Den Europæiske Landbrugsfond for Udvikling af Landdistrikterne:
Danmark og Europa investerer i landdistrikterne

Miljø- og Fødevareministeriet
Landbrugsstyrelsen

Den Europæiske Landbrugsfond
for Udvikling af Landdistrikterne

LDP 2020

Se EU-Kommissionen, Den Europæiske Landbrugsfond for Udvikling af Landdistrikterne

Final Project Report, SEGES 2706, "Sundere jord"

The significance of reduced soil tillage for the occurrence and activity of arbuscular mycorrhizal fungi in the field

Guest Researcher Mayra E. Gavito, Department of Plant and Environmental Sciences, University of Copenhagen and Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México

Associate Professor Ole S Lund, Professor Emeritus Iver Jakobsen, and Associate Professor Ole Nybroe, Department of Plant and Environmental Sciences, University of Copenhagen

Background

Arbuscular mycorrhizal fungi (AMF) live as obligate symbionts of the majority of plants that provide them with carbon compounds they are not able to obtain from other sources. The mycelium of AMF develops both inside and outside the roots. The mycelium extends into the soil beyond the rhizosphere and thereby expands the soil volume that plants scavenge for phosphorous (P). P taken up and transported by the mycelium is rapidly transferred to the plant via arbuscules that are branched mycelium structures formed inside root cortex cells. AMF have a high capacity to transport P from the soil to the plants (Smith and Read, 2008).

The role of AMF in P nutrition of cereal crops is well documented in many controlled pot experiments, but their importance in P uptake and yield under conventional agriculture is currently under debate since their main contribution to plant nutrition becomes superfluous after the constant addition of fertilizers (Ryan and Graham, 2002). Although the high nutrient availability inhibits root colonization by most AMF, opportunistic AMF may still colonize the roots and drain plant C without contributing to plant nutrition and sometimes cause growth depressions. Mycorrhizal associations have thus been claimed as undesirable for conventional agriculture systems (Graham 2000). However, the current environmental crisis puts pressure on designing more sustainable agricultural systems with soil, water and biodiversity conservation practices and reduced greenhouse gas emissions, and this unavoidably requires a reduction and an optimal use of fertilizers, mainly chemical fertilizers, and of machinery. Under those conditions, AMF may have the chance to coexist functionally with the crops and contribute to their nutrition.

Besides high fertility in agricultural fields, another factor that challenges mycorrhizal development and function is the continuous disruption of the extraradical mycelium in the soil by tillage practices (Gavito and Miller, 1998a,b). AMF mycelium forms underground networks that efficiently explore the soil, capture P and transfer it to the plants that become connected. These networks can remain viable for several months, even after crop harvest and when roots are no longer alive and reconnect rapidly after winter or seasonal drought periods when new plants establish and provide C for fungal development (Pepe et al., 2018). Conventional tillage practices disrupting the soil before planting crops may negatively affect the AMF-mediated P uptake, in particular in the early growth stages, because rebuilding a mycelium network costs plant C and takes time before it becomes functional (Gavito and Miller, 1998 a,b; Miller 1999).

Most field studies have been conducted in maize, a crop in which very early P nutrition is critical for final yields. For this crop the negative effects of tillage on early P uptake were ascribed to disruption of the AMF mycelium in the soil (Kabir et al., 2005). Still, direct field evidence for such tillage-induced reduction of P uptake via the AMF mycelium is lacking. Possible effects of tillage on composition of the AMF community should also be considered as P uptake efficiency (Munkvold et al., 2005) and resistance to disruption (De la Providencia et al. 2005) can differ markedly among AMF. Numerous papers have focused on tillage effects on AMF communities (Jansa et al., 2002; Borriello et al., 2012; Bowles et al., 2016), but rather few have investigated AMF composition, abundance and function in long-term tillage experiments under relevant agronomic conditions. The effects of tillage practices on AMF abundance and function are often mixed with the effects of other agricultural practices and are therefore difficult to disentangle from other factors (Entry et al., 1996). To date, despite the thousands of publications on AMF effects on plant nutrition, very few have demonstrated their contribution under field conditions and even less have shown directly their

specific contribution to plant P uptake. Clearly more fieldwork is needed to quantify the direct contribution of AMF to crop nutrition and to understand how management practices can be used to favor the expression of this natural mechanism for plant nutrition in more sustainable agricultural systems.

Objectives and approach

The aim of this project was to determine how long-term reduced soil tillage affects the abundance and activity of AMF in the field.

The experiments were designed to measure the development of arbuscular mycorrhizal associations and the specific contribution of arbuscular mycorrhizal fungi to P uptake during the early development of two crops in two long-term tillage experiments. We measured this by periodically sampling soil, roots and shoots to follow the development of plants and AMF. Further, we used radioactive P to separate the contribution of mycorrhizal mycelium and roots to plant P uptake. In some experiments, soil labeled with ^{33}P was placed in nylon mesh bags that allowed access only to mycorrhizal hyphae or to roots plus mycorrhizal hyphae to compare the amount of ^{33}P transferred to the plants via the mycorrhizal hyphae alone and via roots and hyphae in combination.

Based on previous evidence we hypothesized that more ^{33}P would be transferred in the early growth stages in the reduced tillage treatments than in the conventional tillage treatment. This is because the mycorrhizal hyphal network from the previous season would be preserved so that mycorrhizal colonization in soil and P transfer to roots could be reactivated faster in the reduced tillage treatment. We also hypothesized that most of the ^{33}P transfer from the mesh bags would be performed by mycorrhizal hyphae; and consequently we expected similar P transfer from hyphal and roots+hyphal bags.

Methodology

Field localities, soil properties, and fertilizer regime

The field site in Aulum belongs to Ejgil Andersen and is located at Vestermosevej 40, 7400 Herning. The long-term tillage experiment was established in 2002. The reduced tillage treatment has been without plowing but including harrowing. This is a sandy soil, with pH of 5.7-5.8 and high P availability (43-67 mg kg) in 2017. It was planted with winter barley (byg) cv. Frigg on September 15, 2017. The owner applied no fertilizer in the autumn but applied 100 kg/ha N27-4S on March 18, 37 tones/ha of manure on April 26, and 120 kg/ha N27-4S on May 9. Also Boxer, Sempra, Manganese nitrate, Hussar Plus, Pixaro, Mustang Forte, and Pro Line were applied for for weed or pest control.

The field site in Jerslev belongs to Jørn Jørgensen and is located at Hovvej 43, 4490 Jerslev. The long-term tillage experiment was established in 1999. The reduced tillage has been without plowing and direct sowing without any prior soil tillage. This is a loam soil, with pH 7-4-7.7 and low P availability (10-15 mg/kg) in 2017. It was planted with oats (havre) cv. Domenik on April 22, 2018. The owner applied 250 kg/ha of N-P-K-S 10-7-18-8, and 300 kg/ha N-34 as fertilizers, and Roundup Power Max, Starone, and Ally for weed or pest control.

At both sites, there are two tillage treatments replicated three times as shown in Figure 1. In Aulum 1 indicates conventional tillage (CT, plowing), while 2 indicates reduced tillage (RT, harrowing). There is another tillage treatment, 3, that was not included in the current experiments). In Jerslev the plowed treatment is marked as (CT) and the directly sown treatment as (RT). To simplify writing, we will use the term reduced tillage (TR) for both harrowing and direct sowing in below text. There was a designated sampling area and a no-sampling area that was used to determine yield within each replicate.

(Aulum) (Jerslev)

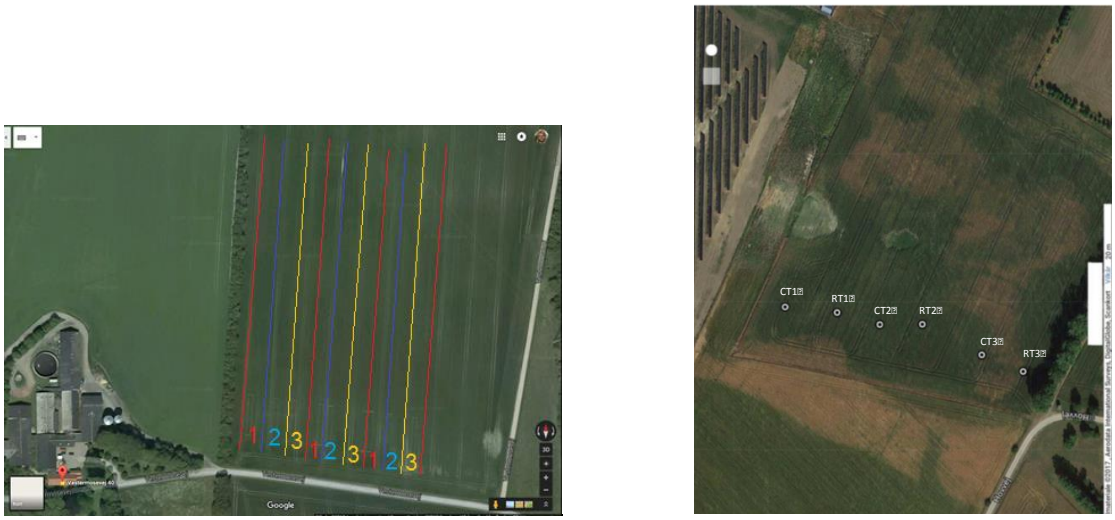


Figure 1. Location of the tillage treatments in Aulum and Jerslev

Experimental design and sampling

Experiments to measure the abundance and activity of arbuscular mycorrhizal fungi in 2017
Activities started in November 2017 in Aulum where winter barley plants were sown by mid-September and were at stages 21-31 on average. Each sampling area was divided into six sections of approximately 10 x 10 m and a marking pin was placed near its center. Samples were taken according to the following general design (Figure 2).

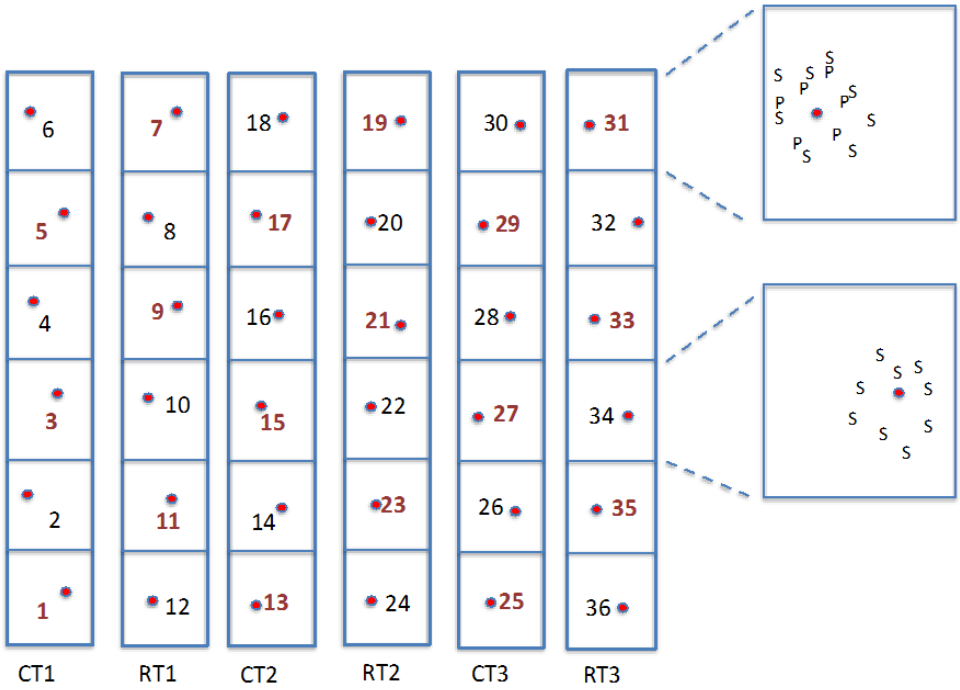


Figure 2. Diagram of a field plot showing the three replicates for the conventional tillage (CT1, CT2, CT3) and reduced tillage (RT1, RT2, RT3) treatments. Each replicate was divided into six sections and these were numbered consecutively. Plant shoots and roots, and soil samples, for molecular and biochemical quantification of mycorrhizal abundance in soil were taken only in all odd number sections (18) to reduce costs of the most expensive determinations. Two sections within one replicate (RT3) are enlarged to show how samples were taken around the central mark in an even and in an odd number section (P: plant samples; S: soil samples)

Samples of plant shoots and roots were obtained by digging up soil monoliths from five random points around the central marking pin. Each monolith contained several plants with their roots and they were taken intact so that the roots could be extracted verifying they belonged to the crop. The shoot and root samples from the same section were pooled together and taken to the lab.

Soil samples were taken with a 5 cm diameter soil borer down to 20 cm depth in six random points around the central marking pin. The soil samples were also composited in a large bag, thoroughly mixed by shaking and a 50 g sample was taken for soil properties. In odd numbered sections we took also a 15 g soil sample for freeze-drying and subsequent fatty-acid analyses. In this way we had spatially representative samples from each replicate: six composite samples for soil properties (coming from the six sections marked in each replicate and within each section from eight soil cores) and three composite samples within each replicate for plant shoots and roots, and for fatty-acid analyses (coming from the three odd-numbered sections marked in each replicate and within each of these sections from eight soil cores and six soil monoliths with intact plants).

The samples were maintained in a cooler with ice blocks until they arrived to the lab. Soil and plant samples for fatty-acid analyses were first frozen at -80 ^{o}C and then freeze-dried. Samples for soil properties were stored in the fridge at 2^{o}C until processed.

Experiments to measure the abundance and activity of arbuscular mycorrhizal fungi in 2018

The experimental designs applied for the experiments in 2018 in Aulum and Jerslev are shown in Figures 3 and 4, respectively. Treatment replicates went vertically from bottom (block 1) to top (block 4) and blocks horizontally from left (CT1) to right (RT3) at both sites. There were five blocks in Jerslev because we included one additional block receiving a fungicide treatment in the mesh bags to estimate P uptake in the absence of mycorrhizae, see below.

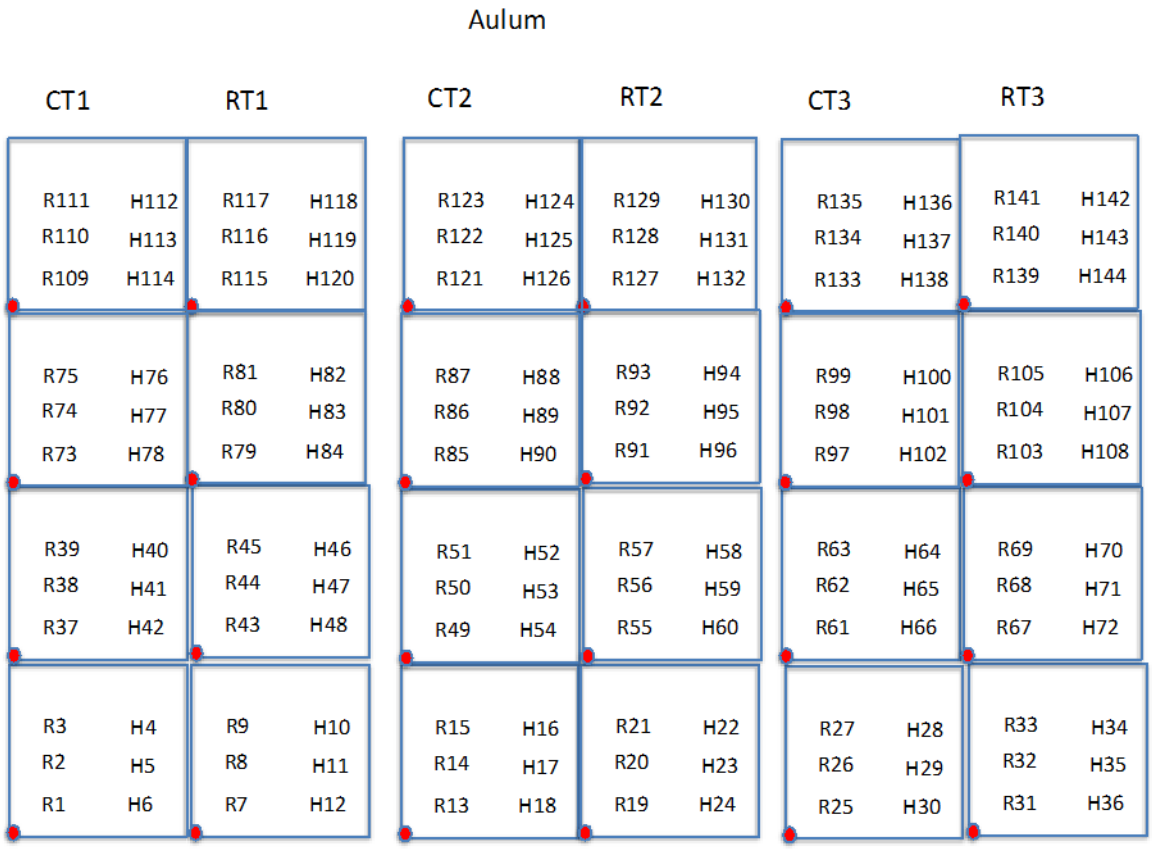


Figure 3. Diagram showing the location of the two treatments, conventional tillage (CT), reduced tillage (RT), and the three replicates of each treatment (CT1, RT1, CT2, RT2, CT3, RT3) in Aulum. There were four blocks from bottom to top and root bags (R) were always on the left and the hyphae bags (H) on the right.

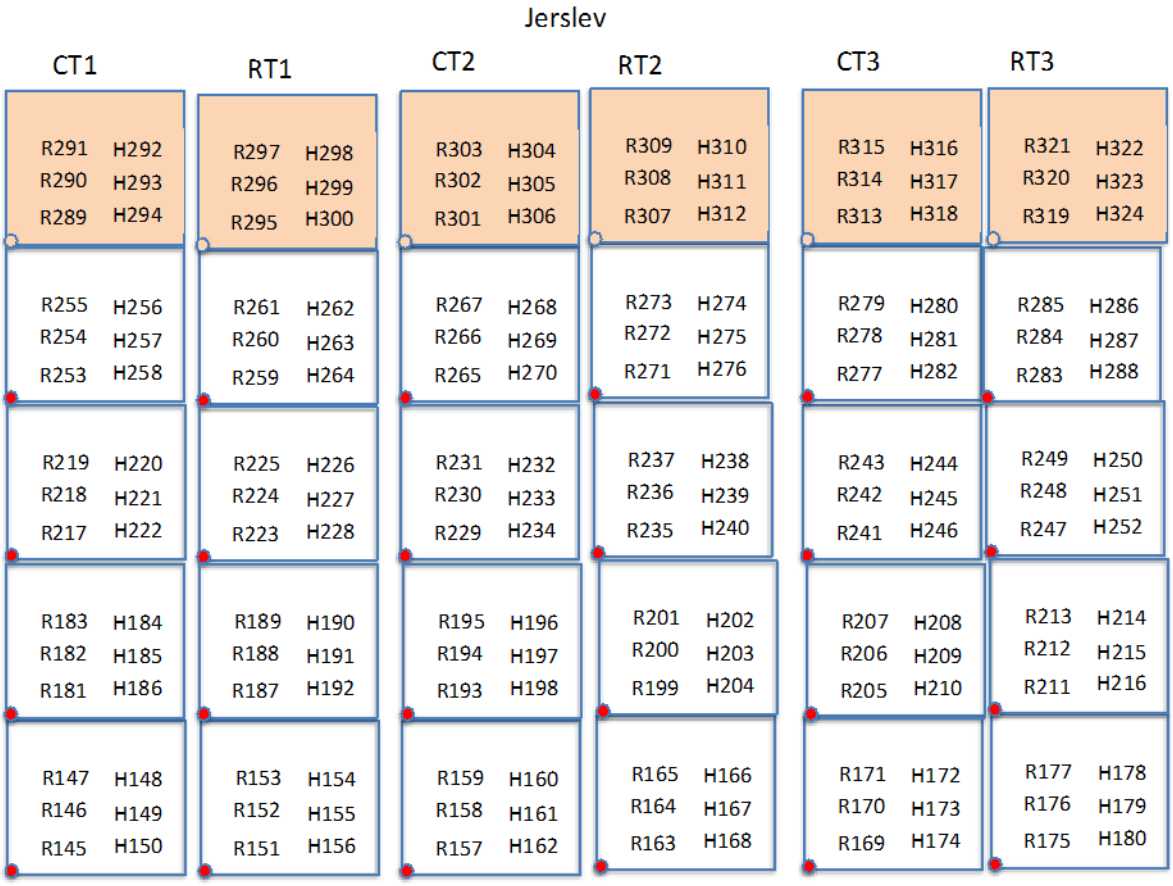


Figure 4. Diagram showing the location of the two treatments, conventional tillage (CT), reduced tillage (RT), and the three replicates of each treatment (CT1, RT1, CT2, RT2, CT3, RT3) in Jerslev. There were five blocks from bottom to top and the root bags (R) were always on the left and the hyphae bags (H) on the right. The fifth block (shading) corresponds to the fungicide (carbendazim) experiment within this experiment.

Mesh bag approach

To compare the amount of ^{33}P transferred to the plants via the mycorrhizal hyphae alone and via roots and hyphae in combination, soil labeled with ^{33}P was placed in nylon mesh bags that allowed access only to mycorrhizal hyphae (hyphal bags) or to roots and mycorrhizal hyphae (root bags).

To establish mesh bags, we collected 30 kg soil from the top 15 cm separately from each tillage treatment within each site as soon as weather allowed it, in early April 2018. The soil was air dried in the lab and a sample was used to determine water content and water retention capacity. We prepared batches of the air-dried soil for each treatment and site combination (CT Aulum, RT Aulum, CT Jerslev, RT Jerslev).

In parallel, we prepared four batches of 200 g of sand dried at 60^0C and added by pipetting homogeneously 20 ml of a solution containing 64.5 MBq ^{33}P prepared with ^{33}P Radionuclide Orthophosphoric acid in 5 mCi/ml HCl-free water (Perkin-Elmer). The sand with the isotope was mixed with a glass spatula, and mixed with its corresponding batch of soil using a large soil mixer. The resulting soil-sand mix was labeled with 10

KBq/g of ^{33}P with the desired activity (depending on the day bags were buried). After mixing thoroughly, 112 g of labeled soil-sand mix were placed directly in mesh bags (approx. 1MBq per bag).

We included 72 bags with 25 microns mesh that prevented the passage of roots (hyphal bags) and 72 bags with 250 microns mesh that allowed the passage of roots and hyphae (root bags) for each site. Three bags of each type (Root bags or Hyphal bags) were buried in four sections within each replicate plot, which were considered as blocks in the experimental design.

In Jerslev an extra block was included where the mesh bags received a fungicide treatment to estimate P uptake in the absence of mycorrhizae. The preparation of soil for these bags was as described above, but in addition they received a solution containing 100 microgram of Carbendazim fungicide per g soil to inhibit the activity of AMF (Larsen et al., 1996; Schweiger and Jakobsen, 1999).

The bags were buried at 10 cm depth and at least 1-m distance from each other in parallel rows (Figure 5) separated also by at least 1-m distance so that there was always a minimum 1-m separation between all bags (Figure 6). In Jerslev the bags were buried as soon as emerging plants were visible to locate the rows. We packed the soil back after burying them and poured a bit of water on top to ensure close contact with soil and to prevent air gaps.

Figure 5. Mesh bag buried between two rows of plants, at a depth between 5 and 10 cm.

All mesh bags were buried at the beginning of the experiment and were retrieved, together with soil, root and shoot samples as indicated in Table 1. The carbendazim bags were removed only at the final date. At each harvest date we harvested a full block, that is one square from each replicate plot. In each experiment, each bag had a unique ID number according to figures 3 and 4.

At harvest, the mesh bags were emptied in a tray and their contents mixed thoroughly. For root bags, we collected the roots with fine forceps, washed them and placed the full sample, or a subsample if there were too many, in a tube filled with 50% alcohol solution for subsequent determination of AMF root colonization. Then we mixed the soil and transferred a sample into a 50 ml tube. For hyphae bags we mixed the soil thoroughly and took soil samples in the same way. The soil weight was registered before and after freeze-drying.

Table 1. Dates for burying and retrieving of mesh bags, soil, root and shoot samples for both field experiments.

	Aulum	Jerslev
Burying bags	27 April	4 May
Collecting bags 1	14 May	22 May
Collecting bags 2	24 May	30 May
Collecting bags 3	6 June	12 June
Collecting bags 4	18 June	25 June

Figure 6. Representative sampling squares in Aulum (left) and Jerslev (right) with red flags signaling the three root bags on the left and the three hyphae bags on the right side.

Figure 7. Example of the method used to define the shoots to be harvested.

Determination of plant dry weight and plant P (total P and ^{33}P)

After pulling out the mesh bags, we harvested the shoots surrounding the place where the bag was buried. We used the hole remaining after removal of the bags to define the center and a 30-cm diameter ring to enclose the shoots to be harvested, as shown in Figure 7. We cut of the shoots of all the plants that were rooted within the frame and stored them in plastic bags. Rhizosphere soil and root samples were obtained from neighboring plants to measure mycorrhizal abundance within each treatment and replicate.

We washed the shoots to eliminate soil particles if necessary, chopped them with strong scissors to facilitate bagging, weighing and subsampling. When plants formed reproductive tissue we separated the vegetative and the reproductive parts and processed them independently. These samples were dried at 65^{o}C to constant weight. After weighing the shoots the three samples close to the root bags and close to the hyphal bags were composited so that we processed only one composite sample for root bags and one for hyphal bags for each of the three replicates of each tillage treatment. The composite samples were finely ground to powder and subjected to acid digestion to measure ^{33}P radioactivity in a scintillation counter (Packard, PerkinElmer, Waltham, MA, USA) and total P concentration colorimetrically in an autoanalyzer.

Quantification of AMF root colonization

Roots from the mesh bags and from the neighboring plants were washed from the 50% ethanol solution, stained with trypan blue (method modified from Phillips and Hayman, 1970) and scored for intraradical colonization under the microscope (McGonigle et al. 1990).

Quantification of AMF in soil

The abundance of mycorrhizal mycelium in soil was measured using the neutral lipid fatty-acid biomarker 16:1w5c, which has been suggested as the best marker for AMF (Olsson et al. 1995). Fatty acids were extracted from four grams of freeze-dried soil and prepared for quantification in a gas chromatograph according to Frostegård and Bååth (1996). Peaks were identified and quantified with MIDI software.

Analyses of fungal community composition in soil

DNA extraction was carried out by phenol chloroform extraction as described in Nicolaisen et al. (2008). Extracts were stored at -70°C. Microbial DNA from the ribosomal 18S rRNA gene (Eukaryotes) was amplified as described in Stokholm et al. (2016) with primer tags targeting Eukaryotes including AMF modified for the amplicon sequencing platform Illumina. Software SCATA software was used for DNA cluster analysis as described by Stokholm et al. (2016). Data from barley roots from field in site Aulum has been processed and presented below. Data from site Jerslev (Oats roots) have been obtained but have not been analyzed. In addition, DNA from AMF fungi was specifically targeted and amplified using primers designed to bind to the ITS region of AMF fungi. Data from AMF primers will be included as soon as the PCR products have been sequenced and data analyzed.

Results

Aulum, sandy high P soil, November 2017

A first evaluation was carried out during autumn 2017. Samples were obtained in mid-November, about two months after sowing of winter barley. Roots were colonized by AMF in both treatments, but interestingly, intraradical mycorrhizal colonization was more than twice as high in the reduced tillage (RT) treatment than in the conventional tillage (CT) treatments (Figure 8A). The amount of AMF mycelium in the soil was not different between the CT and RT treatments and generally low (Figure 8B).

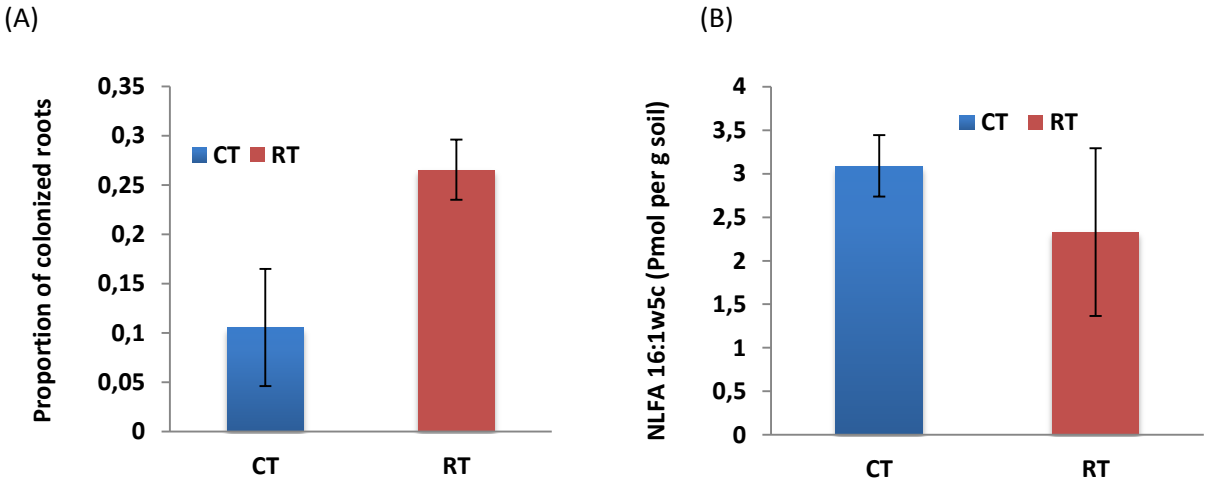


Figure 8. Intraradical (A) and extraradical (soil) (B) mycorrhizal colonization in winter barley plants grown in conventional tillage (CT) or reduced tillage (RT) plots in mid-November 2017.NLFA 16W5c is a fatty acid marker for AMF. Bars represent mean values± S.D., n=3.

Shoot dry mass (Figure 9A) and shoot P concentration (Figure 9B) were similar for both tillage treatments at this point. Before the winter period, crop development was thus similar in both tillage treatments and fungal development was higher in the RT than in the CT treatment.

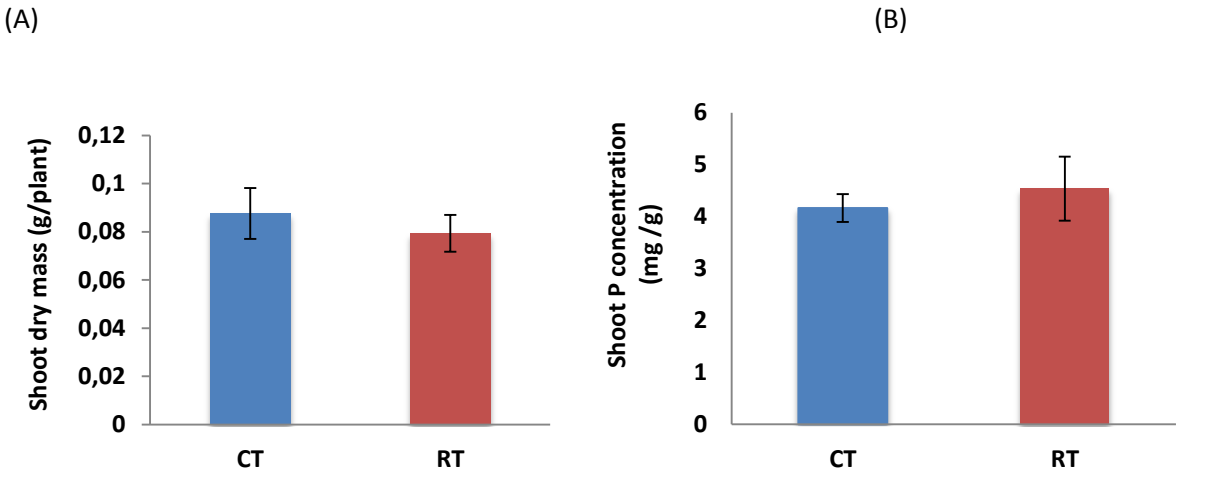


Figure 9. Shoot dry mass per plant (A) and shoot P concentration (B) in winter barley plants grown in conventional tillage (CT) or reduced tillage (RT) plots in mid-November 2017. Bars represent mean values ± S.D., n=3.

Aulum, sandy high P soil, Summer 2018

Mycorrhizal colonization in barley roots after the winter period was, contrary to the observations made in the previous autumn, close to zero in both tillage treatments (Figure 10A). The amount of mycorrhizal mycelium in soil after the winter period dropped to zero in the CT and to 0.29 pmoles per gram soil in the RT (blue and red bars hardly visible, Figure 10B). After 1.5 months, on May 24, mycelium had still not developed in the CT treatment and had not reached 1 pmole per g of soil in the RT treatment (Figure 10B).

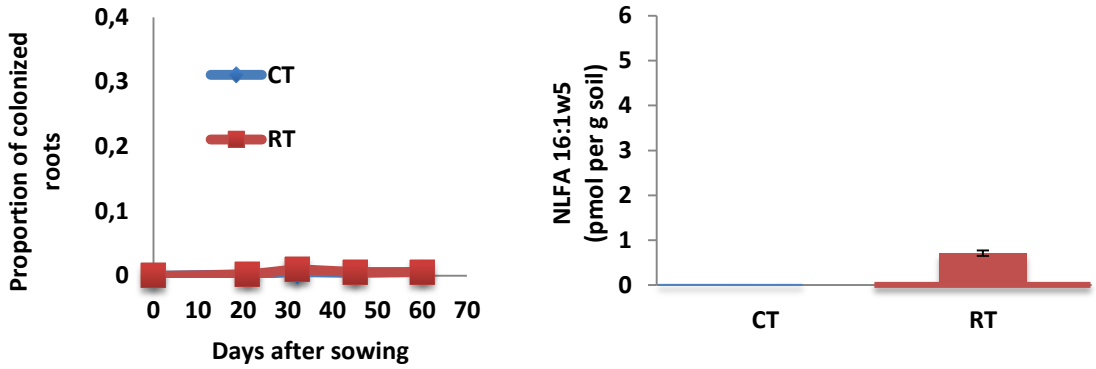


Figure 10. Development of AMF colonization in roots (A) and abundance of mycelium in soil (B) on May 24, approximately 1.5 month after plants reinitiated growth after the winter. Figures are deliberately drawn at the same scale as figures for the autumn evaluation to ease comparison between the two assessments. NLFA 16W5c is a fatty acid marker for AMF.

Shoot total biomass (Figure 11A) and shoot P uptake (Figure 11B) were similar along the measuring period in both tillage treatments. Radioactive P uptake from mesh bags with roots was higher than uptake from bags with only hyphae but again with no differences between tillage treatments (Figure 12A). Despite a trend for higher specific root uptake in conventional tillage roots, the large variation indicated no differences between the roots from both tillage treatments (Figure 12 B).

(A) (B)

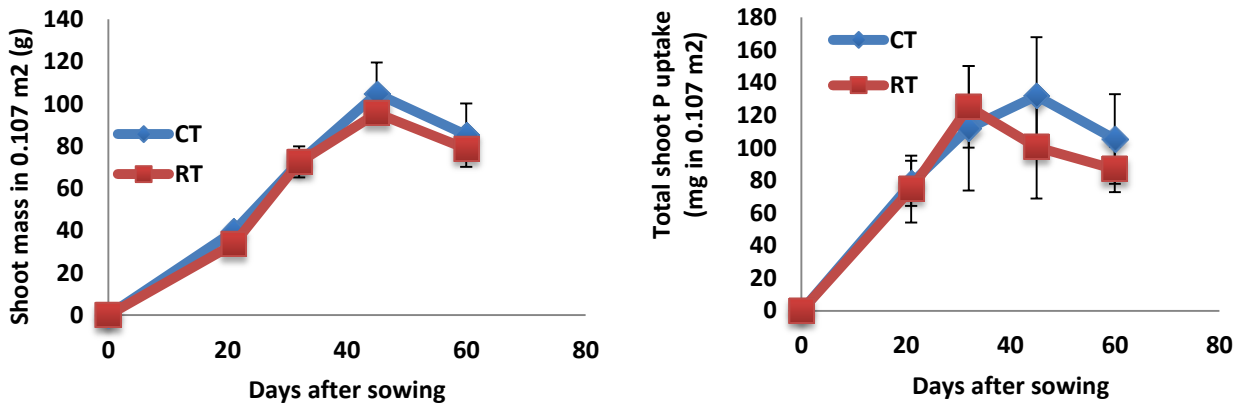


Figure 11. Development of winter barley shoot biomass in the two tillage treatments. Data are presented as mean ± S.D., n=3.

(A) (B)

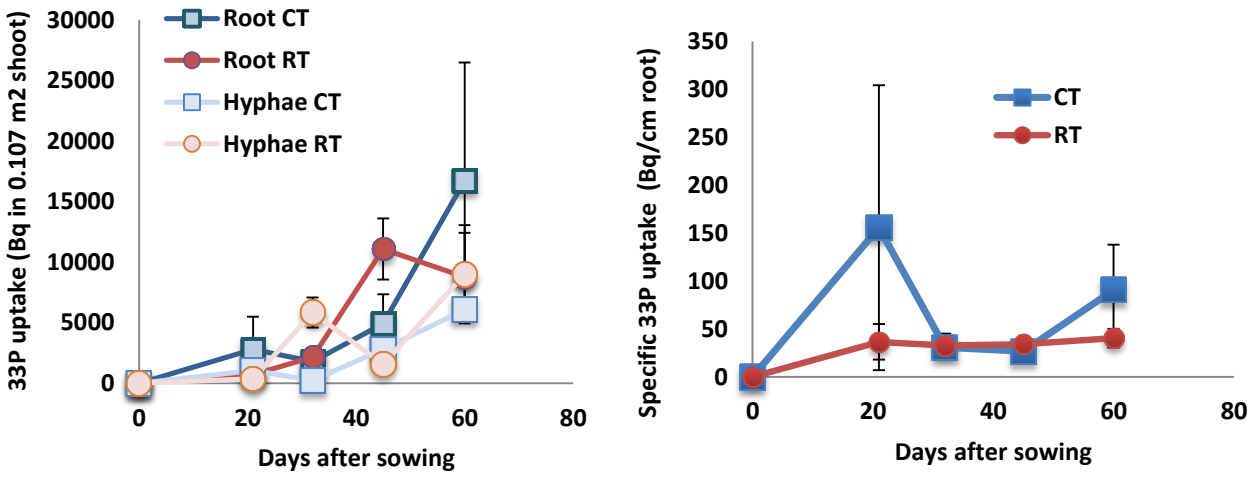


Figure 12. Development of radioactive P uptake in winter barley expressed as total uptake in the shoots (A) and as the capacity of roots to take up P, or root specific uptake (B) in the two tillage treatments. Data are presented as mean ± S.D., n=3.

DNA representing 18S rRNA genes from eukaryotic microbes associated with barley roots in Aulum was amplified from 9 CT samples and 9 RT samples. Samples were collected on 24th May 2018. The fourteen most dominant DNA clusters (OTU´s : Operational Taxonomic Unit) are shown in Table 2 below.

Table 2. Microbial DNA amplified from Barley roots from the Aulum site.

DNA cluster (OTU)	Name	% DNA reads			p-value*
		All samples	Control	Reduced tillage	
#1	Ascomycota_Fusarium	27.01	24.83	27.18	Ns
#2	Nematoda_Acrobeloides/Cephalobus	8.25	11.32	5.19	<0.0005
#3	Zygomycota_Mortierella	6.00	8.54	3.46	<0.025
#4	Rhizaria_Plasmodiophorida_Polymyxa	5.03	4.44	5.61	Ns
#5	Rhizaria_Plasmodiophorida_Woronina	4.75	6.21	3.29	Ns
#6	Chytridiomycota_Chytridiales	4.09	5.28	2.89	Ns
#7	Oomycetes_Aphanomyces-like	3.32	3.73	2.91	Ns
#8	Ascomycota_Glomerellales	3.15	2.83	3.46	Ns
#9	Nematoda_Plectidae	1.79	1.60	1.98	Ns
#10	Basidiomycota_Corticiales	1.71	1.69	1.73	Ns
#11	Ascomycota_Chaetothyriales	1.68	1.67	1.68	Ns
#12	Nematoda_Acrobeloides/Cephalobus	1.64	2.31	0.97	<0.022
#13	Ascomycota_Dothideomycetes	1.45	0.31	2.59	Ns
#14	Rhizaria_Plasmodiophorida_Spongospora	1.15	0.61	1.68	Ns
#15	Other OTU´s	29.0	24.60	33.36	Ns
	All	100	100	100	

*Mann-Whitney test for two samples was used (no requirement for normal distribution of data).

Significant differences were observed between CT and RT samples with respect to the relative abundance of DNA representing two groups/species of Nematode (OTU #2 and #12) and with respect to a cluster of zygomycetes within the genus *Mortierella* (OTU #3). Both the nematodes and the *Motierella* fungi are expected to be non-pathogenic organisms associated with roots and feeding on bacteria and plant root exudate/organic debris respectively.

As can be seen, AMF fungi (Glomeromycota) were not among the 14 most dominant OTU´s and they could not be retrieved even as single sequences from the data sample using reference DNA sequences as baits (Nearest homologues found using AMF DNA from 10 different genera were basidiomycetes with up to 91% identity).

Since the primers have a perfect match to AMF and since we have previously found up to 2% Glomeromycota DNA in roots of 4 years old ray grass (same approach) we believe the relative amount of Glomeromycota DNA in the Barley roots is very low at the time of sampling (May). A second attempt to amplify AMF DNA is ongoing using primers designed to target specifically the ITS region of AMF fungi.

Jerslev, low P soil, summer 2018

The colonization of roots by AMF was comparable for the two tillage treatments along the experiment (Figure 13A). In the control treatment, where the fungicide Carbendazim was applied in the mesh bags, colonization was inhibited in both conventional tillage and the reduced tillage soils (Figure 13A).

(A) (B)

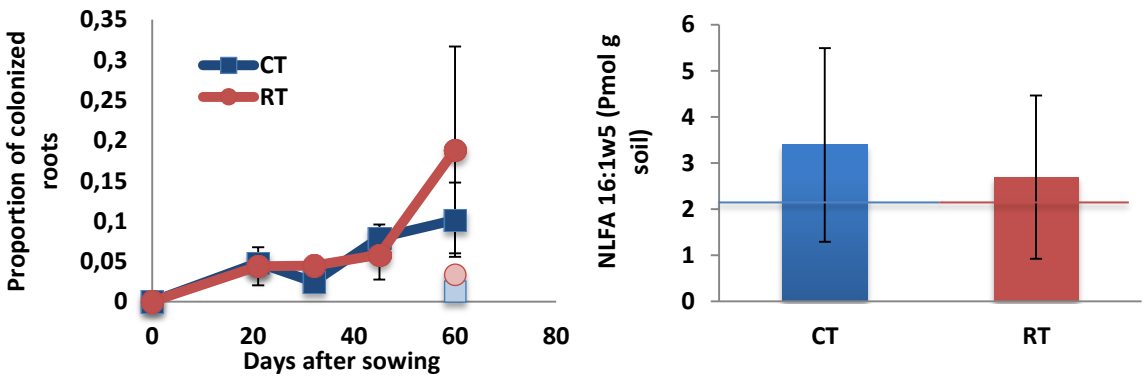


Figure 13. Development of AMF colonization in new roots growing into the mesh bags (A) and abundance of mycelium in soil (B) on June 14, approximately 1.5 month after planting. The pink circle and the light blue square in figure A show that applying Carbendazim fungicide to the mesh bags reduced colonization to basically zero. The blue and red lines in panel B show the amount of mycelium measured in the initial soil collected at the beginning of April, before oats were sown, as baseline for the experiment. Figures are deliberately drawn at the same scale as figures for the experiment in Aulum to ease comparison between the two experiments. NLFA 16W5c is a fatty acid marker for AMF. Data are presented as mean ± S.D., n=3.

Given that root colonization was rather low, mycorrhizal mycelium in soil was examined 1.5 months after sowing oats when plants were already forming grain to see if mycelium in soil was able to develop at the time P uptake was critical. We found that mycelium in soil was similar in the two tillage treatments (Figure 13B) and developed poorly in both treatments, considering that the baseline was 2.09-2.11 pmoles per gram in the soil collected at the beginning of April.

The development of oats plants measured as growth showed minimal differences between conventional tillage and reduced tillage treatments (Figure 14A). Except for one sampling date in the middle of the experiment, shoot total P uptake in oats was not significantly different for the two tillage treatments given the large variation observed, especially during the last two sampling dates (Figure 14B).

(A) (B)

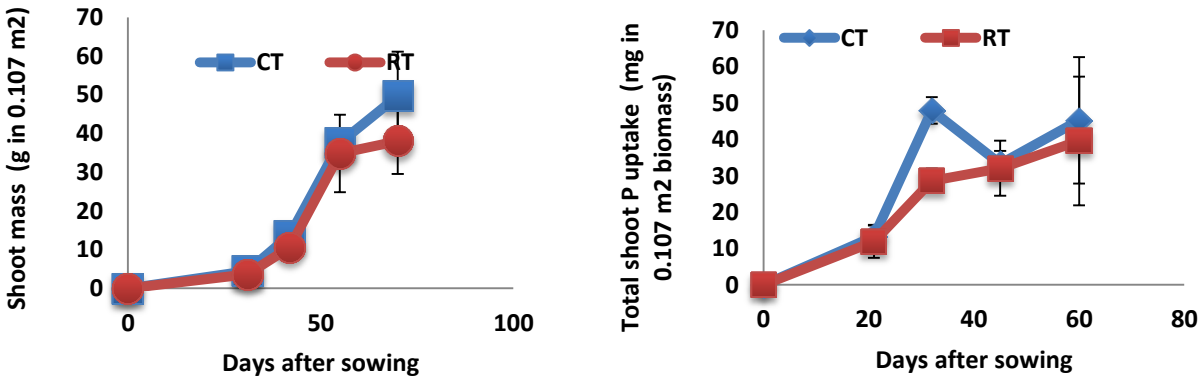


Figure 14. Growth of oats measured as the dry matter content of the shoots (A) and total shoot P uptake (B) along the experiment. Data are presented as mean ± S.D., n=3.

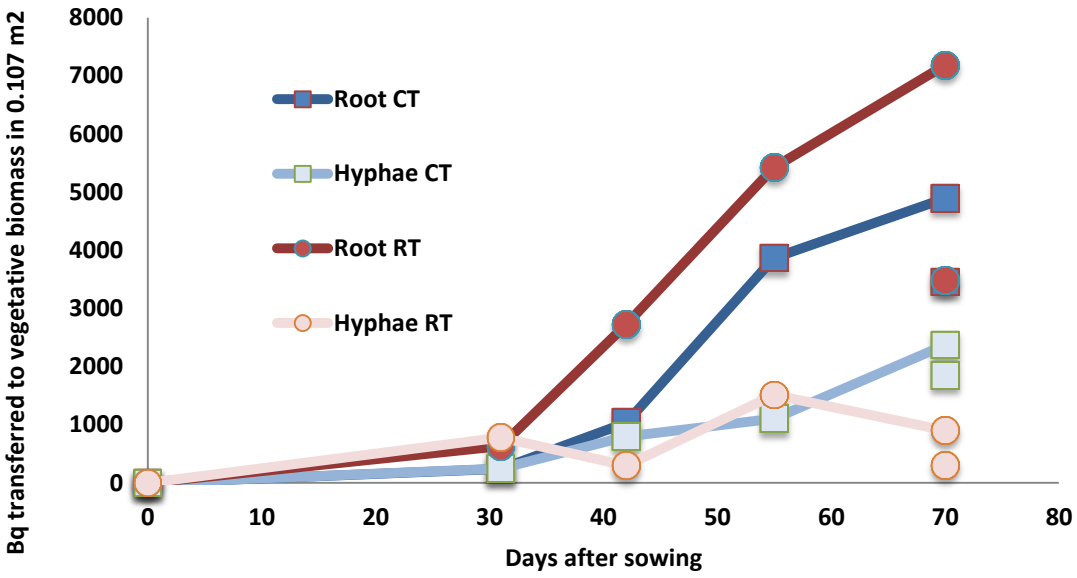
Plant ^{33}P uptake from mesh bags into vegetative and reproductive tissues, respectively, was analyzed (Figure 15 A, B). Uptake from mesh bags allowing ingrowth of hyphae only (hyphal bags) was much lower than uptake from bags allowing ingrowth of roots plus hyphae (root bags). This is in accordance with the poor development of mycelium in soil beyond the background levels measured in the soil collected at the beginning of April. Some of the ^{33}P uptake measured from hyphal bags can be attributed to roots and root hairs surrounding the mesh bags. The amount of radioactive P transferred to vegetative and reproductive tissues was higher from root bags in the RT treatment than from root bags in the CT treatment.

Soil tillage did not change the amount of roots (data not shown) but clearly affected root ^{33}P uptake (Figure 16). Despite the low intraradical colonization and extraradical mycelium development, ^{33}P uptake by RT roots was higher than by CT roots. For CT roots, the ^{33}P uptake was close to zero.

The effect of adding a fungicide to the ^{33}P mesh bag is also shown by the light-red, individual circles in Figure 15 and a pink circle in Figure 16. At the last samplings, transfer of ^{33}P to vegetative and reproductive tissue (Figure 15), and root ^{33}P uptake capacity (Figure 16) were halved by the addition of Carbendazim to

the bags and uptake by RT roots with Carbendazim became comparable to CT root uptake without fungicide.

(A)



(B)

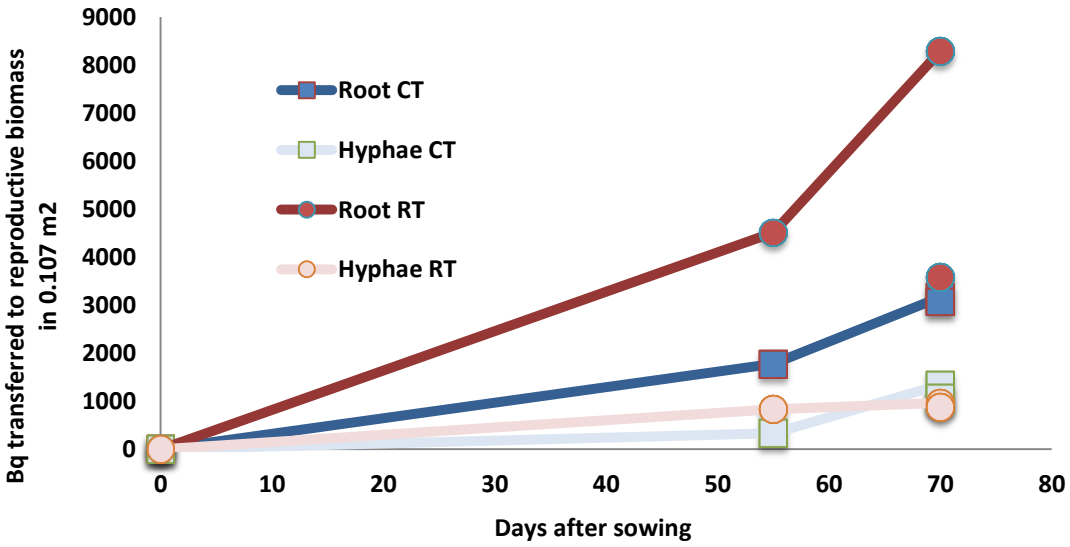


Figure 15. Development of radioactive ^{33}P transferred to shoots (B) or reproductive biomass (B) of oats. The light-red , individual filled circles in (A) and (B) indicate the mean value of the treatment with Carbendazim. Standard deviations are not included to allow visual comparison of the treatments.

Furthermore, specific root transfer of CT roots with and without Carbendazim, and of RT roots with Carbendazim was identical and much lower than specific ^{33}P uptake by RT roots without fungicide. This corresponded to the low colonization determined in roots from plowed soil and with Carbendazim addition (Figure 13A). The effect of the fungicide on ^{33}P uptake and intraradical colonization supports that the high uptake for oats in the reduced tillage treatment is due to the activity of the AMF and to the fact that the integrity of mycelium networks of AMF is better preserved in non-plowed soil than in plowed soil.

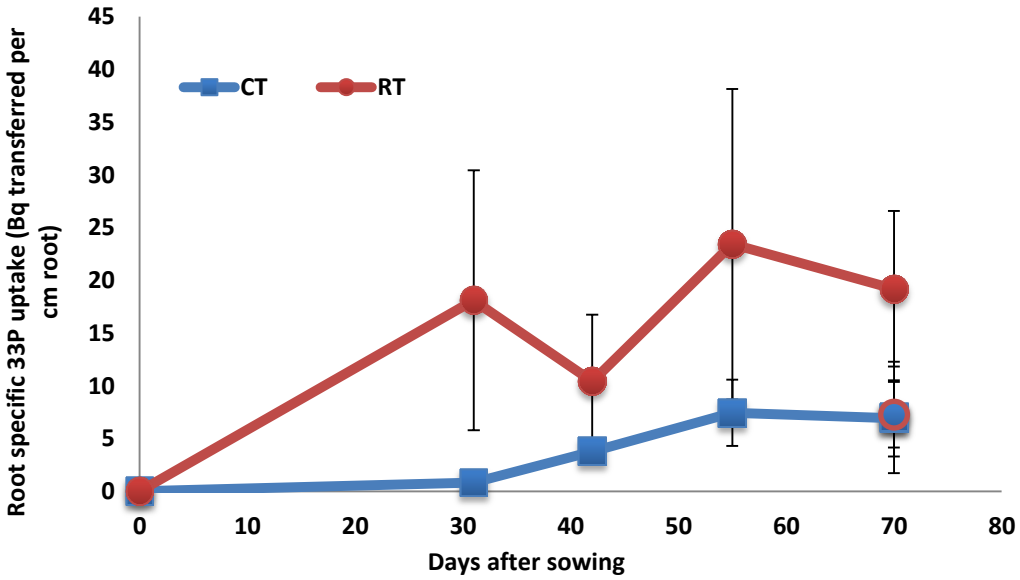


Figure 16. Development of radioactive ^{33}P uptake per cm of root in oats plants. The pink circle and the light blue square behind it show that ^{33}P uptake was halved for reduced tillage samples after addition of the fungicide Carbendazim. Data are presented as mean ± S.D., n=3.

Conclusion and discussion

AMF abundance

The two studies conducted revealed that AMF were present in both study sites, and were slightly more abundant in the low P soil (Jerslev) and in the reduced tillage (RT) treatments, but their abundance and activity were low and heavily hampered by other agricultural practices (fertilization) and unfavorable environmental conditions (drought).

In Aulum, winter barley roots were surprisingly well colonized in the autumn, especially in the RT treatment. Mycorrhizal colonization up to 30% in winter barley roots in November 2017 might be explained by the absence of fertilizer application in this growth period. However, AMF mycelium abundance in soil was very low. The current samples contained only a few picomoles of the marker fatty acid 16W5c, while Scandinavian agricultural soils typically contain in the order of nanomoles per gram soil (Hydbom et al.,

2017). The low amount of AMF in the soil is probably due to the low soil temperature in autumn, which limits growth of AMF in the soil to a larger extent than root colonization (Gavito and Azcón-Aguilar, 2012).

After the winter, mycorrhizal mycelium in soil is expected to drop because of the lack of plant C and the low temperatures, but is expected to recover when both limitations are removed when it warms up and plants can grow again. Our 2018 measurements in Aulum showed, however, that the amount of mycorrhizal mycelium in soil was very low, basically zero in the CT treatment, and hardly measurable in the RT treatment. Further, the root colonization was close to zero and in agreement, AMF were not among the dominating OTUs in barley roots in May 2018. The combination of high fertilization of an already fertile soil and low soil temperature may be related to the very low root and soil colonization by AMF.

Despite the summer of 2018 was one the warmest and driest in the record, soil temperatures in Aulum (10-15^{o}C) was below the threshold for optimal growth observed for several AMF (>15^{o}C for root colonization and >18 ^{o}C for soil colonization (Staddon et al., 2002; Gavito and Azcón-Aguilar, 2012) for most of the experiment. We recorded soil temperatures always around 14:00 h, so the values plotted represent the warmest part of the day, but in continuous data logger measurements (not shown) we observed that most of the day soil temperatures were at least two degrees lower than these values. Soil was shaded by a tight plant cover since the end of April and is likely the reason for the lack of differences in soil temperature between CT and RT treatments. Usually the RT treatment leads to slightly cooler soil temperature than the CT treatment because plant residues shade the soil; but in Aulum the entire field was shaded by the crop from the beginning of the experiment.

In Jerslev, root and soil colonization by AMF was low but in this case the poor development was not attributable to soil temperature since the soil was warmer from the beginning and rose to optimal temperatures rapidly after burying the mesh bags. At this site, the reason is more likely high chemical fertilization and probably also soil toxicity, since one of the preceding crops was a radish. Brassicaceae are well known producers of glucosinolates that are toxic to AMF, and many soil microorganisms, and they inhibit temporarily root colonization (Gavito and Miller, 1998a). Another possibility for this site is that drought prevented higher colonization, since rainfalls during the experiment were extremely rare and the field was not irrigated.

Drought is known to reduce AMF colonization, at least in roots, since plants close their stomata to reduce desiccation. In consequence, C assimilation, and consequently also C allocation belowground, is reduced (Ryan and Ash, 2002). In Jerslev, soil water content dropped gradually from 16% on average (close to field capacity) at the beginning of April, when we collected the initial soil, to 6-7% in the middle of June. Being at approximately 40% of the soil field capacity, oats plants were likely very water stressed. In Aulum, soil dessication was not so severe because of plant shading and lower soil temperature, although this field was not irrigated either. Soil water content dropped from the initial 19% average soil water content to 10% in the middle of June. Therefore, even though soil water content was also low in Aulum, it is unlikely that water stress caused the inhibition of colonization at this site.

AMF activity

According to the extremely low values and the lack of differences in root and soil colonization by AMF in Aulum, ^{33}P uptake from hyphae bags was low, presumably from root hairs passing the mesh and from roots surrounding the bags, and uptake from root bags was slightly higher. There were no differences in radioactive P uptake between CT and RT treatments, either as total ^{33}P transferred or as ^{33}P uptake capacity, since they had a similar root length development in the bags and all roots were basically nonmycorrhizal. Shoot P content and biomass developed similarly as well.

In Jerslev, on the other hand, the presence of AMF in roots and soil (despite being low) resulted in clearer differences in total ^{33}P transferred to the shoot and in root ^{33}P uptake capacity. Contrary to our prediction, uptake from hyphae bags was much lower than from root bags, but AMF abundance in the mesh bags was also very low. Therefore, hyphae activity could not be properly evaluated in both experiments because mycelium development was very poor. However, ^{33}P uptake capacity by mycorrhizal oats roots, even being low mycorrhizal, was much higher than the ^{33}P uptake capacity of nonmycorrhizal roots in the Carbendazim treatment, proving the high capacity of AMF to scavenge and transfer P to plants in field conditions. This capacity makes them ideal to increase fertilizer use efficiency when aiming at reducing and optimizing fertilizer utilization. Unfortunately, the high application of fertilizers in both sites obscured mycorrhizal activity substantially and there were no differences in total shoot P uptake or biomass since all nutrients were in high availability. The results are encouraging to test AMF activity in fields with lower fertilizer applications or in experimental trails where nutrients are not provided in excess, thereby making AMF more abundant and their nutrient transfer activity more relevant.

References

Borriello, R., Lumini, E., Girlanda, M., Bonfante, P., & Bianciotto, V. (2012). Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biology and Fertility of Soils* 48, 911-922

Bowles, T. M., Jackson, L. E., Loeher, M., & Cavagnaro, T. R. (2017). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *Journal of Applied Ecology* 54, 1785-1793

De La Providencia, I. E., De Souza, F. A., Fernández, F., Delmas, N. S., & Declerck, S. (2005). Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *New Phytologist* 165, 261-271

Entry, J. A., Reeves, D. W., Mudd, E., Lee, W. J., Guertal, E., & Raper, R. L. (1996). Influence of compaction from wheel traffic and tillage on arbuscular mycorrhizae infection and nutrient uptake by Zea mays. *Plant and Soil* 180, 139-146.

Frostegård, Å. & Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils* 22, 59-65.

Gavito, M. E., & Miller, M. H. (1998)a. Changes in mycorrhiza development in maize induced by crop management practices. *Plant and Soil* 198, 185-192.

Gavito, M. E., & Miller, M. H. (1998)b. Early phosphorus nutrition**,** mycorrhizae development, dry matter partitioning and yield of maize. *Plant and Soil* 199, 177-186

Gavito, M. E., & Azcón-Aguilar, C. (2012). Temperature stress in arbuscular mycorrhizal fungi: a test for adaptation to soil temperature in three isolates of *Funneliformis mosseae* from different climates. *Agricultural and Food Science* 21, 2-11

Gavito, M. E., Schweiger, P., & Jakobsen, I. (2003). P uptake by arbuscular mycorrhizal fungi: effect of soil temperature and atmospheric CO_2 enrichment. *Global Change Biology* 9, 106-116

Graham, J.H. (2000). Assessing costs of arbuscular mycorrhizal symbiosis in agroecosystems. In: Podila G.K., Douds D.D., eds. *Current advances in mycorrhizae research*. St. Paul, MN, USA: APS Press, 127–140.

Hydbom, S., Ernfors, M., Birgander, J., Hollander, J., Jensen, E. S., & Olsson, P. A. (2017). Reduced tillage stimulated symbiotic fungi and microbial saprotrophs, but did not lead to a shift in the saprotrophic microorganism community structure. *Applied Soil Ecology* 119, 104-114

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I., & Frossard, E. (2002). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza* 12, 225-234

Kabir, Z., O'halloran, I. P., Fyles, J. W., & Hamel, C. (1997). Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. *Plant and Soil* 192, 285-293

Kabir, Z. (2005). Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science* 85, 23-29

Larsen, J., Thingstrup, I., Jakobsen, I., Rosendahl, S. (1996). Benomyl inhibits phosphorus transport but not alkaline phosphatase activity in a Glomus-cucumber symbiosis. *New Phytologist* 132, 127-133

McGonigle, T.P., Miller, M.H., Evans D.G., Fairchild G.L. & Swan J.A**. (**1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 15, 495-501

Miller, M.H. (2000). Arbuscular mycorrhizae and the phosphorus nutrition of maize: a review of Guelph studies. *Canadian Journal of Plant Science* 80, 47-52

Munkvold, L., Kjøller, R., Vestberg, M., Rosendahl, S. & Jakobsen, I. (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* 164, 357-364

Nicolaisen, M.H., Bælum, J., Jacobsen, C.S., & and Sørensen, J. (2008) Transcription dynamics of the functional *tfdA* gene during MCPA herbicide degradation by *Cupriavidus necator* AEO106 (pRO101) in agricultural soil*. Environmental Microbiology* 10, 571-579

Olsson, P.A., Baath, E., Jakobsen, I., & Soderstrom, B. (1995). The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research* 99, 623-629.

Pepe, A., Giovannetti, M., & Sbrana, C. (2018). Lifespan and functionality of mycorrhizal fungal mycelium are uncoupled from host plant lifespan. *Scientific Reports* 8, 10235

Phillips, J.M., & Hayman, D.S. (1980). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi. *Transactions of the British Mycological Society* 55, 158-160

Ryan, M.H., & Ash, J.E. (1996). Colonisation of wheat in southern New South Wales by vesicular-arbuscular mycorrhizal fungi is significantly reduced by drought. *Australian Journal of Experimental Agriculture* 36, 563-569

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., & Graham, J.H. (2018). Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. *New Phytologist* 220, 1092-1107

Schweiger, P.F., & Jakobsen, I. (1999). Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. *Agronomy Journal* 91, 998-1002.

Smith S. E., & Read J. D. (2008). Mycorrhizal Symbiosis. Academic Press, London.

Staddon, P.L., Heinemeyer, A. & Fitter, A.H. (2002). Mycorrhizae and global environmental change: research at different scales. *Plant and Soil* 244**,** 253-261

Stokholm, M.S., Wulff, E.G., Zida, E.P. et al.(2016). DNA barcoding and isolation of vertically transmitted ascomycetes in sorghum from Burkina Faso: *Epicoccum sorghinum* is dominant in seedlings and appears as a common root pathogen. *Microbiological Research* 191, 38-50

Vivekanandan, M., & Fixen, P. E. (1991). Cropping systems effects on mycorrhizal colonization, early growth, and phosphorus uptake of corn. *Soil Science Society of America Journal* 55, 136-140