

## The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments

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**Abstract.** There is an urgent need to develop novel approaches to enhance sustainable agriculture while not reducing crop yields. Arbuscular mycorrhizal (AM) fungi establish symbiotic associations with most crop plants improving plant performance and soil health. This study investigated the extent of colonisation of potato roots by indigenous AM fungi in the arable soil under conventional and organic farming systems. Potato roots had greater AM fungal colonisation levels under organic than conventional farming, though in general, root colonisation levels were extremely low in both farming systems. Potato root AM fungal colonisation was lower with higher soil P content and higher with higher annual C input. Trap plant root AM fungal colonisation was considerably higher than in field potato roots and showed that soil mycorrhizal inoculum potential was higher in organic than in conventional farming. Thus, the positive impact of manure application in organic fields to the potato AM fungal colonisation can be explained by previous higher total annual C fresh organic matter input and lower soil P content under treatment. Furthermore, the natural AM fungal abundance in the soil was sufficient to colonise trap plant roots, suggesting a low mycorrhizal dependence of the studied potato cultivar.

**Key words:** Cropping systems, Glomeromycota, management practices, *Solanum tuberosum* L.

### INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are the most widespread symbionts of plant roots in terrestrial habitats. AM fungi provide a major contribution to plant nutrient uptake in most ecosystems and thus improve plant growth (Smith & Read, 2008; Smith & Smith, 2011). In addition to nutritional benefits, AM fungi protect host plants against root and shoot pathogens, including nematodes, other fungi and viruses, and enhance host plant resistance to various abiotic stresses such as drought, salinity and high heavy

metal concentrations in soil (Augé et al., 2015; Pozo et al., 2015). The ecosystem services provided by AM fungi in agroecosystems make them an important group of soil biota to be managed for crop production both in conventional and sustainable systems (Gianinazzi et al., 2010; Mahmood & Rizvi, 2010; Bender et al., 2016).

Diversity and functioning of AM fungi in agroecosystems are affected by differences in management regimes. Intensive agricultural management approaches are characterised by high N and P inputs (Verbruggen & Kiers, 2010). Environmental conditions with high nutrient input cause a decrease in host plant resource allocation to AM fungi and therefore have a negative impact on AM fungal biodiversity and species richness (Mäder et al., 2000; Verbruggen et al., 2010). The low-input systems, on the other hand, promote AM fungal colonisation, because plants benefit from the AM fungi by increased soil nutrient uptake when these are available at low concentrations (Mäder et al., 2000).

AM fungi are native to agricultural soils and form a mutualistic symbiosis with the majority of crop plants (Douds et al., 2007). Potato is a non-grain crop of global importance but has one of the heaviest production demands for fertilizer and pesticide inputs of all vegetable crops (Wu et al., 2013). For plants such as potato, which have a low root density in soil, the AM symbiosis may be of particular significance in coping with P-deficiency stress in natural ecosystems (McArthur & Knowles, 1993). Previous inoculation studies have reported high levels of AM fungal colonisation in potato roots (Davies et al., 2005; Douds et al., 2007), but the knowledge about AM fungi colonising potato roots in field conditions under common agricultural practices is scarce.

Therefore, it is necessary to determine how the natural AM fungal colonisation in potato roots is affected by different agricultural management regimes.

We specifically asked: (1) How do the conventional and organic farming systems influence potato root AM fungal colonisation? (2) How do the conventional management treatments with different mineral fertilization levels and different organic treatments with cover crop and manure amendment influence potato root AM fungal colonisation? We characterized AM fungal colonisation of potato roots in field grown potato plants and in addition we determined AM fungal inoculum potential (IP) using a trap plant bioassay.

## MATERIALS AND METHODS

### Field experiment

The field site was located in Tartu, Estonia (58°22' N, 26°40' E). This field was established in 2008 as a part of the 5-year crop rotation experiment with two organic (OFS) and four conventional farming systems (CFS). In the crop rotation experiment, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were grown in succession. In both organic management systems, a winter cover crop (CC) for green manure was used. Winter oilseed rape (*Brassica napus* L.) seeds as CC were sown at the rate of 6 kg ha<sup>-1</sup> before the potato cropping in September 2009 and ploughed under in April the year 2010. No cattle manure was added to one treatment (CC), whereas composted cattle manure (M) was added to the second organic treatment (CC+M). No fungicides, herbicides or insecticides were applied under organic systems. Weeds were removed mechanically. In a CFS, four mineral fertilizer treatments were used: N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>,

N<sub>50</sub>P<sub>25</sub>K<sub>95</sub>, N<sub>100</sub>P<sub>25</sub>K<sub>95</sub>, and N<sub>150</sub>P<sub>25</sub>K<sub>95</sub>. Conventional systems were treated with several synthetic pesticides. Field operations and their timings are shown in Table S1.

**Table S1.** Field operations and their timings during year 2010 in the study site

Field operation	Conventional farming system	Organic farming system
Planting date	May 6 <sup>th</sup>	May 6 <sup>th</sup>
Planting rate	3 t ha <sup>-1</sup> 57,000 tubers ha <sup>-1</sup>	3 t ha <sup>-1</sup> 57,000 tubers ha <sup>-1</sup>
Harvest date	August 30 <sup>th</sup>	August 30 <sup>th</sup>
Fertilization	N <sub>0</sub> P <sub>0</sub> K <sub>0</sub> – not fertilized N <sub>50</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>30</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	April 20 <sup>th</sup> Winter cover crop
	N <sub>100</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>60</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0) 3) June 16 <sup>th</sup> N <sub>20</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	April 20 <sup>th</sup> Winter cover crop + composted cattle manure 40 t ha <sup>-1</sup>
	N <sub>150</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>90</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0) 3) June 16 <sup>th</sup> N <sub>40</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	
Herbicide application	June 7 <sup>th</sup> Titus 25 DF (50 g ha <sup>-1</sup> ) (containing 12.5 g ha <sup>-1</sup> rimsulfuron)	No herbicides applied
Insecticide application	July 22 <sup>nd</sup> Fastac 50 (0.3 L ha <sup>-1</sup> ) (containing 15 g ha <sup>-1</sup> alpha-cypermethrin) August 6 <sup>th</sup> Decis 2.5 EC (0.2 L ha <sup>-1</sup> ) (containing 5 g ha <sup>-1</sup> deltamethrin)	No insecticides applied
Fungicide application	June 25 <sup>th</sup> Shirlan 500 SC (0.4 L ha <sup>-1</sup> ) (containing 200 g ha <sup>-1</sup> fluazinam) July 8 <sup>th</sup> and 22 <sup>nd</sup> Ridomil Gold MZ 68 (2.5 kg ha <sup>-1</sup> ) (containing 100 g ha <sup>-1</sup> metalaxyl-M + 1.6 kg ha <sup>-1</sup> mancozeb) August 6 <sup>th</sup> Ranman 400 SC (0.15 L ha <sup>-1</sup> ) (containing 60 g ha <sup>-1</sup> cyazofamid)	No fungicides applied

\*AN – ammonium salpeter.

Treatments were arranged in a systematic block design with each plot in four replications. The size of each test plot was 60 m<sup>2</sup>. Organic and conventional plots were separated by an 18 m long section of mixed grasses to avoid contamination with synthetic pesticides and mineral fertilizers. The distance between seed tubers was 27 cm, and the distance between rows was 70 cm. The soil of the trial field was *Stagnic Luvisol* (LVj) with sandy loam texture with a humus layer of 20–30 cm. No irrigation was used. The data of total annual carbon inputs from cover crops, straw, roots of pea, weeds and cattle manure (kg C ha<sup>-1</sup> y<sup>-1</sup>) to the soil before potato were obtained from earlier publications about this field trial (Kauer et al., 2015; Madsen et al., 2016).

Potato root samples were collected in July 2010 from plants of the locally bred potato cultivar 'Reet' (Tsahkna & Tähtjärvi, 2008). Three potato plants were sampled randomly from each of the four replicate plots of all treatments of both farming systems (a total of 72 samples). Roots were sampled at the potato flowering (BBCH60) stage (Hack et al., 2001). Root samples were dried with silica gel and preserved airtight at room temperature as described by Uibopuu et al. (2012).

### **Trap plant bioassay**

Narrowleaf plantain (*Plantago lanceolata* L.), as an AM host plant commonly used in experiments, was used to evaluate the mycorrhizal inoculum potential (IP) of field soil. Three soil samples were collected randomly from each of the four replicate plots of all treatments before soil tillage in spring 2011, and stored in darkness at 10 °C until use. Before the use in experiment, the three soil samples per plot were pooled and handled as one composite sample. Each composite sample was thoroughly mixed with autoclaved sand in a 1:1 v/v ratio to improve drainage and aeration of the soil mixture. *P. lanceolata* seeds were germinated in Petri dishes on moist filter paper, following Uibopuu et al. (2012). Three seedlings per pot were planted on January 2011 in plastic pots (13 x 15 cm, depth x diameter). One seedling was retained per pot after four weeks of growth. Plants were kept in a greenhouse under controlled conditions, watered as needed with tap water and grown for 3 months. At harvest, shoots and roots were separated and handled like described previously. The infectivity bioassay of Moorman & Reeves (1979) was used to quantify the relative density of colonising propagules of AM fungi.

### **Root staining and assessment of AM fungal root colonisation**

Root samples from both field and pot experiment were stained with Trypan blue according to Koske & Gemma (1989). Briefly, roots were cleared in 10% KOH, acidified with 1% HCl and stained with 0.01% trypan blue in lactoglycerol. Root colonisation by AM fungi was estimated using the magnified grid-line intersections method (McGonigle et al., 1990), by scoring 120 fields of view per sample under the compound microscope at 400x magnification, as described in Uibopuu et al. (2012). Total root length colonised was estimated.

### **Soil chemical analysis**

In mid-April 2010, before tillage, soil samples were collected from the bulk soil at a depth of 0–25 cm. Eight sub-samples were taken from each plot and mixed to obtain a composite sample for each plot. Soils were air-dried and sieved through a 2 mm sieve. Soil pH was determined in a 1 M KCl solution (1:2.5). Soil organic carbon ( $C_{org}$ ) was measured using the Tjurin method (Vorobeva, 1998), and total nitrogen ( $N_{tot}$ ) concentration was measured using the Kjeldahl method (van Reeuwijk, 1995). The concentrations of plant-available nutrients in the soil (P, K, Ca and Mg) were determined by the ammonium lactate (AL) method (Egnér et al., 1960).

### **Statistical data analysis**

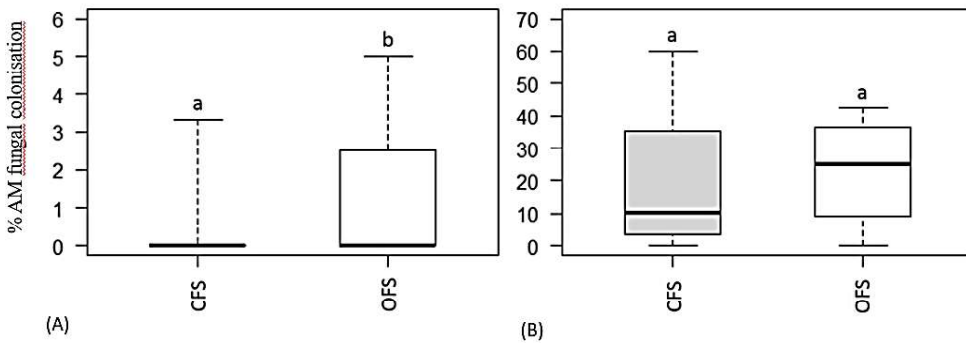
Statistical analysis was performed using R 3.22 (R Core Team 2015) within R-Studio environment (0.99.484; RStudio 2015). First, normal distribution (*Shapiro-Wilk* test) and equality of variances in treatment groups (*Levene* test) of data were explored. Then, the non-parametric *Kruskal-Wallis rank sum* test was conducted to

determine the differences in among treatments followed by the *Dunn's* test for pairwise comparison. For the *Dunn's* test, the *P*-values were adjusted according to the *Bonferroni correction*. When comparing only two groups, the non-parametric *Wilcoxon signed-rank* test was used. To study the relationship between soil parameters and AM fungal colonisation *Pearson's correlation* analysis was carried out.

## RESULTS AND DISCUSSION

### Potato root AM fungal colonisation

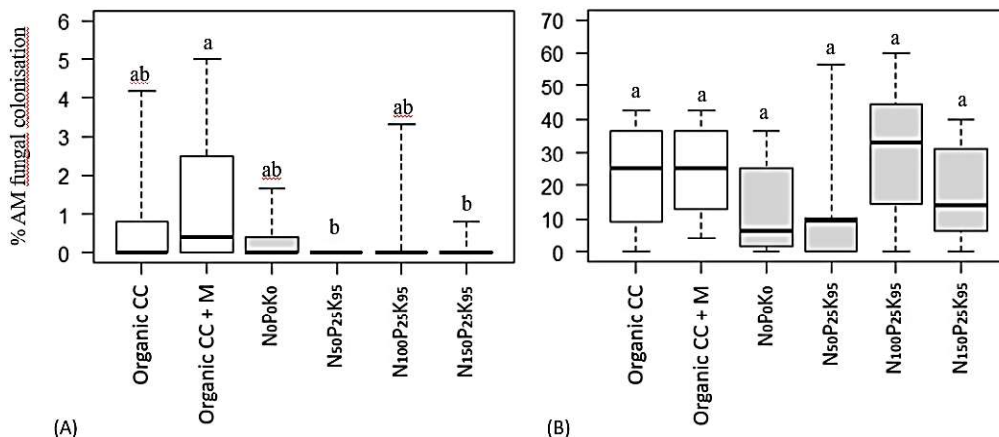
AM fungal colonisation level in roots of the potato cultivar 'Reet' was higher in OFS than CFS, though colonisation levels were very low in both systems (median 0%, maximum 3.3% in CFS and 5.0% in OFS; *Wilcoxon signed-rank* test,  $W = 415.5$ ,  $p = 0.007$ ; Fig. 1, A). Individual treatments influenced root AM fungal colonisation levels differently. AM fungal colonisation was higher in treatment CC+M than treatments N50 and N150 (*Kruskal-Wallis* test,  $\chi^2 = 11.85$ ,  $df = 5$ ,  $p = 0.037$ ; Fig. 2, A). There were no statistically significant differences among other treatments.



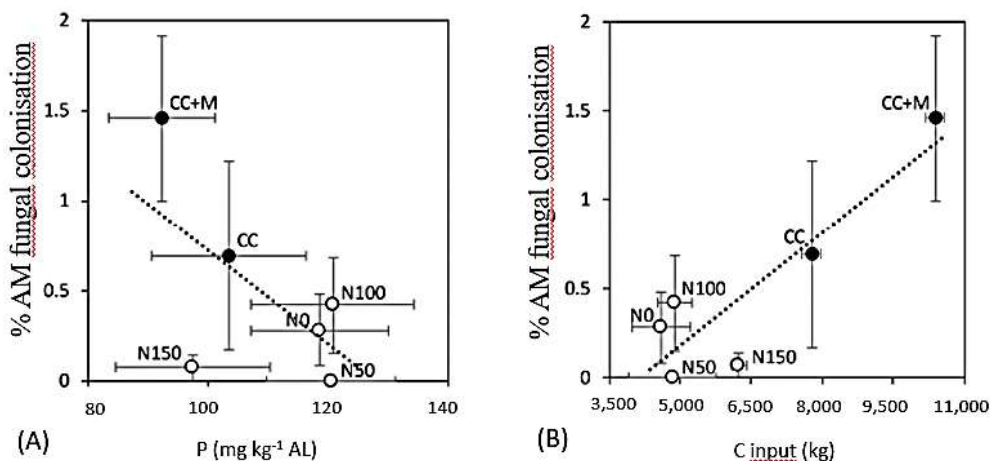
**Figure 1.** Median values ( $\pm$  min, max) of percent of arbuscular mycorrhizal (AM) fungal colonisation of potato roots in conventional (CFS) and organic farming systems (OFS) in the field trial (A) and narrowleaf plantain roots grown in pots in sand inoculated with soil from the same conventional and organic farming systems (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at  $P < 0.05$  among treatments (*Wilcoxon rank sum* test). Grey boxes – CFS, open boxes – OFS.

Potato root AM fungal colonisation level was negatively correlated with soil phosphorus content ( $r = -0.50$ ,  $p = 0.012$ , Fig. 3, A; Table S2) and positively with total annual C input to the soil ( $r = 0.46$ ,  $p = 0.023$ ; Fig. 3, B; Table S2). When exploring these relationships separately by treatment groups, the relationship between soil phosphorus content and potato root AM fungal colonisation level remained for OFS ( $r = -0.78$ ,  $p = 0.022$ ; Table S3), but not for CFS ( $r = -0.11$ ,  $p = 0.67$ ; Table S4). In the case of relationship between total annual C input to soil and potato root AM fungal colonisation level, there were no significant relationships neither for OFS ( $r = 0.26$ ,  $p = 0.52$ ; Table S3) nor CFS ( $r = -0.35$ ,  $p = 0.17$ ; Table S4). Exceptionally, in OFS, potato root AM fungal colonisation level was negatively correlated with  $C_{org}$  ( $r = -0.76$ ,

$p = 0.03$ ; Table S3). There were no significant relationships between soil pH,  $C_{org}$ , K, Ca, Mg and total N content, and potato AM fungal colonisation (Table S2, Table S5).



**Figure 2.** Median values ( $\pm$  min, max) of percent of AM fungal colonisation of potato roots in different agricultural treatments in the field trial (A) and narrowleaf plantain roots in greenhouse trial inoculated with soils from the same agricultural treatments in the field (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at  $P < 0.05$  among treatments (*Kruskal-Wallis rank sum* test, as post hoc test, was used *Dunn* test with *Bonferroni* correction). Open boxes – organic farming systems, grey boxes – conventional farming systems. CC – farming system with cover crops, CC + M – farming system with cover crops and composted cattle manure, N<sub>0</sub>P<sub>0</sub>K<sub>0</sub> – control system with no additional fertilizers used, N<sub>50</sub>P<sub>25</sub>K<sub>95</sub>, N<sub>100</sub>P<sub>25</sub>K<sub>95</sub>, and N<sub>150</sub>P<sub>25</sub>K<sub>95</sub> – systems with different N rates used.



**Figure 3.** Relationships between AM fungal colonisation of potato roots and (A) soil phosphorus content and (B) total annual carbon input in the field trial. The points with whiskers indicate the mean ( $\pm$  standard error) of four replicate samples per treatment; the dotted lines present the linear relationship. Closed circles – organic farming systems, open circles – conventional farming systems. See Fig. 2 legend for treatment coding.

**Table S2.** Linear correlation coefficients between soil characteristics and AMF colonisation in potato roots

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	<b>0.57*</b>	1							
P (mg kg <sup>-1</sup> )	<b>0.63*</b>	0.39	1						
K (mg kg <sup>-1</sup> )	0.27	0.33	<b>0.48*</b>	1					
Mg (mg kg <sup>-1</sup> )	<b>0.78*</b>	<b>0.45*</b>	<b>0.56*</b>	0.38	1				
Ca (mg kg <sup>-1</sup> )	<b>0.86*</b>	<b>0.67*</b>	<b>0.59*</b>	0.4	<b>0.85*</b>	1			
Total N (%)	<b>0.55*</b>	<b>0.74*</b>	<b>0.41*</b>	<b>0.54*</b>	<b>0.44*</b>	<b>0.62*</b>	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.35	<b>0.50*</b>	-0.24	0.12	0.25	<b>0.43*</b>	<b>0.52*</b>	1	
AM fungal colonisation (%)	-0.04	0.09	<b>-0.50*</b>	-0.18	-0.09	-0.00	0.09	<b>0.46*</b>	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S3.** Linear correlation coefficients between soil characteristics and AMF colonisation of potato in OFS

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	0.09	1							
P (mg kg <sup>-1</sup> )	0.67	0.59	1						
K (mg kg <sup>-1</sup> )	0.53	0.35	0.32	1					
Mg (mg kg <sup>-1</sup> )	<b>0.90*</b>	-0.09	0.51	0.34	1				
Ca (mg kg <sup>-1</sup> )	<b>0.96*</b>	0.25	<b>0.79*</b>	0.53	<b>0.87*</b>	1			
Total N (%)	0.37	0.56	0.44	<b>0.81*</b>	0.10	0.46	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.28	0.07	-0.10	<b>0.74*</b>	0.25	0.31	0.67	1	
AM fungal colonisation (%)	-0.31	<b>-0.76*</b>	<b>-0.78*</b>	0.00	-0.31	-0.47	-0.13	0.26	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S4.** Linear correlation coefficients between soil characteristics and AMF colonisation of potato in CFS

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	<b>0.63*</b>	1							
P (mg kg <sup>-1</sup> )	<b>0.82*</b>	<b>0.67*</b>	1						
K (mg kg <sup>-1</sup> )	0.26	<b>0.62*</b>	<b>0.53*</b>	1					
Mg (mg kg <sup>-1</sup> )	<b>0.76*</b>	<b>0.68*</b>	<b>0.87*</b>	<b>0.57*</b>	1				
Ca (mg kg <sup>-1</sup> )	<b>0.81*</b>	<b>0.71*</b>	<b>0.85*</b>	<b>0.57*</b>	<b>0.88*</b>	1			
Total N (%)	<b>0.61*</b>	<b>0.81*</b>	<b>0.72*</b>	<b>0.57*</b>	<b>0.72*</b>	<b>0.62*</b>	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.23	0.30	0.11	0.39	0.02	0.17	0.25	1	
AM fungal colonisation (%)	-0.26	0.04	-0.11	-0.18	-0.20	-0.15	-0.22	-0.35	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S5.** Average ( $\pm$ standard error) soil characteristics by treatments

	Treatment					
	N0	N50	N100	N150	CC	CC $\pm$ M
pH KCl	5.95 ( $\pm$ 0.21)	5.83 ( $\pm$ 0.22)	5.73 ( $\pm$ 0.19)	5.72 ( $\pm$ 0.11)	5.94 ( $\pm$ 0.10)	6.03 ( $\pm$ 0.19)
C <sub>org</sub> (%)	1.22 ( $\pm$ 0.12)	1.17 ( $\pm$ 0.08)	1.30 ( $\pm$ 0.12)	1.34 ( $\pm$ 0.10)	1.46 ( $\pm$ 0.06)	1.43 ( $\pm$ 0.07)
P (mg kg <sup>-1</sup> )	118.6 ( $\pm$ 11.4)	120.6 ( $\pm$ 10.7)	120.8 ( $\pm$ 13.5)	97.5 ( $\pm$ 12.9)	103.4 ( $\pm$ 12.9)	92.3 ( $\pm$ 9.0)
K (mg kg <sup>-1</sup> )	146.4 ( $\pm$ 5.3)	170.8 ( $\pm$ 6.8)	167.4 ( $\pm$ 8.8)	165.0 ( $\pm$ 10.7)	139.1 ( $\pm$ 9.7)	167.1 ( $\pm$ 11.3)
Mg (mg kg <sup>-1</sup> )	127.8 ( $\pm$ 15.1)	147.0 ( $\pm$ 12.6)	147.3 ( $\pm$ 14.6)	120.7 ( $\pm$ 15.0)	143.1 ( $\pm$ 11.3)	161.3 ( $\pm$ 33.4)
Ca (mg kg <sup>-1</sup> )	1,156 ( $\pm$ 143.0)	1,310 ( $\pm$ 133.4)	1,247 ( $\pm$ 84.0)	1,185 ( $\pm$ 105.0)	1377 ( $\pm$ 90.0)	1,452 ( $\pm$ 137.8)
Total N (%)	0.122 ( $\pm$ 0.007)	0.126 ( $\pm$ 0.002)	0.127 ( $\pm$ 0.010)	0.127 ( $\pm$ 0.008)	0.129 ( $\pm$ 0.011)	0.145 ( $\pm$ 0.005)
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	4,604 ( $\pm$ 640.1) <sup>A</sup>	4,841 ( $\pm$ 955.4) <sup>A</sup>	4,900 ( $\pm$ 615.1) <sup>A</sup>	6,241 ( $\pm$ 445.0) <sup>AB</sup>	7,778 ( $\pm$ 195.1) <sup>B</sup>	1,0394 ( $\pm$ 195.1) <sup>C</sup>

Means with different letters are statistically significantly different among treatments ( $p < 0.05$ , *Tukey* test). See Fig. 2 legend for treatment coding.



### **AM fungal inoculum potential of arable soil**

Root AM fungal colonisation of narrowleaf plantain in greenhouse trial was significantly greater ( $p < 0.001$ ) than that of field-grown potato roots in both farming systems (data not shown). The median AM fungal colonisation levels in the narrowleaf plantain roots were 10.6% (range 0–60%) in CFS and 25.0% (range 0–42.5%) in OFS. The AM fungal colonisation of narrowleaf plantain showed an insignificant tendency to be higher in the OFS than in the CFS (*Wilcoxon signed-rank test*,  $W = 451.5$ ,  $p = 0.14$ ; Fig. 1B). AM fungal root colonisation values in soils from individual field treatments varied considerably, but with no significant differences between treatments.

## **DISCUSSION**

By combining a field trial and trap plant greenhouse assay we show that AM fungal colonisation in potato roots in the field conditions was very low, regardless of sufficient amount of AM fungal inoculum in the field soils to support moderate root colonisation levels of trap plant narrowleaf plantain inoculated with these field soils. AM fungal root colonisation tended to be higher in the organic farming systems both in the field grown potato roots and greenhouse-grown plantain roots. The individual fertilizing treatments in the conventional and organic farming systems did not show regular differences in root AM fungal colonisation levels neither in the field potato roots, nor trap plant roots, with the exception of positive effect of manure amendment in organic farming system as compared to inorganic fertilizer addition in the conventional farming system. We found that high soil P content decreased root AM fungal colonisation and higher annual C input to the soil increased root AM fungal colonisation. Therefore, the positive impact of organic farming on the potato AM fungal colonisation in our study system can be explained by previous higher fresh organic matter input and lower soil P content under manure amendment treatment. These results suggest that the potato cultivar studied by us could be a relatively poor AM host, and that AM fungal abundance and functioning is further decreased by higher soil fertility, but can be improved by organic farming practices such as use of manure as fertiliser.

In the present study root mycorrhizal colonisation rate was measured for a locally bred potato cultivar 'Reet'. This relatively new cultivar is described by breeders Tsahkna & Tähtjärv (2008). Breeding programs are generally conducted in experimental stations under high nutrient levels (Philippot et al., 2013). This has resulted in several crops showing lower root mycorrhizal fungal colonisation and lower mycorrhizal growth response than their wild progenitors, though large variations exist (Martin-Robles et al., 2018). It is therefore conceivable that modern potato varieties may have lower mycorrhizal dependence caused by selective breeding under conditions where plants receive little benefit from mycorrhizal symbiosis. This possibility is supported by evidence that newer cultivated plant varieties tend to have lower AM fungal root colonisation (Lehmann et al., 2012). Similar to our results, very low root colonisations have also been reported in earlier field surveys of potato (Cesaro et al., 2008). Furthermore, potato root AM fungal colonisation can vary to a large degree across plant growth phases (Buysens et al., 2017).

Additionally, soil in our field experiment had high to very high plant-available P levels (Schick et al., 2013), which could be one of the reasons for low observed AM fungal root colonisation. Negative relationship between root AM fungal colonisation and

soil P level as observed in this study, is frequently reported in other agricultural and natural systems (Verbruggen et al., 2013). As P availability increases, plants in return become less dependent on AM fungi and down-regulate their mycorrhiza formation (Smith & Read, 2008). Still, not only phosphorus fertilization negatively affects AM fungi, but previous studies have shown that high-input conventional farming as a whole chemical-dependent system negatively affects AM fungi (Kabir, 2005; Verbruggen et al., 2010; Prosser et al., 2015).

However, our root colonisation data verified the previous findings (Jansa et al., 2006) that manure amendment has beneficial effect on plant colonisation by AM fungi compared to the application of inorganic fertilizers. This is in accordance with findings by Gryndler et al. (2006), who showed that in manured soil the concentration of AM fungal spores and mycelial growth increased with mineral fertilization. Furthermore AM fungal colonisation rate in plant roots might also be influenced by other factors related to farming practices like cropping history. A meta-analysis conducted by Lekberg & Koide (2005) showed that avoiding non-mycorrhizal plants in crop rotation has a positive effect on subsequent mycorrhizal colonisation. The cover crop used in the current study was winter oilseed rape, which is a non-mycorrhizal crop plant. Therefore, low AM fungal colonisation rate in potato roots of our study could in part result from usage of non-mycorrhizal crops as a cover crop, which possibly decreased the positive effect of AM fungi for the following crop.

In comparison to the field crop, potato, the trap plant narrowleaf plantain roots showed higher AM fungal colonisation, indicating that the field soils were not exhausted of AM fungi. It is noteworthy that the higher root colonisation of plantains was obtained on the field soil diluted with sand, effectively reducing the amount of available AM fungal propagules for the host plants compared to that available for potato plants in the field. Narrowleaf plantain is a widely used plant species because of its commonly high mycorrhizal colonisation and responsiveness, as well as its broad range of AM fungal partners (Schnoor et al., 2011; Davison et al., 2015). Our comparison of potato and plantain also confirms that host plant–AM fungal relationships depend on both symbiosis partners (e.g., Bever, 2002), whereby the same fungal inoculum may result in very different plant root colonisation levels, fungal community compositions and plant growth in the case of different host plant species. Host-AM fungal compatibility may also influence potato yield, as shown earlier in inoculation trials of micropropagated potatoes (Duffy & Cassells, 2000). Whether a different AM fungal community would result in a different root colonisation (and ultimately, yield) of the potato cultivar studied by us, requires further testing.

## CONCLUSIONS

Our study demonstrated extremely low AM fungal colonisation rate in the roots of potato in field conditions. At the same time, AM fungal colonisation was higher in organic than in conventional farming system, and was related to higher fresh organic matter input and lower soil phosphorus content. Interestingly, both soils from the conventional and organic field had relatively high AM fungal inoculum potential as detected by trap plant assay with narrowleaf plantain. These results suggest that plant species or cultivar can have a strong influence on AM fungal colonisation levels. Further research is needed to clarify whether other varieties of potato show similarly low levels

of AM fungal colonisation as the cultivar used in this study, and to which degree plant growth phase affects this measure. Furthermore, it is necessary to explore how AM fungal species diversity in field potato roots relates to the root colonisation levels and potato yield of different cultivars under the regionally used potato cropping systems. This would provide guidelines for the most efficient management of AM fungi in these cropping systems with regionally used cultivars, fertilization levels, cover crop and pesticide usages considering optimal potato production at the regional scale.

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