; received in revised form 17 June 2015; accepted for publication 23 June 2015

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Shoot N and P content (mixture of *Lolium* and *Trifolium*) for eight different field soils inoculated with *R. irregulare* (black) or left uninoculated (grey). Additionally, N:P ratios of shoot nutrient concentrations are shown. N:P ratios below 14 indicate N-limitation whereas N:P ratios above 16 imply P-limitation (Koerselman & Meuleman, 1996). Depicted are means ± SEM of 6 replicates. Asterisks indicate significant differences between the inoculated and not inoculated field soil according to a two-sample t-test (*p<0.05,**p<0.01).

Figure S2. Relation between total root length colonized per plant species and the number of copies of the nLSU for *R. irregulare* in the roots (N=94). All soils were included in the regression analysis (without control soil H: *Trifolium* r=0.31**, *Lolium* r=0.50***). Grey shades visualize the confidence interval. (p<0.05 *, p<0.01 **, p<0.001 ***).

Table S1. Means and SEMs (6 replicates) of the different above and belowground biomasses (in g per individual plant) for 8 soil types (A-H) and two inoculation treatments C=control, I=mycorrhizal inoculation).

Table S2. Means and SEMs (6 replicates) of the total root length colonized and colonized by arbuscules as well as the copy number of nLSU (presented as log10) specific for *R. irregulare* for 8 soil types (A-H) and two inoculation treatments C=control, I=mycorrhizal inoculation).

Table S3. Means and SEMs (6 replicates) of the aboveground nutrient analysis and N:P concentration ratio for 8 soil types (A-H) and two inoculation treatments C=control, I=mycorrhizal inoculation).

Table S4. t-values of one sample t-tests with the effect size of biomass and mycorrhizal parameters for each of the eight different field soils. Degrees of freedom were 5 except for the change in nLSU copy numbers for soil A (df=4).

Table S5. Results of the correlation between mycorrhizal root length colonized (in %, arbuscular and total) and log10 of the nLSU copy number of R. *irregulare* per mg root. Significance levels are indicated as asterisks (p<0.05 *, p<0.01 **, p<0.001 ***).

Table S6. Correlations between the plant response to mycorrhizal inoculation (expressed as MGR) and the AMF response to inoculation (expressed as difference between inoculated and uninoculated soils). Analyses were done with and without control soil H and were performed for *Trifolium* and *Lolium* and with total root colonization and root colonization with arbusculaes. Significance levels are indicated by asterisks (p<0.05 *, p<0.01 **, p<0.001 ***).

Table S7. Results for the mixed effect models using soil type (eight levels: A-H) and inoculation treatment as well as their interaction as fixed effect and block as random effect. Asterisks indicate significance levels (p<0.05 *, p<0.01 **, p<0.001 ***). One microcosm was taken out of the analysis except for the plant specific root colonization.