

DR. JULIEN ROY (Orcid ID : 0000-0003-2964-1314)

PROF. MATTHIAS C RILLIG (Orcid ID : 0000-0003-3541-7853)

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### Title

Legacy effects of pre-crop plant functional group on fungal root symbionts of barley

### Authors

Julien Roy<sup>1,2,6</sup>, Richard van Duijnen<sup>3</sup>, Eva F. Leifheit<sup>1,2</sup>, Susan Mbedi<sup>4,5</sup>, Vicky M. Temperton<sup>3</sup>, and Matthias C. Rillig<sup>1,2</sup>

1. Institut für Biologie, Freie Universität Berlin, 14195 Berlin, Germany
2. Berlin–Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195 Berlin, Germany
3. Institute of Ecology, Leuphana University of Lüneburg, Lüneburg, Germany
4. Naturkundemuseum Berlin, 10115 Berlin, Germany
5. Berlin Center for Genomics in Biodiversity Research, 14195 Berlin, Germany
6. Corresponding Author. E-mail: roy.julien@live.fr

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## Abstract

Arbuscular mycorrhizal (AM) fungi, a group of widespread fungal symbionts of crops, could be important in driving crop yield across crop rotations through plant-soil feedbacks (PSF). However, whether preceding crops have legacy effect on the AM fungi of the subsequent crop is poorly known. We set up an outdoor mesocosm crop rotation experiment that consisted of a first phase growing either one of four pre-crops establishing AM and/or rhizobial symbiosis or not (spring barley, faba bean, lupine, canola), followed by an AM crop, winter barley. After the pre-crop harvest, carbon-rich organic substrates were applied to test whether it attenuated, accentuated or modified the effect of pre-crops. The pre-crop mycorrhizal status, but not its rhizobial status, affected the richness and composition of AM fungi, and this difference, in particular community composition, persisted and increased in the roots of winter barley. The effect of a pre-crop was driven by its single symbiotic group, not its mixed symbiotic group and/or by a crop species-specific effect. This demonstrates that pre-crop symbiotic group has lasting legacy effects on the AM fungal communities and may steer the AM fungal community succession across rotation phases. This effect was accentuated by saw dust amendment, but not wheat straw. Based on the previous observation of decreased crop yield after AM pre-crops, our findings suggest negative PSF at the level of the plant symbiotic group driven by a legacy effect of crop rotation history on AM fungal communities, and that a focus on crop symbiotic group offers additional understanding of PSF.

**Key-words:** Agro-ecosystems; amplicon sequence variants; arbuscular mycorrhizal fungi; crop rotation; mesocosm experiment; plant-soil feedback; phylogenetic scale; sustainable agriculture

## Introduction

Plants influence abiotic and biotic soil properties, which in turn affect plants, defining plant-soil feedbacks (PSF). Over time and plant generations, soils accumulate species-specific pathogens or symbionts, which consequently colonize surrounding plants and their offspring. PSF are positive when plants of a certain species grow better in soil conditioned by individuals of the same species than in soil conditioned by individuals of a different species, and PSF are negative in the reverse case. PSF scale to the ecosystem level to drive plant diversity in natural ecosystems (Bennet et al., 2017, Teste et al., 2017; van der Putten et al., 2013) and affect crop yield in agricultural systems

(Huang et al., 2013). In agriculture, PSF occur particularly during continuous cropping (Mariotte et al., 2018). In fact, crop rotations have probably been implemented from empirical observations of decreasing yield (i.e, negative PSF) over succeeding monoculture crops. Understanding PSF mechanisms is crucial because they may steer ecological restoration of degraded ecosystems or improve crop yield (Mariotte et al., 2018).

As symbionts, arbuscular mycorrhizal (AM) fungi occur in the roots of most plants including crops (Brundrett and Tedersoo 2018) and are strong drivers of plant diversity and productivity (van der Heijden, Bardgett and Van Straalen 2008). AM fungi provide soil nutrients (mostly nitrogen and phosphorus) to the plant in exchange for sugars and lipids (Smith and Read 2008). AM fungi show important functional variation such as the degree of soil and root colonization, investment in spores, propagules sources, phenology, nutrient preferential uptake and ecosystem specialization (Hart and Reader 2002; Hart, Reader and Klironomos 2002). Different lineages within AM fungi drive positive or negative PSF (Koziol and Bever 2019). Therefore, the community composition of AM fungi is of importance in affecting plant growth (van der Heijden et al., 1998; Hoeksema et al., 2010; Maherli and Klironomos 2007). Cover cropping with AM crops can increase root colonization by AM fungi in the subsequent crop (Bowles, Jackson, Loher, and Cavagnaro, 2016; Lekberg and Koide, 2005; Thompson 1987). However, whether preceding crops (hereafter referred to as pre-crops) have a legacy effect on the AM fungal community associated with the following crop remains poorly understood (Berruti, Bianciotto, and Lumini, 2018; Campos, Carvalho, Brígido, Goss and Nobre 2018).

Designing crop rotation schemes that acknowledge the role of AM fungi requires understanding the factors affecting AM fungal communities. Climate, edaphic factors such as fertility or pH and vegetation characteristics all simultaneously affect AM fungal communities (Davison et al., 2015). Host-specificity of AM fungi is generally low (Davison et al., 2015; Lekberg and Waller 2016), although preference has been observed (Torrecillas, Alguacil, & Roldán 2012; Vandenkoornhuyse et al., 2002). Rather than plant taxonomic identity, plant traits and functional group (such herbs, forbs and grasses or ecological strategies such as resource acquisitive or conservative) drive the recruitment of AM fungi (Chagnon, Bradley and Klironomos 2015; Davison et al., 2015; López-García et al., 2017) to form an association where plant and fungal ecological strategies are tightly coupled (Chagnon, Bradley, Maherli and Klironomos, 2013). A focus on plant functional group

in rotation schemes (Lekberg and Koide, 2005) may offer a predictive and mechanistic understanding of PSF beyond metrics of plant evolutionary relatedness and soil physico-chemical parameters (Mariotte et al., 2018).

Fertilization changes the nutritional demand of plants and therefore its investment into mycorrhiza (Johnson 2010; Treseder et al., 2018). While mineral N and P fertilization has mostly been studied, few studies have addressed the influence of organic amendment on AM fungal communities and their symbiosis with plants (Yang et al., 2020). In parallel with building soil organic matter stocks and increasing soil C storage, organic amendments are promising options for decreasing N leaching over winter and to supply the leftover N to the following crop by stimulating microbial nitrogen immobilization. Undoubtedly, AM fungi (indirectly) contribute to decomposition and the uptake of newly available nutrients (Leifheit, Verbruggen, and Rillig 2015; Yang et al., 2020). As a consequence, organic amendment probably affects the AM fungal community through changes in soil stoichiometry and plant investment into mycorrhiza (Johnson 2010; Treseder et al., 2018). It is unknown whether extrinsic inputs of nutrients, particularly in the form of organic carbon-rich substrate (high carbon amendment, HCA), affect AM fungal community composition independently or in interaction with the crop rotation strategy.

It is still uncertain if ecological or functional specialization of AM fungi occurs below or above the species level, or even at higher taxonomic ranks (Sanders and Rodriguez 2016). On the one hand, a turnover of distantly related AM fungi is observed along broad environmental gradients suggesting deep phylogenetic signal in the ecology of AM fungi (Powell and Sikes 2014; Roy et al., 2019). On the other hand, a high variability for certain traits is observed within AM fungal species (Munkvold, Kjøller, Vestberg, Rosendahl and Jakobsen, 2004; Mensah et al., 2005). For example, differences in functional consequences (plant performance and nutrient uptake) can be large among plants colonised by genetically different populations or individuals of one AM fungal species (Koch, Croll and Sanders 2006). Testing whether dissimilarity in community composition among and between treatments is due to the recruitment of relatively closely or distantly related AM fungi (Roy et al., 2019) is important to understand the level of phylogenetic differentiation in AM fungi that induce different functions in soil or in their effects on plants. This will further help identify the ecologically relevant level of diversity to be conserved or engineered and to define bioindicators (i.e. clades that correlate with plant yield).

We set up an outdoor mesocosm crop rotation experiment that consisted of a first phase growing AM or non-AM and/or rhizobial or non-rhizobial crops followed by an AM crop (Fig. S1). After the pre-crop harvest, carbon-rich organic substrates were applied. Yield results suggested a negative PSF at the level of plant symbiotic group (continuous cropping of AM crops), independently of organic fertilization (van Duijnen, Roy, Härdtle, and Temperton, 2018). Here, we characterized root and soil AM fungal communities using molecular techniques and, coupled with recent bioinformatic and phylogenetic sequence analyses and microscopy techniques, we asked:

1. Does the crop symbiotic functional group imprint (i) composition or (ii) richness of AM fungi associated with a following AM crop, and is it observable in roots and soil?
2. If a pre-crop legacy effect is observed, is this legacy attenuated, accentuated or modified by organic amendment?
3. Do the traits of AM fungi reflect community richness or composition?
4. Which community attribute (richness, composition and traits) best correlates with crop yield?

## Material and Methods

### Mesocosm set-up

The experiment was conducted outside at the Leuphana University garden facilities, Lüneburg, Germany ( $53^{\circ}14'23.8''\text{N}$   $10^{\circ}24'45.5''\text{E}$ ). Mesocosms (rectangular pots; edge length of 37.5 cm at the top; edge length of 26.5 cm at the bottom; height of 37 cm; resulting volume of 38 L) were filled with soil originated from the top 0–30 cm of the experimental farm Hohenschulen of the Christian-Albrechts-University in Kiel ( $54^{\circ}19'05.6''\text{N}$   $95^{\circ}8'38.8''\text{E}$ ). The soil is sandy loam (Cambic Luvisol) and has a history of agricultural practice, including cropping with a mixture of catch crops (such as clover and lupine) without fertilization in the growing season before the experiment started, and with maize the season even before, fertilized with slurry and triple superphosphate. The soil had a total of 1.26% C, 0.14% N, a C/N ratio of 9.2 and a pH of 6.0 at the start of the experiment.

### Crop species choices

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The experiment consisted of two phases: during the first, conditioning phase (hereafter referred to as t1), mesocosms were cultivated with either one of four different pre-crops that varied in their symbiotic associations with root microbes (i.e. plant symbiotic groups). Plant symbiotic groups focused on two plant-microbe symbioses: the rhizobial and AM symbioses. Four plant species were used: spring canola (*Brassica napus* cv. Medicus, NPZ; non-rhizobial / non-AM), white lupine (*Lupinus albus* cv. Energy, Feldsaaten Freudenberg; rhizobial / non-AM), spring barley (*Hordeum vulgare* cv. Barke, Saatzucht Breun; non rhizobial / AM) and faba bean (*Vicia faba* cv. Tiffany, NPZ; rhizobial / AM). Thus, each plant species represents a combination of the two symbiotic groups, and the AM or rhizobial plant symbiotic group is replicated with two plant species. The plant AM status is not correlated with the plant rhizobial status. This allows to test the independent effect of each symbiotic group. The statistical interaction between the AM and rhizobial status therefore represents the pre-crop species-specific effect (see below the statistical analyses section). The pre-crop mixed symbiotic group is confounded with species-specific additional evolutionary history and eco-physiological properties. Therefore, no inference can be drawn on the legacy effect of the pre-crop's mixed symbiotic group. However, inferences can be drawn on the legacy effect of each single symbiotic status (mycorrhizal or rhizobial).

The succeeding crop was winter barley (*Hordeum vulgare*, cv. Antonella, Nordsaat Saatzucht, non-rhizobial / AM). Winter barley was chosen because it is widely used in agriculture in Germany. Winter barley is a close relative to spring barley (specifically, they are the same species). Within-species (Semchenko, Saar and Lepik 2017) and even within-population specialization of soil microbes to different plant genotypes (Eck, Stump, Delavaux, Mangan, and Comita, 2019) may induce PSF. In this study, growing winter barley after spring barley represents a treatment of a plant species being grown in a soil conditioned by the same species (conspecific). Growing a second distantly-related crop to spring or winter barley (for instance, faba bean) but of similar symbiotic functional group (for instance, AM) allows to test legacy effect at the plant symbiotic functional group (i.e. growing an AM crop in soils conditioned by AM or non-AM crops, e.g. Teste et al., 2017). If not observed, having two distantly-related pre-crops would inform whether there is stronger legacy effect between conspecifics than heterospecifics, which could be due to a degree of phylogenetic signal in the AM symbiosis.

### Crop rotation and organic amendment timing

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Pre-crops (n=15 pots per pre-crops) were sown on May 2016 along with a mineral fertilization. Fertilization followed recommendations specific for each crop species and standard agricultural German practices. Following pre-crop harvest (one week during late August / early September 2016), three types of carbon-rich substrates (high carbon amendment, HCA) were applied (n=5 pots per pre-crops): wheat straw (W), sawdust (S), or no amendment (No). The HCA treatment was applied one week after pre-crop harvest by mixing the HCA material within the top 10 cm of the soil. The soil in the no-amendment treatment received the soil disturbance as well. Winter barley was sown on October 2016. Soil of winter barley was fertilized with a total of 160 kg N / ha, separated in equal additions on three time points. A control with no pre-crop and no HCA treatment was also conducted in three additional pots (hereafter referred to as Control), sown with winter barley after pre-crop harvest time. More details of the experiment, climatic data and level of fertilization are reported in van Duijnen et al. (2018).

### **Soil sampling**

Soil samples were taken at three time points: at the beginning of the experiment before sowing any plants (t0, May 2016), after the pre-crop harvest (t1, one week during late August / early September 2016) and at crop harvest (t2, one day during late June 2017). T0 samples (n=6) were randomly cored from the soil pile collected to set up the mesocosms. At t1, five soil cores of 1 cm width and 10 cm depth were randomly cored per pot after the removal of the pre-crops, and pooled to give a composite sample for each pot, yielding 60 t1 soil samples and the additional three samples with no pre-crop. At t2, five soil cores of 5 cm width and 10 cm depth were randomly cored after the removal of the crop plants, and pooled to give a composite sample for each pot, yielding 60 t2 soil samples and the additional three samples with no pre-crop and no HCA. Soil samples for assessing soil AM fungal abundance were air-dried. Soil samples devoted to molecular analyses were kept in liquid nitrogen and at -80°C before lab processing.

### **Root sampling**

Root sampling was performed during the second phase of the experiment (t2, crop cultivation), yielding 60 t2 root samples and the additional three root samples of winter barley with no previous pre-crop and no HCA. The entire root system per pot was sampled. Roots were thoroughly washed with tap water. Root samples for assessing root colonization by AM fungi were air-dried. Root

samples devoted to molecular analyses were kept in liquid nitrogen and at -80°C before lab processing.

### **Molecular analyses of AM fungal communities**

Molecular analyses of AM fungi were conducted on a total of 195 samples: six t0 soil samples, 60 t1 soil samples (four pre-crops \* 15 replicates) and three control pots, 60 t2 soil samples (four pre-crops \* 3 HCA treatments \* 5 replicates) and the three control pots, 60 t2 root samples (four pre-crops \* 3 HCA treatments \* 5 replicates) and the three control pots.

**DNA extraction, PCR amplification and Illumina sequencing library preparation.** For the soil samples, DNA was extracted from 250 mg of the pooled soil cores using the PowerSoil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA), following manufacturer's instructions. For the root samples, we use the same procedure as for the soil samples except that we used 100 mg of crushed freeze-dried roots. The protocol for DNA extraction, PCR amplification and Illumina sequencing library preparation followed Roy, Reichel, Brüggemann, Hempel, and Rillig (2017). Briefly, using a nested-PCR approach, we amplified the D1-D2 region of the large subunit (LSU) of rDNA using universal fungal primers bound to a 8 nt-long index for sample multiplexing, first targeting a region spanning the small subunit-internal transcribed spacer-large subunit (SSU-ITS-LSU) region using AM fungi-specific primer mixtures (Krüger, Stockinger, Krüger, and Schüssler, 2009). The purified final PCR products were pooled on an equimolar basis and sequenced on an Illumina MiSeq platform at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Berlin, using  $2 \times 300$  bp paired-end sequencing. Raw reads are available at ENA under study accession number PRJEB36419.

**Bioinformatics sequence processing.** Amplicon reads were analyzed using DADA2 (Callahan et al., 2016) in R (R core team 2017) to obtain denoised, chimera-free, nonsingleton, AM fungal exact sequence variants (ESVs) as implemented in Roy et al., (2019). AM fungal ESVs were identified using BLAST search against reference AM fungal rDNA sequences (Krüger, Krüger, Walker, Stockinger, and Schüßler, 2012) with a minimum of 90% coverage and of 90% to our targeted region. Sequences without this requirement were discarded. Additional putative PCR errors were identified using LULU in R (Frøslev et al., 2017) and were discarded. ESV sequences are available at ENA under accession numbers LR761341-LR761569. The ESV contingency table

and ESV taxonomy (<https://doi.org/10.20387/bonares-sea4-n5sp>) and related sample metadata (<https://doi.org/10.20387/bonares-xnhz-xw6e>) are freely accessible.

### **Statistical analyses of AM fungal community richness and composition**

All statistical analyses were conducted in R 3.4.1 (R core team 2017). Before community analyses, ESV read counts per sample were normalized by rarefying sample read count to the lowest number of reads. The ESV richness per sample before and after rarefaction were highly correlated (Pearson's R=0.97). Similarly, pairwise sample Bray-Curtis dissimilarities before (ESV read count per sample normalized by sample read count) and after rarefaction were highly correlated (Mantel Pearson's R=0.99).

**AM fungal richness.** AM fungal richness was calculated by the number of ESVs and analyzed using ANOVA. We tested the effect of crop rotation phase, pre-crop symbiotic group, HCA and plant compartment (roots versus soil). All factors could not be tested in one single model given some factors were restricted to a particular rotation phase (e.g. HCA and plant compartment factors are only relevant at t2). Therefore, we ran separate models using different sample sets to test the effects of these factors. We used all samples to test whether there was a difference in AM fungal richness between crop rotation phases; we used the t1 samples (conditioning phase) to test whether there was a difference in AM fungal richness between pre-crop symbiotic groups at t1; we used the t2 samples (PSF phase) to test whether there was a difference in AM fungal richness between pre-crop symbiotic groups, HCA treatments or plant compartments at t2. In addition, mixed linear models implemented in lme4 (Bates, Mächler, Bolker and Walker 2015) were used to test the differences of richness between t1 and t2 crop rotation phases, and to test the effect of plant compartment at t2, setting the pot identity as random factor. The significance of these factors was tested compared to a model without fixed factors using log-likelihood ratio tests.

**AM fungal community composition.** The change in the composition of the AM fungal community composition was measured using Bray-Curtis dissimilarity. We used permutational multivariate ANOVA (PERMANOVA, Anderson 2001) to estimate the part of variance explained by each factor. Statistical departure from a null model of random treatment assignation of samples was assessed using 999 Monte-Carlo permutations of samples. As for AM fungal richness, different sets of samples were used to run separate models in order to test for effects of crop

rotation phases (all samples), of pre-crop symbiotic group (t1 samples), and of pre-crop symbiotic group, HCA and plant compartment (t2 samples). In addition, a distance-based redundancy analysis (Legendre and Anderson 1999) was used to test for differences of community composition between crop rotation phases (t1 and t2 samples) and to test for differences between plant compartments (t2 samples) while partialling out the variance of pot identity. Similarly to richness analyses, the control pots were removed from the community composition analyses because of low sample size ( $n=3$ ) and to focus on treatment differences. Bray-Curtis dissimilarities among samples were visualized using non-metric multidimensional scaling (NMDS) ordination. Control pots were included along with the other samples to visualize their genetic compositional similarity to other treatments. The direction and strength of the correlation of phylotypes defined at different phylogenetic distances (see below) to the community dissimilarities were measured using multivariate linear regression between phylotype relative abundance and sample coordinates in the NMDS space (Oksanen et al., 2016). Multivariate community analyses were conducted in vegan (Oksanen et al., 2016).

**Phylogenetic dissimilarity of AM fungal communities** We analyzed the dissimilarity of AM fungal communities across phylogenetic depths in order to test whether dissimilarity in community composition among and between treatments is due to the recruitment of relatively closely or distantly related AM fungi. We screened community dissimilarities across phylogenetic depths from the ESV- to order-level and tested at which phylogenetic depth the correlation between community composition and treatments was the highest. To infer the phylogenetic relatedness between ESVs, reference partial SSU-ITS-LSU rDNA sequences (Krüger et al., 2012) were first aligned, followed with the alignment of ESVs to the reference backbone alignment using MAFFT (Katoh, Misawa, Kuma, and Miyata, 2002). A phylogenetic tree was built using all ESVs and all reference sequences, by conducting a bootstrap analysis (100 bootstraps) and searching for the best-scoring maximum-likelihood tree under a GTRGAMMA model of nucleotide substitution in RAxML (Stamatakis 2014). Reference sequences were pruned and ESVs were clustered into phylotypes at phylogenetic distances (in substitution per site, hereafter referred to as subs/site) spanning the entire phylogenetic tree and keeping cluster monophyly using BDTT in R (Grousson et al., 2017). A phylotype-by-sample count matrix was generated at each phylotype resolution and the PERMANOVA models were fitted as described above. We generated a null distribution of PERMANOVA  $R^2$  across phylogenetic depths by randomizing ESV phylogenetic relatedness

while keeping constant community composition and repeating the analysis. Uncertainty in phylogenetic relatedness between ESVs was accounted for by repeating the analysis for the 100 bootstrapped phylogenetic trees. We accepted a genuine phylogenetic signal when explained variance was higher than the 5%-95% null distribution obtained from phylogenetic randomizations and that the PERMANOVA p-value was below 0.05 for more than 90% of the bootstrapped phylogenetic trees.

### Analyses of AM fungal traits

**Root colonization and structural investment.** Different intra-radical AM fungal structures were recorded separately, including arbuscules, hyphae, vesicles and spores. Dried root systems were cut into 1-2 cm fragments, placed in 10% KOH and left in a water bath at 80°C for 30 min. Bleached roots were rinsed with tap water and acidified with 1% HCl for 10 min and stained with 0.05% Trypan Blue (Phillips and Hayman, 1970). Approximately 20 root fragments were mounted on glass slides. The percentage of roots colonized by AM fungi, as well as separate AM fungal structures, was quantified at 200 magnification using the magnified root intersections method (McGonigle, Millers, Evans, Fairchild, & Swan, 1990). The percentage of microsclerotia and dark-septate pigmented hyphae, reflecting the colonization by non-mycorrhizal root-colonizing fungi, was also reported.

**Soil hyphal colonization.** Soil colonization by AM fungi was determined as hyphal length in m g<sup>-1</sup> soil (modified from Leifheit, Verbruggen, and Rillig, 2015). Briefly, hyphae were extracted from 4.0 g of soil, stained with 0.05% Trypan Blue and quantified following the line-intersect method at 200x magnification (Jakobsen, Abbott, and Robson, 1992). Hyphae were identified as AM fungi if they were aseptate, dark- to light-blue stained and with characteristic unilateral angular projections according to Mosse (1959). All other hyphae were counted as non-AM fungi. Very short pieces of hyphae were discarded because a reliable morphological identification was not possible. The effects of pre-crop symbiotic group and HCA were tested using ANOVA.

**Statistical analyses of community traits.** The effects of pre-crop symbiotic group and HCA on hyphal root colonization, other intra-radical structures and soil hyphal length were tested using ANOVA. Furthermore, the direction and strength of the correlation of the different intra-radical AM fungal structures and soil hyphal length to the community dissimilarities at different

phylogenetic distances (see above) was measured using multivariate linear regression between the AM fungal structures and sample coordinates in the NMDS space.

### **Correlation of AM fungal community attributes with winter barley yield**

Winter barley yield data were originally described in van Duijnen et al. (2018), from which we extracted total grain yield (weigh) and total grain N uptake (or yield, the N concentration of the seeds multiplied by grain yield). We used multivariate and univariate analyses to identify which community attributes (richness, composition and traits) best correlate with winter barley yield. The direction and strength of the correlation of yield to the community dissimilarities at different phylogenetic distances (see above) was measured using multivariate linear regression between yield and sample coordinates in the NMDS space. We also summarized covariation among all community attributes and winter barley yield i) using Principal Component Analysis (PCA) of centred and scaled data implemented in ade4 (Dray and Dufour 2007) and ii) using pairwise Pearson's correlations. The analyses were assessed at phase t2 (where the AM symbiosis takes place, although one could argue that the abundance of phylotypes associated to pre-crops in the conditioning t1 phase can affect crop biomass at t2), and to root compartment (to focus on a likely active symbiosis).

## **Results**

### **General description of the sequencing results and AM fungal diversity**

AM fungal ESVs were assigned to three orders (Archaeosporales, Diversisporales, Glomerales), five families (Archaeosporaceae, Acaulosporaceae, Diversisporaceae, Claroideoglomeraceae, Glomeraceae), and six genera (*Archaeospora*, *Acaulospora*, *Diversispora*, *Claroideoglomus*, *Rhizophagus*, *Funneliformis*) (Fig. 1a). The number of phylotypes strongly decreased from 229 ESVs to 7 phylotypes at 0.15-0.20 subs/site. These phylotypes were composed of ESVs always annotated to the same species, or to the same genus (Fig. 1b). Four clades were composed of several lineages annotated to different species. For instance, this was the case for *Rhizophagus* node 338 (*R. irregularis* and a second unknown lineage), *Funneliformis* node 392 (*F. caledonius*, *F. mosseae* and *F. constrictus*), *Claroideoglomus* node 306 (*C. claroideum*, *C. etunicatum*, *C. luteum*, and an unknown lineage), *Diversispora* node 276 (*D. eburnea*, *D. celata*, *D. epigaea* and two unknown lineages). The *Rhizophagus* node 324, the *Acaulospora* node 267 and the

*Archaeospora* node 231 were represented by ESVs annotated to a single species, respectively *Rhizophagus cerebriformis*, *Acaulospora cavernata* and *Archaeospora schenckii*. All phylotypes at 0.2 subs/site were composed of a minimum of 10 ESVs (*Diversispora* node 276) to a maximum of 67 ESVs (*Funneliformis* node 392). At 0.4 subs/site, all Glomerales EVS grouped into one phylotype while the other phylotypes (*Diversispora*, *Acaulospora cavernata* and *Archaeospora schenckii*) remained delineated. Overall, *Acaulospora cavernata* node 267 was the most abundant phylotype in terms of read number, whereas *Rhizophagus* node 338 and *Funneliformis* node 292 were the most ESV-rich phylotypes (Fig. 1c).

### **Does the crop symbiotic group imprint the succeeding crop's AM fungal composition?**

Overall, community variance was better explained at the species to genus-level (0.15-0.2 subs/site) than at the within-species level (ESVs) for all factors investigated (Fig. 2; Appendix S1: Table S1). In the following, we therefore focused on the dynamics of clades delineated at this phylogenetic level.

The original soil was mostly composed of *Diversispora* node 276 (66% of the reads) and *Acaulospora cavernata* node 267 (33%). *Archaeospora schenckii* node 231 (0.4%), *Rhizophagus* node 338 (0.1%) and *Funneliformis* node 392 (0.1%) were present but rare, while *Rhizophagus cerebriformis* node 324 and *Claroideglomus* node 306 were not detected (Fig. 3; Appendix S1: Fig. S2, Table S2). Overall, we observed differences in community composition among rotation phases (0.2 subs/site:  $R^2=0.095$ ,  $p=0.001$ ) due to the selection of different phylotypes by different pre-crops and the persistence and accentuation of this effect, even when accounting for pot autocorrelation (0.2 subs/site:  $R^2=0.095\%$ ,  $p=0.001$ ).

At t1, after pre-crop conditioning, we observed an effect of the pre-crop mycorrhizal group on the AM fungal community composition left in soil (ESV:  $R^2=0.04$ ,  $p=0.01$ ; 0.2 subs/site:  $R^2=0.1$ ,  $p=0.002$ ), but not of its rhizobial group ( $p > 0.05$  in all cases) (Fig. 2b; Appendix S1: Table S1). At the species to genus level (0.2 subs/site), community composition strongly converged between the two pre-crops with mycorrhizal associations, and diverged from the two pre-crops with no mycorrhizal associations (Fig. 4a, b). *Rhizophagus* node 338, *Funneliformis* node 392 and *Acaulospora cavernata* node 267 were the main clades driving the differences in community composition after the conditioning by AM or non-AM pre-crops (Fig. 4b; Appendix S1: Table S3).

Compared to t0, *Rhizophagus* node 338 and *Funneliformis* node 392 strongly increased in abundance, especially in pots conditioned with AM pre-crops (Fig. 3a; Appendix S1: Table S2). Conversely, *Acaulospora cavernata* node 267 was slightly depleted in soil conditioned by AM pre-crops. *Claroideoglomus* node 306 sporadically appeared after AM pre-crops (Fig. 3a). *Archaeospora schenckii* node 231 was only abundant in control pots (i.e. without pre-crop) and depleted from the other soils (Fig. 3a, Fig. 4b; Appendix S1: Table S2).

At t2 (PSF phase), the recruitment of AM fungi of different species and genera between AM and non-AM pre-crops persisted and was accentuated in the roots of winter barley (0.2 subs/site:  $R^2=0.07$ ,  $p=0.004$ , Appendix S1: Table S1). Root communities grouped according to the mycorrhizal group of the conditioning pre-crop, with a particularly strong convergence of the winter barley root community in the pots conditioned by the two AM pre-crops (Fig. 4d).

*Rhizophagus* node 338 and *Acaulospora cavernata* node 267 were again the main drivers of these differences (Fig. 4d; Appendix S1: Table S3). *Rhizophagus* node 338 strongly persisted and even increased in abundance in the roots of winter barley in soils conditioned by AM pre-crops whereas *Acaulospora cavernata* node 267 further decreased (Fig. 3a; Appendix S1: Table S2). Other distantly related fungi from different families and even orders were more abundant in the roots of winter barley when grown in soils conditioned by non-AM than AM pre-crops, e.g. *Claroideoglomus* node 306 and *Diversispora* node 276 (Fig. 3a, Fig. 4d; Appendix S1: Table S2).

In soil, the imprint of pre-crops was less evident (at 0.2 subs/site:  $R^2=0.03$ ,  $p=0.136$ ) and soil communities largely retained clades abundant in the original soil (Fig. 3a). We observed differences between root and soil communities from the ESV level ( $R^2=0.267$ ,  $p=0.001$ ) to the selection of fungi from different families or orders (0.4 subs/site:  $R^2=0.137$ ,  $p=0.001$ ) (Fig. 2b; Appendix S1: Table S1). *Acaulospora cavernata* node 267 and *Rhizophagus* node 338 were the main drivers of the differences between root and soil communities (Fig. 3; Appendix S1: Fig. S2; Table S3). *Acaulospora cavernata* node 267 was relatively more abundant in soil than roots (Fig. 3a; Appendix S1: Table S2). This clade was the dominant clade in all samples, both in the original soil and throughout the experiment irrespective of the treatments, including in the control pots and has opposite trends to *Rhizophagus* node 338 and *Funneliformis* node 392 (Fig. 3; Appendix S1: Fig. S1). Significant community dissimilarities remained when accounting for pot autocorrelation indicating consistent differences across pots ( $R^2=0.152$ ,  $p=0.001$  at 0.2 subs/site) as observed by

the distinction in the centroids of soil and root samples due to the non-overlap of numerous soil and root samples (Appendix S1: Fig. S3).

Generally, the impact of the plant mixed symbiotic group and/or the crop species-specific effect was statistically absent after accounting for a plant single symbiotic group (i.e. rhizobial or mycorrhizal), at any phylogenetic level, time (t1, t2), and plant compartment (root, soil) (Appendix S1: Table S1). Nonetheless, for t1, a separation of community composition for each pre-crop was observed at the ESV level (Fig. 4a). At t2, root communities conditioned by faba bean (AM / rhizobial) differed from all others (i.e. conditioned by spring barley, canola or lupine, and the control) (Fig. 4c).

### **Does the crop symbiotic functional group imprint on the richness of AM fungi associated with winter barley?**

On average there were  $12.6 \pm 8$  (mean  $\pm$  sd) ESVs per sample. ESV richness increased with time ( $F=11.08$ ,  $p=2.83e-05$ ; Appendix S1: Table S4) from t0 ( $4.7 \pm 2.9$  ESVs) to t1 ( $9.5 \pm 5.8$  ESVs) to t2 ( $14.4 \pm 8.3$  ESVs) due to the recruitment of AM fungi barely detected at t0 (Fig. 5). This effect remained when accounting for pot autocorrelation ( $\chi^2=16.368$ ,  $p=5.217e-05$ ). At t1, ESV richness in soil after pre-crop harvest was affected by the pre-crop mycorrhizal group ( $F=33.484$ ,  $p=3.55e-07$ ), but not its rhizobial group ( $F=1.362$ ,  $p=0.248$ ) (Appendix S1: Table S4): ESV richness was higher after AM pre-crops (spring barley and faba bean,  $13 \pm 5.6$  ESVs) than non-AM pre-crops (lupine and canola,  $6 \pm 3.4$  ESVs) (Fig. 5a). This was driven by increasing *Rhizophagus* node 338 and *Funneliformis* node 392 ESV richness, whereas *Acaulospora* node 276 and *Archaeospora* node 231 showed opposite patterns (Appendix S1: Fig. S2). This observation did not hold at t2, after crop (winter barley) harvest ( $F=1.980$ ,  $p=0.162$ ; Appendix S1: Table S4): we observed an increase in richness after non-AM pre-crops to a similar level as after AM pre-crops (Fig. 5a), due to the selection of *Claroideoglomus* node 306 and *Diversispora* node 267 (Appendix S1: Fig. S2). In general, root communities ( $16.5 \pm 10.4$  ESVs) had higher ESV richness than soil communities ( $12.4 \pm 4.8$  ESVs) (Appendix S1: Table S4); this effect remained when accounting for pot autocorrelation ( $\chi^2=10.157$ ,  $p=0.001437$ ).

### **Is the pre-crop legacy effect attenuated, accentuated or modified by organic amendment?**

High carbon amendment (HCA) explained a higher part of variance than the mycorrhizal group of the pre-crops in the community dissimilarities at the harvest of winter barley at t2 (Fig. 2b; Appendix S1: Table S1). We observed strong community differences between the HCA treatments from the ESV level ( $R^2=0.034$ ,  $p=0.003$ ) to the selection of fungi from different families or orders (0.4 subs/site:  $R^2=0.175$ ,  $p=0.003$ ) (Fig. 2b; Appendix S1: Table S1). The HCA effect was mostly driven by the selection of a *Rhizophagus cerebriformis* clade (node 324) but also by the above-mentioned *Funneliformis* (node 392) and *Rhizophagus* (node 338) clades after sawdust amendment (Fig. 3b; Fig. 4e, f; Appendix S1: Table S3). These three clades, *Rhizophagus cerebriformis* node 324, *Rhizophagus* node 338 and *Funneliformis* node 392 were more abundant in roots (Fig. 3b; Appendix S1: Fig. S4; Table S2), so that the soil community was less effected by HCA than the root community (Appendix S1: Table S1).

At t2, HCA strongly affected ESV richness ( $F=21.032$ ,  $p=2.74\text{e-}08$ ; Appendix S1: Table S4): ESV richness increased after sawdust amendment ( $20.3 \pm 11.7$  ESVs) compared to wheat straw ( $10.3 \pm 3.8$  ESVs) and no amendment ( $13.1 \pm 4.4$  ESVs) (Fig. 5b). This effect was more pronounced in roots compared to soil ( $F=3.096$ ,  $p=0.049$ ; Appendix S1: Table S4), with a higher richness in roots than soil (Fig. 5).

### **Do the traits of AM fungi reflect community richness or composition?**

None of the community traits correlated with community richness or composition (Fig. 6; Appendix S1: Fig. S4; Table S5). Accordingly, neither AM hypha in roots (root colonization:  $18.6\% \pm 12.5\%$ ) or in soil (AM hyphal length:  $1.4 \pm 0.6 \text{ m g}^{-1}$  soil), nor other fitness-related features such as vesicle ( $4.7\% \pm 7.7\%$ ) and spore production ( $8.6\% \pm 10.9\%$ ) varied with pre-crop mycorrhizal (all  $p>0.05$ ) or rhizobial (all  $p>0.05$ ) group, or with HCA (all  $p>0.05$ ) (Appendix S1: Fig. S5, S6, Table S6, S7). The soil colonization by non-AM fungi did not vary among treatments either ( $3.2 \pm 1.1 \text{ m g}^{-1}$  soil), but the percentage of microsclerotia in roots was slightly higher in the control ( $24.4\% \pm 12.7\%$ ) than wheat straw ( $15.3\% \pm 9.4\%$ ) or sawdust ( $21.3\% \pm 12.4\%$ ) treatments (Appendix S1: Fig. S5, S6, Table S6, S7).

### **Which community attribute (richness, composition and traits) best correlates with crop yield?**

Winter barley yield showed a correlation with the community composition in roots, decreasing with communities conditioned with AM pre-crops (Appendix S1: Fig. S4). The correlation was weak and only significant for N yield, not grain yield (Appendix S1: Table S5). The PCA and pairwise correlations revealed overall weak correlations between any of the AM fungal community attributes in winter barley and its yield (Fig. 6). The only correlation was a negative correlation between the abundance of *Rhizophagus* node 338 and winter barley grain yield (Pearson's  $r=-0.317$ ,  $p=0.014$ ) and total N yield (Pearson's  $r=-0.36$ ,  $p=0.005$ ) (Fig. 6c). For instance, mean grain yield was  $659.4 \pm 113.1$  and  $733.1 \pm 107.5$  g m<sup>-2</sup> for presence or absence of *Rhizophagus* node 338. In contrast, the presence/absence of two other clades, *Claroideoglomus* node 306 (correlation with N yield: Pearson's  $r=0.255$ ,  $p=0.052$ ) and *Diversispora* node 276 (correlation with N yield: Pearson's  $r=0.255$ ,  $p=0.051$ ), correlated with higher crop yield (Appendix S1: Fig. S7, Table S8).

## Discussion

### **Does the crop symbiotic functional group imprint on the composition or richness of AM fungi associated with a succeeding AM crop, and is it observable in roots and soil?**

The pre-crop mycorrhizal status affected the richness and composition of AM fungi available to the succeeding crop. This difference, in particular community composition, persisted and increased in the roots of the succeeding crop. The effect of the pre-crop on AM fungal communities was driven by its single symbiotic group (for instance, mycorrhizal), not mixed symbiotic group and/or crop species-specific or conspecific effect. This demonstrates that pre-crop symbiotic group has lasting legacy effects on the AM fungal communities associated with the succeeding crops and may steer the AM fungal community succession across rotation phases. Our previous findings showed a 23% decrease in winter barley yield when grown in soil conditioned by AM pre-crops, N-fixing or not (van Duijnen et al., 2018). Our findings suggest negative PSF at the level of the plant symbiotic group, here of a continuous AM cropping, driven by a legacy effect of crop rotation history on AM fungal communities.

Our result may appear surprising, as it is well established that AM fungi often increase plant growth (Smith and Read 2008). Microbial symbionts do not necessarily imply positive PSF, even when the presence of these symbionts increases plant growth (Bever 2002; Bennet et al., 2017). In

fact, it has long been recognized by farmers that the colonization of crop roots by AM fungi can be detrimental to yield. This was experimentally demonstrated (Hoeksema et al., 2010; Koch, Antunes, Maheral, Hart, and Klironomos, 2017), pointing to parasitism by AM fungi (Kirkegaard, Christen, Krupinsky, and Layzell, 2008). Farmers implement crop rotation schemes that may involve a breaking crop, i.e. a non-AM host such as from the *Brassicaceae* family, to decrease the load of AM fungi to the next AM crop, and this has been shown to benefit the following crop in terms of yield (e.g. wheat, Angus et al., 2015; Kirkegaard et al., 2008). Legumes, which establish an atmospheric N-fixing rhizobial symbiosis and most of which are mycorrhizal (e.g. faba bean), are also used during crop rotation with yield benefits to the next crop (Angus et al., 2015). This was not the case here when legumes were the AM host. While several crop species, including cereals (Zhang, Lehmann, Zheng, You, and Rillig, 2019), generally respond positively to inoculation with AM fungi, this is often less so for cereals (Eo & Eom 2009). Indeed, cereal responses to AM fungi are generally positive but weak, highly crop-specific and tend to be negative for crops released after the 1950s (Zhang et al., 2019).

High N content, such as in our study, could favour mutualistic interactions but not under high P content (Johnson 2010). We likely had high soil P here, since pre-crops were fertilized with triple superphosphate and because soils used here had long history of agriculture. Even in P-limited soils, AM fungi with tolerance to high N enrichment may not be able to intensively forage for P, since plants can reduce the investment into AM fungi (Treseder et al., 2018). In this study, we do not have AM fungal data under low N input. Unsurprisingly, winter barley yield was strongly reduced in low compared to high N fertilization (van Duijnen et al., 2018). However, in the low N fertilization treatment there was no difference in yield between plants grown after AM or non-AM pre-crops (van Duijnen et al., 2018). Therefore, it is unlikely that the AM fungal community parasitized winter barley under low N input. This is a crucial point for further research, to test whether AM fungi tend to form parasitic associations with crop roots under higher N, but mainly have positive interactions under low N.

Specifically, we identified one *Rhizophagus* clade (node 338) in particular and one *Funneliformis* clade (node 392) that were selected after AM pre-crops. However, the original soil, which has a long history of agricultural practice, was mostly composed of an *Acaulospora cavernata* clade, a *Diversispora* clade and an *Archaeospora schenckii* clade, as observed in 50-year old re-cultivated

barren soils (Roy et al., 2017). *Rhizophagus* and *Funneliformis* were almost undetected in the original soil, indicating the strong colonization ability of these fungi. *Rhizophagus* fungi possess life-history traits of ruderal fungi (Hart & Reader 2002; Chagnon, et al., 2013) and are often observed in early stages of ecosystem succession (Nielsen, Kjøller, Bruun, Schnoor, and Rosendahl, 2016; Roy et al., 2017). Ruderal AM fungi may colonize roots faster after a first crop is sown, giving them an advantage over slow-growing fungi to colonize the succeeding crop (Verbruggen and Kiers 2010).

Nonetheless, the legacy effect was stronger in roots than in soil. Soil retained members of fungal clades abundant in the original soil (*Acaulospora cavernata*, *Diversispora*, and *Archaeospora schenckii*). Root colonization is intrinsically determined by ability to germinate, propagule sources, growth rate, competitive ability, host and habitat preference, and phenology. It is possible that the time scale of agricultural practices (e.g. rotations) and the time scale of fungal root colonization are not matched. The spore-bank and/or outcompeted AM fungi could better colonize roots if conditions are favourable or if time to colonize roots is sufficiently long. Indeed, AM fungal communities are highly dynamic in agricultural systems (Roy et al., 2017) with seasonal succession being observed in natural (Dumbrell et al., 2011) and agricultural ecosystems (Berutti et al., 2018).

#### If a pre-crop legacy effect is observed, is this legacy attenuated, accentuated or modified by organic amendment?

We did not observe an interaction between HCA and the pre-crop symbiotic group, indicating that their respective influences on AM fungal communities were independent. HCA induced the most important shifts in AM fungal communities, including strong change in community composition and richness, especially in roots, which was mostly due to sawdust amendment. Sawdust increased AM fungal richness, especially of ruderal fungi. It strongly positively selected *Rhizophagus* node 338, *Funneliformis* node 392 and *Rhizophagus* node 324, and negatively selected *Acaulospora cavernata* node 267. A 6.3% decrease in yield was observed after sawdust, but not wheat straw (van Duijnen et al. 2018). Therefore, HCA may have an additive effect with crop mycorrhizal group, either positive in the case of sawdust amendment following AM pre-crops, or antagonistic in the case of wheat straw. It is interesting to note that the strongest effect on AM fungal

communities (sawdust) was not related to the strongest effect on yield (pre-crop mycorrhizal group).

### **Do the traits of AM fungi reflect community richness or composition?**

The dissimilarities between AM fungal communities were due to recruitment of distantly related lineages, not variants within species. This suggests that relatively ancient evolutionary divergences in AM fungi, at the species level and above, are related to observable ecological and/or functional specialization (Lekberg, Gibbons, and Rosendahl, 2014; Roy et al., 2019) with consequences for crop yield. Given phylogenetic conservatism of morphology and growth traits in AM fungi (Hart and Reader 2002, Koch et al., 2017) and the congruent deep phylogenetic dissimilarities between AM fungal communities of different treatments (e.g. AM versus non-AM pre-crops), we expected soil and root colonization to be lower and higher after AM pre-crops, respectively. Higher soil and lower root colonization should induce a low C cost to the plant to support AM fungi while increasing nutrient foraging by AM fungi (Chagnon et al., 2013). This was not observed, and trait data did not reflect community composition. Similarly, a recent study showed that host performance cannot be predicted from AM fungal morphology and growth traits (Koch, Antunes, Maherali, Hart, and Klironomos, 2017). They concluded that divergent effects on plant growth among isolates within an AM fungal species may be caused by coevolution between co-occurring fungal and plant populations. Contrary to sterile conditions and single inoculum addition (Hart, Reader and Klironomos 2002), competition among the microbial community may blur the correlation between AM fungal life history traits and community composition in natural settings (Maherali and Klironomos 2007). Soil colonization rates by AM fungi were also comparatively low in this experiment (e.g. Rillig, Wright, and Eviner, 2002), probably due to the overall high nutrient levels in the collected soil and the N fertilization (Treseder 2004). Enhanced phenotyping of AM fungi and of the symbiosis would help understand why the communities that are favoured after AM pre-crops negatively affect the succeeding crop yield (Montero, Choi and Paszkowski, 2019).

### **Which community attribute (richness, composition and traits) best correlates with crop yield?**

The correlation between winter barley yield and the abundance of *Rhizophagus* node 338 was the best and only one. *Rhizophagus* fungi, but also *Funneliformis*, poorly colonize soil but mostly roots (Hart and Reader 2002, Chagnon et al., 2013; Koch et al., 2017). They may not provide nutritional benefits given the poor nutrient foraging and transfer capacity (Chagnon et al., 2013). It is possible that competition and low functional complementarity between coexisting individuals of *Rhizophagus* and *Funneliformis* may have reduced the benefit of the symbiosis: while the coexistence of AM fungi from different families can increase plant growth due to functional complementarity (Maherali and Klironomos 2007), coexisting AM fungi within species, genus or family may have opposite effects (Roger, Colard, Angelard and Sanders, 2013; Yang et al., 2017). The relatively low phylogenetic divergence of coexisting *Rhizophagus* and *Funneliformis* fungi might have led to enhanced competition and reduced functional complementarity, with less benefit to the plants (Maherali and Klironomos 2007).

## Conclusion

Our findings suggest negative PSF at the level of the plant symbiotic group driven by a legacy effect of crop rotation history on AM fungal communities, suggesting that a focus on crop symbiotic group offers additional understanding of PSF. Differences in AM fungal community composition may have functional consequences that should not be neglected in agriculture (Rillig et al., 2019), although this debate is open (Ryan and Graham 2018). Crop rotation design, but also nutrient input strategies, could foster the AM symbiosis towards targeted services. Overall, differences in AM fungal communities among treatments were due to recruitment of relatively phylogenetically distant AM fungi, with *Rhizophagus* correlated with decreasing crop yield. These findings suggest that knowing how and when certain AM fungal phylotypes are favoured could provide inroads towards better managing the symbiosis in agroecosystems. More experiments with a range of crops of different symbiotic groups, of different succeeding crops and with various farming regimes are now needed to expand the generality of our findings.

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## Supporting Information

Additional supporting information may be found online at: [link to be added in production]

## Open Research

Raw Illumina sequencing reads of LSU amplicons are available on the European Nucleotide Archive under accession no. PRJEB36419. Amplicon sequence variants obtained after bioinformatics are available on the European Nucleotide Archive under accession nos. LR761341 through LR761569. The sequence contingency matrix and the taxonomy of the sequences (<https://doi.org/10.20387/bonares-sea4-n5sp>), and the sample metadata (<https://doi.org/10.20387/bonares-xnhz-xw6e>) are freely available through the BONARES data portal.

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## Figure captions

**Figure 1. Overview of AM fungal diversity.** (a) Maximum likelihood phylogenetic tree of AM fungal ESVs. Clades delineated at a 0.2 genetic distance (mean substitution/site, subs/site) are highlighted with alternating grey and blue rectangles. The taxonomic annotation of subclades within clades at 0.2 subs/site is the majority consensus annotation based on the taxonomic identity at the species level. Scale bar indicates 0.1 mean substitution/site. (b) Number of clades (operational taxonomic units, OTUs) across phylogenetic distances. (c) Overall number of ESVs and relative abundance of clades at 0.2 subs/site.

**Figure 2. Variance partitioning of AM fungal community dissimilarities across phylogenetic depth.** (a) Variance explained ( $R^2$ ) by mycorrhizal (AM) and rhizobial (N2) plant symbiotic groups at t1, at each phylogenetic depth used to cluster ESVs into broader OTUs. (b) Variance explained ( $R^2$ ) by plant mycorrhizal (AM) and rhizobial (N2) groups, high carbon amendment (HCA) and plant compartment at t2, at each phylogenetic depth used to cluster ESVs into OTUs. Filled and open symbols respectively indicate  $p < 0.05$  or  $p > 0.05$  after 1000 sample permutations for  $>90\%$  of the 100 phylogenetic trees obtained with bootstrapping. The grey area represents the 95% percentiles interval of the  $R^2$  for 100 randomizations of ESVs phylogenetic relatedness while keeping community composition constant.

**Figure 3. Distribution of AM fungal clades across rotation phases, pre-crops, high carbon amendment and plant compartment.** Distribution across (a) pre-crop species and (b) high carbon amendment treatments is emphasised. The size of the point represents clade relative read abundance. The colour of the points represents clade relative occurrence. T0: original soil communities; t1: soil communities after pre-crop conditioning phase; t2: root communities at crop harvest. Can: canola; Lup: white lupine; Bar: spring barley; Fab: faba bean; Control: control; No: no amendment; W: wheat straw; S: sawdust; Control: control.

**Figure 4. Unconstrained ordination analysis of AM fungal community dissimilarities among pre-crops and high carbon amendment at t1 and t2.** Dissimilarities (Bray-Curtis) are split among rotation timing, (a, b) t1 = pre-crop harvest, (c-f) t2 = winter barley harvest, prior to ordination analysis. Dissimilarities were computed at two phylogenetic depths, (a, c, e) ESV level,

and (b, c, f) 0.2 subs/site. The centroid of sample distribution per treatment is shown with bigger dots that connect to the respective samples. For t2 ordinations, centroids for (c, d) pre-crops and (e, f) high carbon amendment (HCA) are shown separately. At t2 (c, f) the analysis presents root communities. Supported correlations of ESVs ( $p=0.001$ ) or clades at 0.2 subs/site ( $p=0.01$ ) to dissimilarities are overlayed as vectors. Statistical differences among treatments are presented in Table S1. All correlations of clades at 0.2 subs/site to dissimilarities are presented in Table S3.

**Figure 5. ESV richness of AM fungal communities across rotation phases (t0, t1, t2), pre-crops, high carbon amendment, and plant compartment.** Richness across (a) pre-crop species and (b) high carbon amendment treatments is emphasised. The boxplots represent the 25th and 75th percentiles, the median and outlying points. Data points are shown on the boxplots. Y-axes of all panels are on the same scale for comparability among rotation phases and treatments. Statistical comparisons are presented in Table S4. Can: canola, Lup: white lupine, Bar: spring barley, Fab: faba bean, No: no amendment, W= wheat straw, S: sawdust, Control: control.

**Figure 6. Correlation overview between AM fungal community richness, composition, traits and winter barley yield at winter barley harvest t2 phase.** (a) and (b) Principal Component Analysis of AM fungal community richness, composition, traits and winter barley yield. The centroid of sample distribution per treatment is shown with bigger dots that connect to the respective samples. Centroids of treatments are shown separately for (a) pre-crops and (b) high carbon amendment (HCA). (c) Pairwise Pearson's correlations. Only significant correlations at  $p < 0.05$  are shown. Community composition (clade relative abundance and NMDS axes) and richness variables are those calculated at t2 in roots of winter barley, similarly to community traits and winter barley yield.











