

# Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota

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**Abstract.** It is well established that agricultural practices alter the composition and diversity of soil microbial communities. However, the impact of changing soil microbial communities on the functioning of the agroecosystems is still poorly understood. Earlier work showed that soil tillage drastically altered microbial community composition. Here we tested, using an experimental grassland (*Lolium*, *Trifolium*, *Plantago*) as a model system, whether soil microbial communities from conventionally tilled (CT) and non-tilled (NT) soils have different influences on plant productivity and nutrient acquisition. We specifically focus on arbuscular mycorrhizal fungi (AMF), as they are a group of beneficial soil fungi that can promote plant productivity and ecosystem functioning and are also strongly affected by tillage management.

Soil microbial communities from CT and NT soils varied greatly in their effects on the grassland communities. Communities from CT soil increased overall biomass production more than soil communities from NT soil. This effect was mainly due to a significant growth promotion of *Trifolium* by CT microorganisms. In contrast to CT soil inoculum, NT soil inoculum increased plant phosphorus concentration and total plant P content, demonstrating that the soil microbial communities from NT fields enhance P uptake. Differences in AM fungal community composition resulting, for instance, in twofold greater hyphal length in NT soil communities when compared to CT, are the most likely explanation for the different plant responses to CT and NT soil inocula.

A range of field studies have shown that plant P uptake increases when farmers change to conservation tillage or direct seeding. Our results indicate that this enhanced P uptake results from enhanced hyphal length and an altered AM fungal community. Our results further demonstrate that agricultural management practices indirectly influence ecosystem services and plant community structure through effects on soil biota.

**Key words:** agricultural practice; AMF; arbuscular mycorrhiza; conservation tillage; ecosystem services; extraradical hyphae; Glomeromycota; grassland; soil management; soil microbial communities; tillage.

## INTRODUCTION

The increasing need for more environmentally friendly and sustainable agriculture is driving the search for alternative strategies to reduce the use of fertilizer. One way to enhance agricultural sustainability is by using management practices to manipulate soil microbial communities for increased service provisioning. But thus far, it is an option that seldom has been discussed and much less utilized (Verbruggen and Kiers 2010, Barber et al. 2013). This is surprising, as soil microorganisms facilitate numerous soil-related processes like nutrient uptake, soil aggregate stability, organic matter formation and decomposition, and water regulation, all

of which are of fundamental importance for agroecosystem functioning and plant productivity.

An increasing number of studies demonstrate that agricultural practices, such as tillage regime, fertilization, crop rotation, intercropping, and management type have a significant impact on the diversity, activity, and abundance of soil biota (Altieri 1999, Brussaard et al. 2007, Postma-Blaauw et al. 2010, Verbruggen et al. 2010, Mulder et al. 2011, de Vries et al. 2013). However, until now, the consequences of agriculturally induced shifts in soil communities have rarely been investigated (Corkidi et al. 2002, Verbruggen et al. 2012, Barber et al. 2013).

A farming practice that has a big impact on soil microbial communities is soil tillage (Feng et al. 2003, Mathew et al. 2012). Typically, two contrasting tillage regimes can be considered. Conventional tillage (CT), the most widespread tillage regime, disrupts the upper 20–35 cm of soil and causes a change in the physical and

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chemical soil conditions (Peigné et al. 2007). Consequently, the soil habitat for microorganisms is changed, and thus the microbial communities are altered. In contrast, in no-till farming (NT) the soil is not disturbed as the seeds are inserted directly into the soil without tillage. In order to enhance the sustainability of agricultural systems, no-tillage has recently been promoted to reduce soil erosion and energy use as well as to increase organic matter content (Peigné et al. 2007). Several reports have shown that soil microbial biomass and activity is higher in NT fields (for review see Andrade et al. [2003], Miura et al. [2008]), and the abundance of several functionally important groups of soil organisms, such as earthworms (Jossi et al. 2011, Castellanos-Navarrete et al. 2012) and arbuscular mycorrhizal fungi (AMF; Borie et al. 2006), is enhanced in the absence of soil tillage.

Analogous to the nature of the tillage practice applied, effects on the soil biota differ with soil depth. Negative impacts of tillage in soil layers below the tillage zone can be smaller for some microbes (Miura et al. 2008). For example, mycorrhizal populations change with both soil depth and tillage practice (Douds et al. 1995). Oehl et al. (2005) observed that AMF communities in deeper soil layers vary from communities in the topsoil. As tillage disturbs only the upper 20–35 cm, a compensational effect of AMF in soil layers below the tillage zone could be considered.

In this paper we focus on AMF. These fungi form symbiotic associations with the majority of land plants, including many crops (Smith and Read 2008). AMF facilitate nutrient uptake in return for plant carbon (Smith and Read 2008). Up to 80% of plant P can be derived from AMF (Li et al. 1991), showing that they are important for plant nutrition, especially if soil nutrient availability is low. Mycorrhizal diversity and abundance has been shown to be highly affected by agricultural management such as fertilization, crop sequence, fallow periods (for review see Douds and Millner [1999]), and tillage practices (for review see Kabir [2005], Alguacil et al. [2008]).

Soil tillage is not tolerated by all AMF species equally, and several reports have shown that a number of AMF species are highly sensitive to soil tillage and disappear in tilled fields (Boddington and Dodd 2000, Jansa et al. 2003, Castillo et al. 2006, Yang et al. 2012). As a consequence, tillage-induced changes in AMF communities result in AMF community structures specific to each soil practice and often lead to a reduced mycorrhizal diversity in tilled fields (Boddington and Dodd 2000, Schnoor et al. 2011, Brito et al. 2012, Yang et al. 2012). The soil-tillage-induced shift of the mycorrhizal community structure may have consequences for their functioning, as AMF functional traits differ considerably among and within species (Raju et al. 1990, McGonigle et al. 2003, Smith et al. 2003). A prevalent characteristic of no-till AMF communities is that they produce more extraradical hyphae (Kabir et al. 1997,

Kabir et al. 1998b, Borie et al. 2006, Curaqueo et al. 2011) and usually colonize the roots of their host plants to a greater extent than those AMF exposed to soil disturbance (Miller et al. 1995, McGonigle and Miller 1996, Galvez et al. 2001).

Thus while it is well known that tillage influences AMF abundance and the composition and diversity of AMF communities, the functional consequences of such altered AMF communities have not been investigated so far. Soil disturbance created by tillage may select fast-growing AMF species that are less mutualistic and less efficient in improving host-plant nutrient uptake (Smith and Smith 1996, Johnson et al. 1997, Scullion et al. 1998). Moreover, soil tillage destroys mycorrhizal hyphal networks, the main structures for nutrient uptake by AMF. As a consequence, soil tillage may select for AMF taxa that are resistant to disturbance and that acquire lower amounts of nutrients. As a result, we expect that nutrient uptake is reduced when plants are colonized by AMF communities from tilled soils rather than communities from non-tilled soils.

In this study we tested whether soil communities from tilled and non-tilled soil have different impacts on plant productivity and nutrient uptake, two soil ecosystem services being of key importance for agricultural productivity. Our main research questions were (1) Are there differences in biomass production and nutrient uptake in the presence of soil communities from tilled and non-tilled fields? and (2) Do soil communities isolated from 30–40 cm soil depth have different effects on biomass production and nutrient uptake than soil communities isolated from 0–10 cm soil depth? These research questions were tested using model grassland systems that were inoculated with soil communities originating from tilled and non-tilled fields from 0–10 cm and 30–40 cm soil depth. Specific attention was given to AMF as these soil fungi are known to be strongly affected by tillage management. We hypothesized that soil communities from non-tilled fields enhanced biomass production and nutrient uptake compared to soil communities from tilled fields. Furthermore, we expected that soil communities from deeper soil layers will substitute for functional losses by plowing. The aim of this study was to investigate the microbial-driven consequences of different soil management practices for agroecosystem functioning.

## METHODS

### *Soil inoculum*

Soil inoculum was sampled from a long-term field trial where conventional tillage (CT) and no-tillage (NT) systems have been compared since 1991. This trial (named “Oberacker”), was located at the Inforama Ruetti in Zollikofen, Switzerland (46°59'17.19" N, 7°27'47.80" E, 527 m above msl) and was performed on a Cambisol with 15% clay and 3% organic matter (for a description of the trial and soil tillage practices see Sturny et al. [2007] and Nemecek et al. [2011]). Soil cores

from four tilled and four non-tilled plots were taken in 2009 from plots cultivated with winter barley. For each tillage treatment, soil cores were taken at two soil depths (0–10 cm and 30–40 cm). We chose these soil depths because we wanted to include a soil layer that is affected by tillage and one that is below the plowing zone. As plowing homogenizes the soil and arbuscular mycorrhizal fungi (AMF) propagules, we did not expect distinct differences associated with the plowing regime in the zones below the tillage zone (e.g., at 30–40 cm soil depth). AMF trap cultures were set up as described in Oehl et al. (2005). Briefly, largely undisturbed soil pieces (~4 cm in diameter) were taken from the soil cores and used as inoculum for the AMF trap cultures. The soil core pieces were placed at four defined locations in pots with a sterile 3:1 (w/w) mixture of Terragreen (American aluminum oxide, Lobbe Umwelttechnik, Iserlohn, Germany) and Loess (Tegerfelden, Switzerland). The soil inoculum comprised 5% of the total substrate mass. Four trap plant species (*Lolium perenne*, *Trifolium pratense*, *Plantago lanceolata*, and *Hieracium pilosella*) were sown in the pots above the added soil core pieces. Additionally, four pots were set up as a control and received a sterilized mix of “Oberacker” soil. The trap cultures were established in April 2009 and maintained during 20 months in the greenhouse under natural ambient light and temperature conditions. During this period, the perennial trap plants were cut repeatedly 3 cm above the ground.

After 20 months, the four pot culture substrates obtained per soil depth and tillage system were air-dried and pooled together in order to use them as inocula. This approach enabled us to propagate AM fungal communities characteristic for specific soil or management practices (Oehl et al. 2009).

Three 25-g soil samples were collected from each pooled inoculum, and AM fungal spores were isolated using wet sieving and sugar gradient centrifugation procedures of Sieverding (1991). AM fungal spores were subsequently identified morphologically and counted per species on prepared slides (Hawksworth 2011, Oehl et al. 2011; for spore communities see Appendix B: Table B1). No AMF sporulation was detected in the nonmycorrhizal controls over 20 months.

#### Soil substrate

Soil was collected from a permanent grassland at Agroscope in Zurich, Switzerland (47°25'38.71" N, 8°31'3.91" E). The soil was 5-mm sieved and mixed with quartz sand to a ratio of 1:1 (v/v). The mixture was autoclaved at 121°C for 99 min and was subsequently stored at room temperature for six weeks. The autoclaved substrate had a pH of 6.9 and contained 1% humus, 1% clay, and 11% silt. The substrate was phosphate poor with plant available P<sub>2</sub>O<sub>5</sub> (extracted with CO<sub>2</sub>-saturated water) of 0.68 mg/kg, and it contained 3.88 mg NH<sub>4</sub><sup>+</sup>/kg and 2.93 mg NO<sub>3</sub><sup>-</sup>/kg. The cation exchange capacity of the substrate was low

(2.87 cmd/kg), while the base saturation was classified as saturated (81.78%).

#### Experimental system

Grassland microcosms were established in polyvinyl chloride tubes with a diameter of 15.2 cm and a height of 40 cm (Appendix A: Fig. A1). A total of 8.6 kg sterilized substrate (dry mass) was added to each microcosm, covering 32 cm of the height (5.8 L). The substrate contained 3.5% (v/v) soil inoculum, which was placed in two layers 3 cm and 20 cm below the surface. Each microcosm was terminated by a 500- $\mu$ m polypropylene mesh allowing excess water to leach through. For better drainage 675 g of an autoclaved sand–gravel mixture was added to the bottom of the tubes.

The microcosms were inoculated with soil microbial communities from tilled or non-tilled soil from 0–10 cm and 30–40 cm soil depth, respectively, or microcosms received soil inoculum from nonmycorrhizal control pots. Each treatment was replicated eight times, adding up to a total of 40 microcosms (experimental units).

The microcosms were planted with a model grassland community consisting of *Trifolium pratense* L. “Formica” (red clover), *Lolium multiflorum* Lam. “Oryx” (Italian ryegrass), and a Swiss ecotype of *Plantago lanceolata* (ribwort plantain). We chose this plant community as it is widespread in both agricultural and natural grassland ecosystems where these species commonly coexist (Nyfeler 2009). Moreover, these three plant species belong to three different functional groups (a legume, a grass, and a forb) and they respond differently to AMF, with *Trifolium* and *Plantago* as highly responsive species (Hart and Reader 2002, Wagg et al. 2011) and *Lolium* as an unresponsive species (Wagg et al. 2011). Before planting, plant seeds (propagated by Agroscope, Zurich, Switzerland) were surface sterilized with 5% household bleach for 5 min and 70% ethanol for 10 min and rinsed thoroughly with sterilized water. Plants were germinated on sterile 1.5% water agar. Of each plant species, six individuals were planted, for a total of 18 seedlings per microcosm.

To equalize differences in nonfungal microbial communities between the different soil inocula, and to include microbes from natural grassland, a microbial wash was added to each microcosm. The microbial wash was created from the same fresh grassland soil used for the experimental substrate and from the soil inocula used in the experiment. 900 g of the fresh field soil and 120 g of each soil inoculum, including the control inoculum, were suspended in 6 L deionized water and filtered through filter paper (N°598, Schleicher and Schuell, Dassel, Germany) via vacuum filtration. Every microcosm received 100 mL of the microbial wash.

#### Growth conditions

The plants were grown in a greenhouse with an average daily temperature of 24°C, a nightly temperature of 18°C, and 16 h of light per day. Supplemental

light was provided by 400-W high-pressure sodium lights when natural irradiation was lower than 300 W. Plants were kept in the greenhouse for 35 weeks between April and December 2011.

Plants were watered with deionized water three times a week to 80% field capacity. Blocks were rotated randomly in the greenhouse when pots were watered. Grasslands were fertilized 11, 13, 19, and 21 weeks after planting with 30 mL of a nutrient solution devoid of phosphorus (6 mmol/L  $\text{KNO}_3$ , 4 mmol/L  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 4 mmol/L  $(\text{NH}_4)_2\text{SO}_4$ , 1 mmol/L  $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$ , and micronutrients [50  $\mu\text{mol/L}$   $\text{KCl}$ , 25  $\mu\text{mol/L}$   $\text{H}_3\text{BO}_3$ , 2  $\mu\text{mol/L}$   $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 2  $\mu\text{mol/L}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5  $\mu\text{mol/L}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.5  $\mu\text{mol/L}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 20  $\mu\text{mol/L}$   $\text{Fe}(\text{Na})\text{EDTA}$ ). This was equivalent to a nitrogen fertilization of 20 kg/ha in total. Pest management was applied when necessary and according to Swiss regulations for organic farming (predatory mites *Amblyseius cucumeris* and *A. swirskii* against thrips, and a fungicide consisting of 20% sulfur and 2.5% copper against powdery mildew).

The effect of soil microbial communities on nutrient leaching was investigated twice using a rain simulator (Knacker et al. 2004, van der Heijden 2010). In short, after six and eight months of plant growth, 20 mL fertilizer (323.9 mmol/L  $\text{NH}_4\text{NO}_3$ , 1 mmol/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 29.3 mmol/L  $\text{KH}_2\text{PO}_4$ , 2 mmol/L  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) were added to each microcosm corresponding to 100 kg N/ha and 10 kg P/ha for each fertilization event. Two days after fertilization microcosms were exposed to 1 L artificial rain provided by a rain simulator. The leachate draining off the microcosms was collected, weighed, and analyzed (see Appendix E).

#### Harvest

After two, four, and six months, shoots were cut 5 cm aboveground to simulate hay-making or grazing, which is typical for most grasslands in Switzerland. After eight months, at final harvest, shoots were cut at soil surface. Plants were separated per species, dried at 60°C for 48 h, and weighed. Microcosms were emptied, and roots were collected and weighed. In order to obtain finer roots, the soil substrate was homogenized, and a 500-g sample was taken and washed by repeatedly decanting the watered subsamples onto a 250- $\mu\text{m}$  mesh. Weighed subsamples of both root samples were dried at 60°C for 48 h, and total root biomass per microcosm was calculated. Subsamples of both root samples were cut into pieces < 1 cm, mixed in water, and stored in 50% ethanol for root colonization analysis. In addition to this, soil substrate samples were collected for nutrient analysis and hyphal length quantification. Soil water content was determined gravimetrically to standardize the results for all microcosms.

#### Analyses

*Arbuscular mycorrhizal fungal parameters.*—For the analysis of mycorrhizal root colonization, roots were cleared with 10% KOH and stained with 5% ink-vinegar

(Vierheilig et al. 1998). The percentage of root length colonized and the frequency of hyphae, arbuscules, and vesicles were quantified microscopically at a magnification of 200 $\times$  with the intersect method (McGonigle et al. 1990) using 100 intersections. Extraradical hyphae in the substrate were extracted with water on a filter membrane (Jakobsen et al. 1992a) for three replicates per microcosm, and hyphal length was quantified using the modified Newman formula (Tennant 1975).

*Plant nutrient analysis.*—Shoots were pooled across harvests for each species and ground for nutrient analysis. Total nitrogen shoot concentration was determined using a CHNSO analyzer (Euro EA, HEKAtech, Wegberg, Germany). For plant P determination, ground biomass was ashed at 600°C and digested using 6 mol/L hydrochloric acid. Digests were diluted, and the total amount of P was quantified colorimetrically according to the molybdenum blue method (Watanabe and Olsen 1965).

*Experimental design and statistical analysis.*—The experiment was set up as a complete randomized block design in the greenhouse, where each soil inoculation treatment was replicated eight times. Each replicate was assigned to one block, making a total of eight blocks. There were two factors “tillage” (soil communities from CT or NT plots) and “soil depth” (soil communities from 0–10 cm or 30–40 cm depth). The soil depth factor was nested within the tillage treatment. In addition to this, a nonmycorrhizal control treatment was included as a fifth treatment.

Statistical analyses were conducted using the software R 2.14.1 (R Development Core Team 2011). For each variable (shoot and root biomass, shoot nutrient content, mycorrhizal root colonization, hyphal length, and N:P ratio) an analysis of variance (ANOVA) with inoculum identity as a factor was performed to test whether treatments varied from each other. Moreover, in order to test whether the nonmycorrhizal control treatment varied from the four treatments with soil inocula, a contrast was created and tested using ANOVA. The effect of tillage and soil depth was evaluated with a nested ANOVA, excluding the control from the data set. In all analyses “block” was included as an error term. *Plantago* biomass was log-transformed to fulfill model assumptions. Correlations between two variables were assessed using Pearson’s correlation. In the text, all figures and tables presented show estimates of the means  $\pm$  SE. *Plantago* and *Trifolium* shoot P content contained one missing value each due to insufficient biomass amount for analysis. Root biomass data comprise one missing value.

## RESULTS

### *Mycorrhizal communities, root colonization, and hyphal length*

*Funneliformis mosseae*, *Claroideoglossum claroideum*, and *Glomus intraradices* showed an increased spore density in inoculum from conventionally tilled (CT)

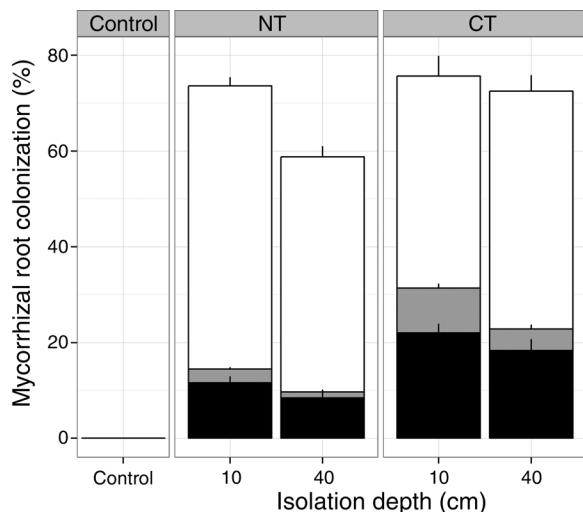


FIG. 1. Percentage of root length colonized by arbuscular mycorrhizal fungi (AMF) in microcosms without AM fungi (control) or in microcosms inoculated with soil microbes from conventionally tilled (CT) or non-tilled (NT) plots for two different isolation soil depths (0–10 cm and 30–40 cm). Total root length colonized is depicted as the sum of root length colonized by arbuscules (black), vesicles (gray), and hyphae (white). Bars are means of eight replicates  $\pm$  SE.

compared to non-tilled (NT) soil inoculum (Appendix B: Table B1). Moreover, *Funneliformis caledonius* was detected only in CT inoculum. In contrast, *Diversispora celata* was specific for NT plots. In addition to this, *Septoglomus constrictum* and a *Glomus* sp. BE12, resembling *Gl. microaggregatum* were also much more abundant in NT inoculum compared to CT inoculum. The abundance of all arbuscular mycorrhizal fungi (AMF) species in tillage treatment and soil depth combinations can be found in the supplemental material (Appendix B: Table B1).

The degree to which the plant roots were colonized by AMF varied among the treatments ( $F_{4,28} = 149.6$ ,  $P < 0.0001$ ; Fig. 1). Plant roots in microcosms inoculated with soil inoculum from CT plots had a higher total root colonization as compared to those with soil inoculum from NT plots ( $F_{1,21} = 7.44$ ,  $P = 0.013$ ). Root colonization by arbuscules and vesicles was also significantly higher in microcosms with soil inoculum from CT plots than those from NT soils (vesicular  $F_{1,21} = 60.41$ ,  $P < 0.0001$ ; arbuscular  $F_{1,21} = 30.68$ ,  $P < 0.0001$ ). The soil depth from where the soil inoculum was generated also affected the percentage of root length colonized. Total root colonization was decreased by soil depth ( $F_{1,21} = 6.9$ ,  $P = 0.005$ ). AM fungal communities from 30–40 cm produced significantly less intraradical hyphae ( $F_{1,21} = 5.52$ ,  $P = 0.012$ ) and vesicles ( $F_{1,21} = 16.68$ ,  $P < 0.0001$ ) than communities from 0–10 cm soil depth. After eight months, the nonmycorrhizal control treatment had no root colonization and remained uncontaminated by AMF. Root nodules were observed in all treatments.

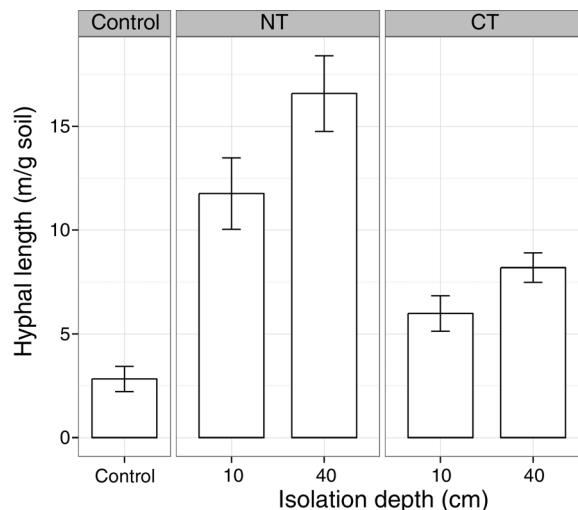


FIG. 2. Length of extraradical hyphae per gram of soil dry matter in control microcosms without AM fungi (control) or in microcosms inoculated with soil communities from tilled (CT) or non-tilled (NT) plots for two different isolation soil depths (0–10 cm and 30–40 cm). Bars depict means of eight replicates  $\pm$  SE.

The length of the extraradical hyphae varied among the different treatments ( $F_{4,28} = 20.33$ ,  $P < 0.0001$ ; Fig. 2). Hyphal length in microcosms inoculated with soil communities from NT soils was twofold greater than in microcosms inoculated with soil communities from CT soils ( $F_{1,21} = 29.49$ ,  $P < 0.0001$ ). Microcosms inoculated with soil communities from deeper soil layers (30–40 cm) produced more extraradical hyphae than AMF from the surface (0–10 cm) ( $F_{1,21} = 4.13$ ,  $P = 0.031$ ). Hyphal length in the nonmycorrhizal control microcosms was lowest and probably reflects dead hyphae or hyphae from nonmycorrhizal soil fungi. The extent of the extraradical hyphal network was significantly negatively correlated with the percentage of root length colonized ( $r = -0.42$ ,  $t_{30} = -2.56$ ,  $P = 0.016$ ; control excluded from analysis).

#### Plant biomass

Total aboveground biomass production of the grassland microcosms varied significantly among the soil inoculation treatments ( $F_{4,28} = 17.57$ ,  $P < 0.0001$ ; Fig. 3). Most of the variance (87.98%) in the total aboveground biomass was explained by the control treatment differing from the other treatments ( $F_{1,28} = 61.85$ ,  $P < 0.0001$ ). Aboveground biomass production was highest in grassland microcosms inoculated with soil inoculum from CT plots and lowest in noninoculated control microcosms (reduction of 22.75%). The biomass of *Plantago* and *Trifolium* was on average 4.96- and 21.14-fold higher, respectively, in microcosms receiving soil inoculum compared to noninoculated microcosms. In contrast, *Lolium* growth was suppressed by soil inoculation, and it was highest in noninoculated

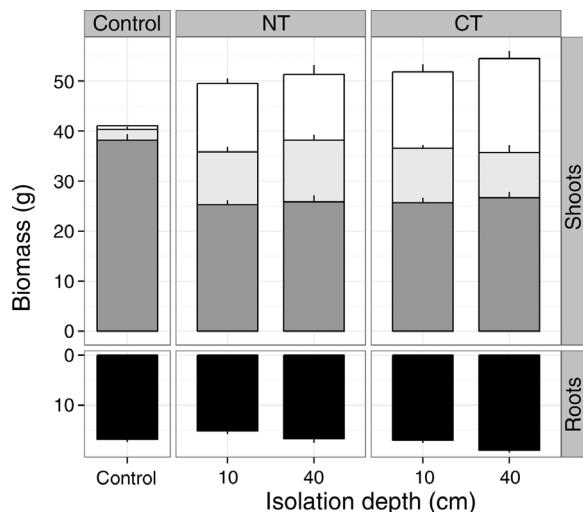


FIG. 3. Shoot and root biomass (dry mass) in microcosms without AM fungi (control) and in microcosms inoculated with soil microbial communities from tilled (CT) or non-tilled (NT) plots for two different isolation soil depths (0–10 cm and 30–40 cm). Aboveground biomass is depicted for *Lolium* (dark gray), *Plantago* (light gray), and *Trifolium* (white). Bars depict means of eight replicates + SE (for root biomass of NT plots of 40 cm soil depth,  $N = 7$ ). Total aboveground biomass is significantly higher in the CT treatment compared to the NT treatment ( $F_{1,21} = 5.97$ ,  $P = 0.023$ ). Fewer roots are produced in the NT treatment compared to the CT treatment ( $F_{1,21} = 16.61$ ,  $P = 0.0006$ ).

microcosms. Aboveground biomass production in grassland microcosms inoculated with soil communities from CT fields was significantly higher compared to inoculation with inoculum from NT soil ( $F_{1,21} = 5.97$ ,  $P = 0.024$ ). This effect resulted mainly from increased *Trifolium* biomass in grassland microcosms inoculated with soil communities from CT plots ( $F_{1,21} = 5.56$ ,  $P = 0.028$ ). Biomass of *Lolium* and *Plantago* did not differ significantly between microcosms receiving soil inocula from tilled or non-tilled plots (*Lolium*  $F_{1,21} = 0.37$ ,  $P = 0.55$ ; *Plantago*  $F_{1,21} = 2.70$ ,  $P = 0.12$ ). The soil depth from which the soil communities were isolated did not explain variation in the total aboveground biomass ( $F_{2,21} = 2.11$ ,  $P = 0.15$ ) nor in the shoot biomass of the different species. The biomass of each plant species for each individual harvest is given in Appendix C: Fig. C1.

Root biomass differed significantly between inocula ( $F_{4,27} = 7.04$ ,  $P = 0.0005$ ) with lower root biomass in the treatment with NT inoculum compared to CT inoculum ( $F_{1,20} = 16.61$ ,  $P = 0.0006$ ). Soil depth affected the root biomass with higher root growth with inoculum from 40 cm soil depth as compared to those from the 10 cm soil depth ( $F_{2,20} = 4.86$ ,  $P = 0.019$ ).

#### Plant P and N uptake

Inoculum source had a significant effect on total aboveground plant P and N content ( $P$   $F_{4,26} = 101.3$ ,  $P < 0.0001$ ;  $N$   $F_{4,28} = 6.92$ ,  $P = 0.0005$ ; Figs. 4 and 5).

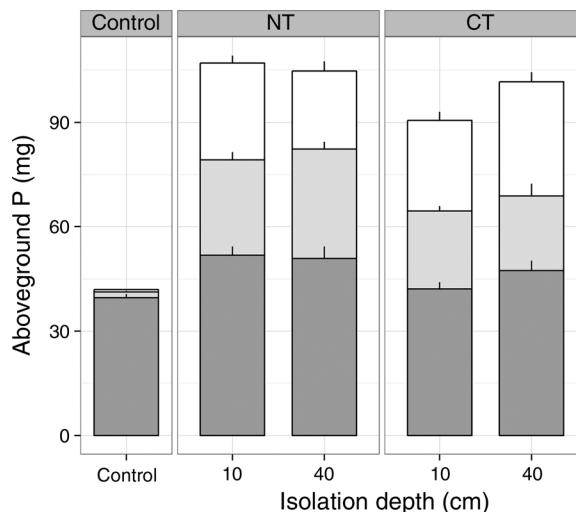


FIG. 4. Total aboveground phosphorus of *Lolium* (dark gray), *Plantago* (light gray), and *Trifolium* (white) per microcosm without AM fungi (control) and in microcosms inoculated with soil microbial communities from tilled (CT) or non-tilled (NT) plots for two different isolation soil depths. Means + SE of eight replicates are shown (for *Plantago* and *Trifolium* only seven replicates are in the control treatment for the P content).

Foliar P and N content was significantly increased in grassland microcosms receiving soil inoculum ( $P$   $F_{1,26} = 376.9$ ,  $P < 0.0001$ ;  $N$   $F_{1,28} = 19.74$ ,  $P = 0.00013$ ) as compared to those in the nonmycorrhizal control. *Lolium* accumulated the highest amount of P and N due to the highest biomass production. In contrast, *Plantago* showed the highest P concentration and

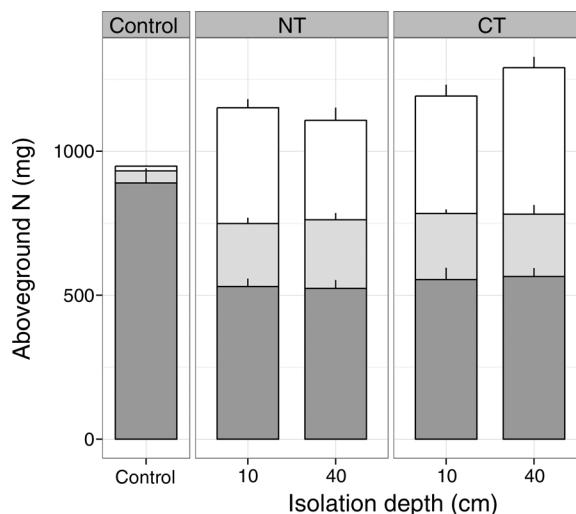


FIG. 5. Total aboveground nitrogen of *Lolium* (dark gray), *Plantago* (light gray), and *Trifolium* (white) per microcosm without AM fungi (control) and in microcosms inoculated with soil microbial communities from tilled (CT) or non-tilled (NT) plots for two different isolation soil depths. Means + SE of eight replicates are shown.

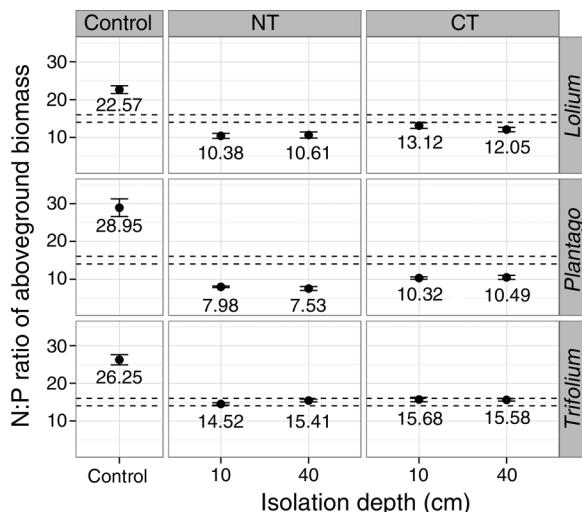


FIG. 6. Shoot N:P ratios of *Lolium*, *Plantago*, and *Trifolium* in microcosms without AM fungi (control) and in microcosms inoculated with microbial soil inoculum from tilled (CT) or non-tilled (NT) plots for two different isolation soil depths. Depicted are means  $\pm$  SE of eight replicates (for *Plantago* and *Trifolium* only seven replicates are in the control treatment). Dashed lines indicate threshold values for N limitation (<14) and P limitation (>16). At N:P ratios between 14 and 16, either N or P can be limiting or co-limiting for plant growth (Koerselman and Meuleman 1996).

*Trifolium* the highest N concentration (Appendix C: Table C1). The effect of soil inoculum on the P and N content was highly dependent on the plant species (significant “soil inoculum”  $\times$  “plant species” interaction: P  $F_{8,96} = 5.92$ ,  $P < 0.0001$ ; N  $F_{8,98} = 35.07$ ,  $P < 0.0001$ ). Plant P and N content of *Plantago* and *Trifolium* as well as P of *Lolium* was increased when inoculated compared to the control; *Lolium* N content decreased compared to the control. Regardless of plant species, the P concentration was always increased by soil inoculum.

Inoculum source had contrasting effects on aboveground P and N content. NT soil inoculum increased total shoot P content as well as the plant-species-specific P content of *Lolium* and *Plantago* compared to CT inocula (total P  $F_{1,21} = 15.6$ ,  $P = 0.0007$ ; *Lolium*  $F_{1,21} = 5.5$ ,  $P = 0.029$ ; *Plantago*  $F_{1,21} = 11.1$ ,  $P = 0.0032$ ). In contrast, total and *Trifolium* N content was higher in pots with CT inoculum (total  $F_{1,21} = 5.69$ ,  $P = 0.027$ ; *Trifolium*  $F_{1,21} = 5.3$ ,  $P = 0.032$ ). Regardless of plant species, the P concentration was always higher in microcosms inoculated with NT soil than those with CT soil (Appendix C: Table C1).

Total P content of the aboveground biomass was higher with inoculum from 30–40 cm soil depth than with inoculum from the surface (nested ANOVA  $F_{2,21} = 5.17$ ,  $P = 0.015$ ). Plant species-specific P content as well as N content was not affected by soil depth (Appendix D: Table D1).

Aboveground P concentration and hyphal length correlated positively in treatments receiving soil inoculum, excluding the control treatment ( $r = 0.55$ ,  $t_{30} = 3.61$ ,  $P = 0.001$ ). As the total aboveground biomass was negatively correlated with hyphal length ( $r = -0.38$ ,  $t_{30} = -2.28$ ,  $P = 0.03$ ), there was no correlation between hyphal length and total aboveground P content ( $r = 0.29$ ,  $t_{30} = 1.67$ ,  $P = 0.11$ ). There was a negative correlation between aboveground N concentration and N content, respectively, with increasing hyphal length (N concentration  $r = -0.48$ ,  $t_{30} = -2.98$ ,  $P = 0.0057$ ; N content  $r = -0.53$ ,  $t_{30} = -3.41$ ,  $P = 0.002$ ). Total root colonization did not explain any variation in aboveground P and N concentration and content when the control was excluded from the analysis (P concentration  $r = -0.14$ ,  $t_{30} = -0.78$ ,  $P = 0.44$ ; N concentration  $r = 0.34$ ,  $t_{30} = 1.99$ ,  $P = 0.06$ ).

The shoot N:P ratio can be used as diagnostic tool to evaluate the nature of nutrient limitation (Koerselman and Meuleman 1996). An N:P ratio <14 indicates N limitation, whereas a ratio >16 is indicative of P limitation. N:P ratios of the three plant species in noninoculated control microcosms were >22, suggesting that plant productivity in these microcosms was P limited (Fig. 6). Inoculation with soil communities decreased shoot N:P ratios significantly for each of the investigated plant species ( $P < 0.000$ ; *Lolium*  $F_{1,28} = 219.56$ , *Trifolium*  $F_{1,27} = 187.16$ , *Plantago*  $F_{1,27} = 322.63$ ). Both *Plantago* and *Lolium* were N limited in grassland microcosms receiving soil inoculum (as indicated by an N:P ratio <14), whereas no indications about nutrient limitations were observed for *Trifolium* (which had an N:P ratio between 14 and 16 in each of the treatments receiving soil inocula from CT or NT soils). NT soil communities intensified N limitation for *Lolium* ( $F_{1,21} = 13.71$ ,  $P = 0.0013$ ) and *Plantago* ( $F_{1,21} = 44.94$ ,  $P < 0.0001$ ) compared to CT soil inoculum as reflected by a lower N:P ratio in NT soil (Fig. 6).

#### Nutrient leaching

The grassland microcosms received a simulated rain after six and eight months of plant growth. This was done to assess whether the different soil communities varied in their ability to retain nutrients. In this paper we focused on the effects of the different microbial communities on plant productivity and nutrient uptake. Thus leaching data are presented very shortly. Soil communities from CT soil reduced phosphate losses via leaching by 27.0% (corresponding to  $13.09 \pm 6.52$  g P/ha) compared to the NT soil community ( $F_{1,21} = 4.38$ ,  $P = 0.049$ ; Appendix E: Fig. E1). No differences were found after eight months of plant growths ( $F_{1,21} = 1.49$ ,  $P = 0.24$ ). Unreactive P leaching did not differ between NT and CT treatments (six months  $F_{1,21} = 1.90$ ,  $P = 0.18$ ; eight months  $F_{1,21} = 0.68$ ,  $P = 0.42$ ; Appendix E: Fig. E1). Both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  leaching losses were determined by the origin of the inoculum (Appendix E: Fig. E2). Soil communities from CT significantly

reduced ammonium and nitrate losses after six months of plant growth by 66.5% and 37.4%, respectively ( $\text{NO}_3^- F_{1,21} = 33.73, P < 0.0001$ ;  $\text{NH}_4^+ F_{1,21} = 4.96, P = 0.037$ ), and by 28.9% and 43.2%, respectively, after eight months ( $\text{NO}_3^- F_{1,21} = 22.63, P = 0.00011$ ;  $\text{NH}_4^+ F_{1,21} = 0.33, P = 0.57$ ).

#### DISCUSSION

A wide range of studies have shown that soil tillage alters the composition and diversity of microbial communities (e.g., Jansa et al. 2002, Borriello et al. 2012). The effect of this change in community structure on agroecosystem processes has, to our knowledge, not been investigated so far. The present study shows, for the first time, that soil microbial communities conditioned by distinct tillage practices provide different and partly contrasting ecosystem services. Soil communities from NT field plots increased P uptake of the grassland communities (Fig. 4) as compared to soil communities from tilled plots. In contrast, overall biomass production tended to be higher in grassland microcosms inoculated with soil communities from conventionally tilled (CT) soil than those from non-tilled (NT) soil (Fig. 3).

The increase in shoot P content in grassland microcosms inoculated with soil communities from NT soil can be explained by substantially higher amounts of extraradical hyphae in those microcosms. Extraradical hyphae have been identified before to determine the uptake and transport of P to the plant (Jakobsen et al. 1992a, b, Jansa et al. 2005, Avio et al. 2006). The correlation between shoot P concentration and extraradical hyphae in this study provides further evidence that extraradical hyphae are important for P uptake. In this study, increased P uptake did not result in increased productivity, possibly because the grassland microcosms were N limited, rather than P limited, as indicated by N:P ratios  $< 14$  (Koerselman and Meuleman 1996; Fig. 6). Thus excessive fungal P facilitation could not be used for further biomass buildup. Other studies have shown that enhanced P uptake (e.g., by mycorrhizae) does not necessarily lead to enhanced plant growth (Smith et al. 2003, Lekberg and Koide 2005), indicating that there can be a sort of superfluous P consumption (Chapin 1980). Furthermore, higher microbial biomass (Appendix F: Fig. F1) and higher hyphal length in microcosms inoculated with NT soil inoculum indicate that more plant C was allocated below ground but could not be invested in plant shoot biomass.

It has been estimated that global phosphate stocks will be depleted within the next 50–100 years, with the quality of the mined material already decreasing and mining costs rising (Cordell et al. 2009). Hence, in the future, there will be a need to develop new methods of fertilizing crops with phosphorus. Enhanced phosphorus uptake from the soil is one way to increase phosphorus availability, especially because many soils contain large amounts of plant unavailable P (e.g., P adsorbed to mineral clay particles; Fe-, Al-, or Ca-phosphates; or P

in organic complexes; Bünemann and Condron 2007). Moreover, in many soils large amounts of phosphorus have accumulated as a result of long-term inputs of fertilizer. Our study shows that the manipulation of microbial communities (e.g., by adapting agricultural management) can help to enhance phosphorus availability to plants by using inherent phosphorus pools and to reduce fertilizer input.

Plowing usually affects a soil depth of 20–35 cm (Peigné et al. 2007), and thus we hypothesized that soil biotic communities from 30–40 cm soil depth would substitute for soil disturbance effects (Miura et al. 2008). Although spore communities, hyphal length, and root colonization levels differed between 0–10 cm and 30–40 cm, no effect could be detected in various ecosystem functions. This suggests that soil depth is not the dominating factor in determining how soil biotic communities perform.

Our objective was to assess the functioning of soil biotic communities without the influence of existing environmental context. This was achieved by propagating predominantly specific arbuscular mycorrhizal fungi (AMF) communities for two years in the greenhouse and testing their impact on plant productivity and nutrient uptake in grassland microcosms grown in sterile standard soil substrate. We attributed the differences between CT and NT soil inoculum mainly to differences in AMF communities and not to other soil biota. We assumed this because (1) we used relatively small amounts of soil from the field to prepare the inocula (e.g., no earthworms were present); (2) we applied a microbial filtrate to each microcosm to equalize bacterial and fungal communities (propagules  $< 10 \mu\text{m}$ ); and (3) our propagation system was specifically designed to propagate AMF from the field, which is also reflected by the high number of AMF species found in the inocula. Moreover, plant biomass and P uptake was correlated with hyphal length and root colonization, providing further evidence that differences in AMF communities, at least in part, explained our results. However, we cannot exclude that soil organisms other than AMF could be responsible for the observed effects.

*Trifolium* biomass production was most affected by the tillage inoculum treatment (Fig. 3). Furthermore, *Trifolium* was the only plant species where total biomass differed significantly between the CT and the NT treatments (Appendix D: Table D1). Previous work has shown that legumes can vary in their response to inoculation with different AMF taxa (Owusu-Bennoah and Mosse 1979, Drew et al. 2003, Scheublin et al. 2007). Hence, the differences in AMF community composition between the CT and NT treatments could explain differences in *Trifolium* biomass production. Differences in *Trifolium* performance will, in turn, affect biological nitrogen fixation, as the fixation rate is positively related to biomass production (Carlsson and Huss-Danell 2003, Pimratch et al. 2008). Therefore, the increased plant nitrogen content in treatments harboring

CT soil inoculum may be related to enhanced *Trifolium* productivity. This would constitute an indirect mycorrhizal effect on overall nutrient cycling, which cannot be uncoupled from the N-fixing microbial community. Although we applied a standardized microbial wash to each microcosm to reduce variation in the bacterial communities between treatments, it is possible that differences in nitrogen-fixing communities associating with *Trifolium* partly explain the varying biomass response between the CT and NT treatments. This is because plant growth responses can be dependent on the AMF–*Rhizobium* combination (Xavier and Germida 2002). Furthermore, because *Trifolium* exhibited the strongest response to the different microbial communities, the effects of the microbial communities on plant productivity would likely be lower if *Trifolium* was absent. Despite this, differences in P uptake between CT and NT were also prevalent in *Plantago* and *Lolium* (Fig. 4), indicating that microbial communities from CT and NT provided different ecosystem services irrespective of effects on *Trifolium*.

All soils we used to propagate inoculum originated from a long-term, replicated field experiment with the same abiotic conditions and the same crop rotation for each plot (Nemecek et al. 2011). Thus differences between the soil communities are exclusively due to the specific tillage treatment and not due to other factors such as soil type or location. Using our trap culture system we were able to propagate tillage-dependent soil communities. Earlier work showed that AMF community composition in trap cultures can change according to cultivation duration, substrate, and the host plant species (Sýkorová et al. 2007, Oehl et al. 2009). This might also be the case in our experiment. Despite this caveat, we successfully propagated different spore communities (Appendix B: Table B1) and found 16 to 20 different AMF species in the inocula from each tillage treatment, which is a representative species number for many AMF communities in the field (Oehl et al. 2005). Moreover, the observed differences in AMF communities between the CT and NT treatments resemble some species responses that depend on soil tillage practice that have been described before in the field. For instance, *Funneliformis mosseae* was increased in CT (Jansa et al. 2002, Schalamuk et al. 2006) as well as *Glomus intraradices* (Jansa et al. 2002). In contrast, in this study and in another study (Borriello et al. 2012), NT enhanced *Diversosporaceae* abundance. The number of AMF species present in our inocula is comparable to the species richness often found in the field (Oehl et al. 2003, Oehl et al. 2010). Despite this, it is possible that some nonsporulating or rarely sporulating AMF taxa, which might be especially abundant under NT conditions (Rosendahl and Stukenbrock 2004), might not have been detected, as we did not use molecular tools to characterize the AMF communities.

In our study, important ecosystem services (plant productivity, biomass production by individual plant

species, and nutrient uptake) differed between soil communities from tilled and non-tilled fields. These differences are likely to be determined by specific functional traits of the AMF (such as the size of the hyphal network and the intensity of root colonization). Several previous studies have shown that different AMF communities alter plant growth (Johnson 1993, Moora et al. 2004) and other ecosystem functions such as nutrient leaching (Verbruggen et al. 2012). The AMF spore communities used in these studies shared a lot of common species. However, a subset of specialists (*Funneliformis mosseae*, *Claroideoglomus claroideum*, *Glomus intraradices*, *Funneliformis caledonius*, *Diversispora celata*, *Septoglomus constrictum*, and a *Glomus* sp. BE12) varied in abundance between the two tillage treatments or were specific for soil inoculum from tilled or non-tilled plots. These specialists were therefore most likely responsible for observed differences in productivity and nutrient uptake. A focus on AM fungal specialists might be of great importance for future inoculation trials, especially in fields with P deficiency.

In this greenhouse study, we assessed the functioning of soil biotic communities independently of other abiotic and biotic factors. Differences detected in the greenhouse might not persist under field conditions as tillage systems vary not only in the soil microbial communities but also in other parameters. Differences in soil temperature, soil mineralization, vertical nutrient distribution, soil moisture, and weed pressure (for a review see Peigné et al. [2007]) between NT and CT systems will affect biomass production as well as nutrient uptake. These different conditions may, in part, overrule the described effects here. Verbruggen et al. (2012) reported that the effects of soil biota on agroecosystems are field site specific. Interestingly though, several field studies confirm our findings that P uptake (Gavito and Miller 1998, Kabir et al. 1998a, Galvez et al. 2001) and fungal hyphal length (Kabir et al. 1997, Boddington and Dodd 2000, Borie et al. 2006) are increased under NT, whereas biomass production is often reduced (Miller et al. 1995, Gavito and Miller 1998, Galvez et al. 2001). This indicates that the driving mechanism behind tillage effects in the field may be of microbial, and especially of mycorrhizal, nature.

### Conclusion

In this study we demonstrated that (1) agricultural management practice, in particular tillage, influences the community composition of soil biota; and (2) that such tillage-induced changes in soil biota alter a number of ecosystem services (plant productivity, nutrient uptake). The observed differences in ecosystem functioning between NT and CT soil biotic communities imply that belowground soil biodiversity has to be taken into consideration when choosing a soil management system. Beyond that, our results also indicate that soil biota should be deliberately manipulated by agricultural practices to reduce fertilizer input and increase sustain-

ability. As NT soil communities are superior in P supply, NT might be especially interesting for fields with a P-deficient condition and reduced P fertilizer input. Further studies should investigate the contribution of single-specialist AMF within the AMF community, especially regarding field inoculum application. Long-term effects of specific microbial communities have to be described, as well as field inoculation studies at field sites under more natural conditions.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Picture of the experimental system (microcosms) ([Ecological Archives A024-209-A1](#)).

### Appendix B

Spore community composition of the different soil inocula determined by morphological AMF spore analysis ([Ecological Archives A024-209-A2](#)).

### Appendix C

Development of the individual shoot biomasses between the four harvests and a summary table of all means of the responses including the standard error ([Ecological Archives A024-209-A3](#)).

### Appendix D

Results for statistical analyses of the effect of soil inocula on plant biomass, shoot nutrient content and concentration, and mycorrhizal parameters, as well as correlation matrix for plant responses and mycorrhizal parameters ([Ecological Archives A024-209-A4](#)).

### Appendix E

Nutrients leached from microcosms after the simulation of rain, including a summary of the statistical analysis and a description of the method ([Ecological Archives A024-209-A5](#)).

### Appendix F

Microbial biomass C and N, including a summary of the statistical analysis and a description of the method ([Ecological Archives A024-209-A6](#)).