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Shaping of soil microbial communities by plants does not translate into specific legacy effects on organic carbon mineralization

Eve Hellequin^{1*}, Françoise Binet¹, Olivier Klarzynski², Sara Hallin³, Jaanis Juhanson³, Virginie Daburon¹, Cécile Monard^{1*}

1. University of Rennes, CNRS, ECOBIO [(Ecosystèmes, biodiversité, évolution)] - UMR 6553, F-35000 Rennes, France.

2. BIO3G Company, 7 rue du Bourg-Neuf 22230 Merdrignac, France.

3. Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Box 7026, 75007, Uppsala, Sweden

* Corresponding authors:

-Eve Hellequin (current address: University of Sorbonne, CNRS, EPHE, PSL, UMR METIS, F-75005 Paris, France. (eve.hellequin@upmc.fr)

-Cécile Monard (cecile.monard@univ-rennes1.fr)

Abstract

Plants shape soil microbial communities through their root architecture, their rhizodeposits and return of dead plant material to the soil. These interactions can have a strong influence on the soil organic carbon dynamics. However, it is unclear whether the plant species effects on the soil microbial community could influence the organic carbon mineralization through plant legacy effects. Therefore, we examined how and to what extent a short-term plant growing phase affected the total and active soil microorganisms and through a possible plant legacy, also the mineralization of soil organic carbon, a central ecosystem function. Using a controlled pot experiment, we first showed that the two phylogenetically distinct plants, *Arabidopsis thaliana* and *Triticum aestivum*, differently shaped the soil microbial communities when recruiting from the same soil community. Although both plants recruited plant-growth promoting bacteria in the vicinity of their roots, *A. thaliana* had a stronger effect than *T. aestivum* and also recruited saprophytic fungi, while inhibiting fungal pathogens. Due to plant legacy effects on the soil microbial communities, different microbial successions occurred in the two previously planted soils when subjected to plant litter. By contrast, plant legacy effects on soil basal respiration were not plant-specific, with basal respiration increasing similarly in both cases and moreover did not translate to changes in litter carbon mineralization in the short-term of 49 days. Our results suggest that the soil nutrient dynamics rather than changes in soil microbial community composition drive the organic carbon mineralization of added litter. The present study brings new insights in how

the relationships between plants, microorganisms and soil nutrient dynamics affect litter carbon cycling.

Key-words: plant legacy, litter mineralization, soil bacteria, soil fungi, *Arabidopsis thaliana*, *Triticum aestivum*, microbial succession

Introduction

Soil harbors a large amount of phylogenetically and functionally diverse microorganisms (Torsvik and Øvreås, 2002) that interact with plants and contribute to above-below ground exchanges. The “plant-soil feedbacks” concept is defined as plant-mediated changes in soil microbiota and soil nutrient availability that influence the fitness of the same or other plants in return (Perkins et al., 2011a; Van der Putten et al., 2013). Plants shape the composition and diversity of soil microbiota through rhizodeposits and roots architecture, litter C:N ratio and litter carbon quality (Heal et al., 1997; Grayston et al., 1998; Zak et al., 2003; Gu et al., 2012). By providing new habitats and resources in the vicinity of their roots, plants induce the colonization of microorganisms, therefore the rhizosphere is known to be a microbial hotspot (Lynch and Whipps, 1990 ; Lundberg et al., 2012; Reviewed in Muller et al., 2016). The microbial diversity declines sequentially from bulk soil to rhizosphere, suggesting increasingly stronger competition among microorganisms (Muller et al., 2016) or a selection of specific microorganisms induced by the plant. Thus, plant roots shape the microbial community directly or indirectly by enriching and/or activating specific microorganisms, but also by depleting and/or inhibiting other ones (Berg and Smalla, 2009).

The modifications induced by plants on soil microbiota may persist after their disappearance (i.e. legacy effects) (Van der Putten et al., 2016), for example, via their rhizodeposits or litter. Such legacy effects of plants have been shown to influence soil carbon storage (Lange et al., 2015) and soil respiration (de Oliveira et al., 2020), and could therefore affect the mineralization of organic carbon and nutrients from litter. The capacity of the soil to mineralize organic matter is attributed to the production of specific enzymes that allow specialized microorganisms to use carbon and nutrients of organic matter with low nutritional value, known as “metabolic ability” (Keiser et al., 2011). Depending on the functional capacity of the decomposer communities, soils have different metabolic abilities (Keiser et al., 2014, Veen et al., 2018). Thus, for the same litter, differences in its

decomposition rate can occur depending on the microbial community present and its active fraction. While active soil microorganisms have been shown to only contribute 2% of the total microbial biomass in soils, they are linked to soil metabolic ability (Blagodatskaya and Kuzyakov, 2013) and may be more relevant than the total microbial community to assess plant legacies on soil function. Hence, by shaping the microbial communities, plants may indirectly also influence the soil organic matter decomposition and, in the long-term, potentially accelerate its own litter decomposition by selecting an adapted decomposing microbial community (Ayres et al., 2009a; Veen et al., 2015a).

In the present study, we asked whether plant species shape the soil microbial community and if such plant-specific effects could influence, the organic carbon mineralization through plant legacy effects. For this purpose, we conducted an experiment with two phases, with the first phase denoted the “plant-soil experiment”, in which we aimed at examining how a short-term (two months) planted agricultural soil affects the total and the active microbial communities and to what extent it depends on plant species. In the second phase referred to the “litter-carbon mineralization experiment”, we determined if such changes induce plant legacy effects that affect the litter-carbon mineralization. Two phylogenetically distinct plants, a common plant model (*Arabidopsis thaliana*) and a crop (*Triticum aestivum*) were grown in the plant-soil experiment and followed by the litter-carbon mineralization experiment using the soils from phase one to which litter of *T. aestivum* was added. In this experiment, differences in the daily and cumulative mineralization rates of organic carbon from litter and subsequent nutrient release were determined. To identify early plant legacy effects on soil microbiota, both DNA (total microbial community) and RNA (active microbial community) were used to quantify microbial abundances (quantitative PCR) and identify the changes in microbial diversity using a 16S rRNA gene and ITS amplicon sequencing approach.

We hypothesized that i) specific microbial communities would develop according to plant species, through specific recruitment from the soil and ii) specific plant-induced changes in microbial communities, soil nutrient and carbon contents would then translate into plant-specific legacy effects on the mineralization of added litter to soil, despite the generalist feature of this function.

2. Materials and methods

2.1. Plant-soil experiment (phase 1)

The soil was an agricultural topsoil (0-20 cm depth) from the agronomic experimental site of the Brittany regional chamber of agriculture at Kerbernez (Plomelin, France; 47°56'39.3"N / 4°07'47.9"O), collected in April 2018 in the field border where grass was growing. It was air-dried at ambient room temperature for 48 h and sieved at 4 mm prior to storage in the dark at 4 °C. The soil was used for the plant-soil experiment, performed in May 2018. Soil physical, chemical and microbial properties were determined following sieving at 2 mm and according to Carter and Gregorich (2008) and are presented in Table 1. The soil was planted with either *A. thaliana* or *T. aestivum* or not planted (bare soil). The *A. thaliana* seeds from the Ecotype Columbia-O and the *T. aestivum* L. cv. "Baldus" seeds were kindly provided by IsoLife BV (Wageninigen, the Netherlands). The seeds were surface-sterilized, directly sown on 10 kg equivalent dry soil in sterile planters (length: 50 cm, width: 17 cm, height 15 cm) at a density of 2.6×10^{-6} and 1.22×10^{-5} seeds per m^2 for *T. aestivum* and *A. thaliana*, respectively. The planters were placed in an environmental growth chamber (Percival scientific, Perry, Iowa, USA) with the following conditions: 16 hours light (day), 8 hours dark (night) and light intensity of $160 \mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature of 20 °C and relative humidity of 80%. Soils were watered with sterile water periodically to maintain WHC around 60% by weighting the planters. After 63 days, which corresponded to the flowering stage of *A. thaliana*, the bare soil from the not planted planter and the soil in the vicinity of the roots of *A. thaliana* and *T. aestivum* were harvested. Four replicates of the original soil (blank) and four soil replicates (30 g of dry weight equivalent for each replicate) from the same bare and planted planters (Bare soil, *A. thaliana* and *T. aestivum*) were sampled and stored at -20 °C and -80 °C for further chemical and microbial DNA- and RNA-based analyses, respectively (n=16). The remaining soil was used for setting-up the second experimental phase.

2.2 Litter-carbon mineralization experiment (phase 2)

In the second experimental phase, we used soil microcosms to study the plant legacy effects on C mineralization based on a 3 x 2 factorial design (three soils from phase 1 without and with *T. aestivum* litter addition). For each of the six treatments, four replicates were independently designed for each of the five incubation times (0, 3, 7, 21 and 49 days) after

litter addition to soil, allowing destructive soil sampling. The design corresponded to a total of 120 soil microcosms. Each soil (e.g. bare soil (control), and previously planted soils with *A. thaliana* or *T. aestivum*) was sieved at 2 mm according to the AFNOR standard (AFNOR XP U 44–163) and soil aliquots of 30 g weight equivalent dry soil were mixed with or without 184 mg of *T. aestivum* sterile chopped straw, corresponding to an input of 40 mg of organic carbon and 0.3 mg of organic nitrogen. The straw was previously chopped into fine pieces of 1 mm. To avoid soil contamination by external microorganisms, the straw was sterilized by Ionisos (Dagneux, France) with gamma irradiation at 25 KGy. The sterilization efficiency was checked by none amplification of the 16S rRNA gene with a polymerase chain reaction (PCR). Each soil microcosm was placed in a hermitically closed 1 L glass jar that was incubated in the dark at 28 °C. At each incubation time, except the initial time point, the CO₂ produced and accumulated in the headspace was measured by micro gas chromatography (Agilent, Santa Clara, California, USA) and expressed as mg of C-CO₂ g⁻¹ dry soil. Before each measurement, the air of the headspace was homogenized with a 50 ml syringe. After each measurement, the air of the headspace was entirely renewed, to ensure that the headspace did not become anoxic, and the soil moisture was maintained at 60% WHC by weighting and adding sterilized water if needed. At day 0 and after each CO₂ measurement (days 3, 7, 21 and 49) a time series of microcosms was destroyed and the soil was stored at -20 °C and -80 °C for further chemical and microbial RNA-based analyses, respectively.

2.3 Chemical analyses

The soil chemical analyses are detailed in Hellequin et al. (2020). Briefly, the total organic carbon and nitrogen were determined with an elemental analyzer (Elementar, Vario PYRO Cube, Lyon, France). The dissolved organic carbon and the dissolved inorganic nitrogen (NO₃⁻ and NH₄⁺) were measured by an automated photometric analyzer following K₂SO₄ extraction. The Olsen phosphorus concentrations were determined according to Olsen et al. (1954). The microbial carbon and nitrogen biomass (MBC and MBN, respectively) were determined according to Vance et al. (1987).

2.4 Microbial quantification and metabarcoding

The DNA and RNA extractions were performed following the protocol described by Griffiths et al. (2000) with some modifications according to Nicolaisen et al., (2008) and Monard et al. (2013). Further details on DNA extraction and subsequent quantitative PCR (qPCR) are available in supplementary materials (section 1). Half of the extract was stored at 20 °C before further DNA-based analyses (original soil and 0 day samples). The other half was subjected to a DNase treatment for further RNA-based analyses (original soil and samples from day 0, 3, 7, 21 and 49). RNA (200 ng) were used for reverse transcription (RT). Half of the cDNA products was stored at –20 °C to prepare 16S rRNA and ITS1 amplicons for the quantification of the total bacteria and fungi by qPCR and the other half was used for 2 X 300 Illumina sequencing. The libraries were constructed using a two-step PCR approach "16S metagenomics sequencing library preparation" protocol provided by Illumina.

2.5 Data analyses

The microbial sequences are available on the sequence read archive (SRA) database (Bioproject PRJNA540147). The microbial sequence analyses were performed according to Hellequin et al. (2020) with the following changes: i) only the bacterial and fungal OTUs present in at least 4 out of 128 samples and representing at least 0.0005% of all sequences were retained, which corresponded to OTUs with a minimum of 12 and 29 sequences for bacteria and fungi, respectively. In the final data set, a total of 1,253,087 bacterial sequences corresponding to 8,911 OTUs and 3,113,807 fungal sequences corresponding to 1,351 OTUs were obtained.

All the statistical analyses were performed using R (v3.4.3, Core Team, 2017). The number of sequences per sample was randomly subsampled to the lowest number, with bacterial and fungal DNA and cDNA amplicons being normalized separately using the *rarefy* function from the vegan package version 2.5-4 (Oksanen et al., 2019); 6 399 and 15 589 for bacterial and fungal DNA, and 7 000 and 6 000 for bacterial and fungal cDNA. Twenty-one out of the 256 samples were below these thresholds and were removed before further analyses. The rarefaction curves at each sampling date and before and after normalization were performed using the *rarecurve* function from the vegan package (Figures S1 and S2). For each sample, the abundance of each OTU was calculated based on its relative abundance from the sequencing analysis and to the bacterial 16S rRNA and fungal ITS quantifications

performed simultaneously. The richness (number of OTUs) (Figure S11) and Shannon diversity index were calculated to estimate the alpha diversity using the *phyloseq* package (McMurdie and Holmes, 2013).

To test for statistical differences between plant-soil treatments, we used analysis of variance (ANOVA) and Tukey's post-hoc test on the following endpoints: emissions, bacterial and fungal Shannon diversities, microbial carbon and nitrogen biomasses, soil nutrient (N and P) contents, and total and active bacterial and fungal abundances. To test for significant difference in the composition of the total and active bacterial and fungal communities at the end of the plant growth phase, powered partial least squares discriminant analysis (PPLS-DA) were generated using DNA-based OTU or RNA-based OTU tables using the *RVAideMemoire* package version 0.9-72 (Hervé, 2019) and the *p/s* package version 2.7-0 (Mevik et al., 2018). ANOVAs were carried out on each OTU to identify significant differences in their abundances. The significant DNA-based or RNA-based OTUs were determined as enriched or activated *versus* depleted or inhibited by the plants, respectively. Over the incubation period in the second experiment, PPLS-DA were generated with the RNA-based OTUs and at each sampling date to compare the active bacterial and fungal community compositions between soils derived from bare soil, *A. thaliana* or *T. aestivum* and amended or not with litter. ANOVAs were carried out on each OTU to identify significant differences in their abundances according to the PPLS-DA results. In parallel, we used co-inertia analyses to identify the relationships between the OTUs that contributed significantly to the differences in community composition between soils and the soil dissolved organic carbon and nutrient contents. Only the sampling times (i.e. day 7 for bacteria and days 0 and 49 for fungi) at which both the PPLS-DA and the co-inertia resulted in significant differences were kept to discuss the results. To identify microbial successions during the incubation, additional PPLS-DA on the RNA-based OTUs were generated to compare the composition of active bacterial and fungal communities over time.

3. Results

3.1 Planting effects on soil carbon and nutrients (N, P) contents

Without plants, the total content of dissolved mineral nitrogen increased significantly after 63 days due to a higher amount of NO_3^- , whereas the content of NH_4^+ remained the same (Figure 1). In soils with plants, dissolved soil organic carbon content increased significantly, whereas the dissolved NO_3^- significantly decreased compared with the bare soil. Both plants tended to increase the microbial carbon (MBC) (significant for *A. thaliana*) and nitrogen biomass (MBN). The soil P content was significantly depleted by *A. thaliana*, but not by *T. aestivum* (Figure 1). Furthermore, *A. thaliana* increased the ratio of carbon from soil microbial biomass to total soil organic carbon compared to the original soil.

3.2. Planting effects on total and active soil microorganisms

Both bacteria and fungi were affected after 63 days of incubation without plants present. A decrease in diversity of the total and active community of both bacteria and fungi was observed as well as a decrease in the abundances of the active bacterial and fungal communities and the RNA:DNA ratio (Figure 2). The active bacteria and fungi were even less abundant in soils with plants. With *T. aestivum*, the original levels of total and active bacterial diversity were maintained, whereas they increased in soil with *A. thaliana*. For the total fungal diversity, the original level was maintained with both plant species, but the diversity of the active fungal community decreased even though *A. thaliana* showed higher diversity levels than bare soil. Interestingly, the copy numbers of the 16S rRNA was 3-fold higher than its gene (Figure 2).

All soils were dominated by the same main bacterial phyla, i.e. *Alphaproteobacteria*, *Acidobacteria*, *Actinobacteria* and *Planctomycetes*, but *A. thaliana* enriched for *Zygomycota*, *Ascomycota* and some unknown fungi (Figure 3). The growth of *A. thaliana* and *T. aestivum* induced significant changes in the structure of the total bacterial and fungal communities and the active fungal community in the vicinity of their roots (Figure 4 A, E & G, $P = 0.004$, 89.81 % of variance explained; $P = 0.028$, 76.36 %; $P = 0.003$, 98.17 %, respectively), while the structure of the active bacterial community was similar between planted and bare soils (Figure 4 C, $P = 0.215$, 88.09 % of variance explained).

We further identified the OTUs driving the observed differences, either because they were enriched or depleted (from DNA) and activated or inhibited (from cDNA) by the plants (Figure 4 B, E, D, H). While the plants induced depletion or inhibition of a high number of bacterial OTUs, they mainly induced enrichment or activation of specific fungal OTUs (Figure

4). *Bacillus* (OTUs 1 and 7) and *Methylobacteriaceae* (OTU 17) were the most depleted bacteria by the two plants while the *Xanthobacteraceae* (OTUs 5, 4 and 9) was specifically depleted by *A. thaliana* (Figure 4B). Among the most abundant bacterial OTUs inhibited by the two plants, some were depleted by both plants (OTUs 1, 4 and 7), and the bacterial OTUs specifically inhibited by *A. thaliana* were all affiliated to *Bradyrhizobium* (OTUs 5, 3 and 2) (4D). For fungi, the only OTU inhibited by the two plants was affiliated to *Sordariomycetes* (OTU 1) (4H).

On top of these general trends, we found a stronger enrichment by *A. thaliana* than *T. aestivum* that was more pronounced for fungi than bacteria (Figure 4 B & E). A total of 46 and 70 DNA-based bacterial and fungal OTUs, respectively, were enriched with *A. thaliana*, against 32 and 14 with *T. aestivum*. *Proteobacteria* (delta, beta and alpha) were mainly enriched by *A. thaliana* while *T. aestivum* mainly enriched *Actinobacteria*, *Acidobacteria*, *Bacteroidetes* and *Planctomycetes*, (data not shown). For fungi, the enriched OTUs by *A. thaliana* were highly abundant and mainly affiliated to *Mortierella* (OTUs 5, 11 and 13) (4F). We detected a stronger activation of bacteria with *T. aestivum* than with *A. thaliana*, which instead activated more fungi when compared with the total microbial communities (4 D & G). The bacterial and fungal OTUs that were activated were also different from those that were enriched (data not shown).

3.3. Plant legacies on basal respiration and carbon-litter mineralization

The modifications observed in the bacterial and fungal communities after 63 days of plant-growth were plant specific. In the following litter-carbon mineralization experiment these changes were associated with functional changes in the soil basal respiration rate. In soil without litter addition, the basal respiration rate in soils that previously had plants, regardless of plant species, was always higher than in the bare soil (Figure 5). The daily emissions were peaking at day 7, with *T. aestivum* having the strongest effect, before decreasing until day 49 independent of the previous treatment in phase 1.

Following soil C-enrichment (litter addition), the soil respiration rate increased in all soils as expected (Figure 5). By contrast to soil without litter addition, the respiration rate and cumulative C-respired were higher in the bare soil than in soils that previously had plants, at days 7 and 21. At day 49, these observations reverted, the respiration rate being higher in the soils that had plants than in the bare soil, with no differences between plants (Figure 5).

3.4. Plant legacies on active microbial successions

The temporal dynamic of abundances and composition of active bacterial and fungal communities varied according to the soils being either C-enriched or not by litter (Figure 3). In the previously bare soil without litter, the abundances of active bacteria and fungi gradually decreased over time. In previously planted soils without litter addition we observed an increase in the abundance of active fungi and bacteria at day 3. For fungi, this was due to an activation of *Ascomycota* (Figure S8). At day 49, the bacterial and fungal abundances were similar to those at the beginning of the second phase experiment.

In soils with litter addition, bacterial and fungal abundances gradually increased until day 21, but then reverted to their initial abundance at day 49. The DNA-based OTUs significantly enriched by the growth of *A. thaliana* or *T. aestivum* (Figure 4 B & F) were compared to their corresponding RNA-based OTUs at all time points of the litter-carbon mineralization experiment. Among the bacterial OTUs enriched by *A. thaliana* or *T. aestivum*, some were activated at the beginning of the incubation (days 3 and 7), but did not remain activated until day 49 (Figures S4 & S5). The fungal OTUs enriched by *A. thaliana* were not more activated along the litter-carbon mineralization experiment (Figure S6), but for those enriched by *T. aestivum*, we observed higher abundances at day 21 (Figure S7).

The soil C-enrichment with litter did not conceal the plant legacy effects detected without litter addition. In *A. thaliana* soil, similar microbial dynamics as without litter were observed, however, with litter, the changes in abundances were less pronounced. The increase in microbial abundances observed at day 3 was due to an activation of *Alphaproteobacteria* (*Rhizobiales*) and *Ascomycota* (*Sordariomycetes*) (Figure S9). Further, we observed an activation of *Deltaproteobacteria* (*Myxococcales*) and *Zygomycota* at day 49. In the *T. aestivum* soil, the community dynamics were different with litter than without, with high bacterial and fungal abundances at day 21 and lower abundances at day 49 compared to day 0. The decreased fungal abundance at day 49 was mainly due to a decrease in active *Ascomycota* (*Sordariomycetes*) (Figure S9).

When the structure of the active bacterial or fungal communities was different between soils, we used co-inertia analyses to identify if there was any relationships between the OTUs that contributed significantly to the differences between soils and the measured soil variables. The NO_3^- soil content was the sole variable explaining the differences between soils. Such significant relationships between planted soil history and soil parameters were identified for bacteria at day 7 with litter and for fungi at day 0 and 49 with and without litter, respectively (Figure S10). For bacteria, OTUs mainly affiliated to *Alphaproteobacteria* (*Caulobacteraceae* and *Sphingomonadaceae*), *Bacteroidetes* (*Cytophagaceae* and *Microscillaceae*) and *Gammaproteobacteria* (*Burkholderiaceae* and *Cellvibrio*) were more abundant in the bare soil and positively correlated with the NO_3^- soil content (Figure S10). For fungi, the OTUs that were more abundant in the bare soil and positively correlated with the NO_3^- content were affiliated to *Ascomycota* (*Sordariomycetes* and *Dothideomycetes*). Lastly, at day 49, the OTUs that were more abundant in the *A. thaliana* soil compared to the bare soil, were negatively correlated with NO_3^- soil content and affiliated to *Zygomycota* (*Mortierella*) and *Basidiomycota* (*Solicoccozyma* and *Tremellales*).

4. Discussion

4.1. Plant-specific effects on the total and active soil microorganisms and soil nutrients

As hypothesized, the growth of both *A. thaliana* and *T. aestivum* induced the selection of specific soil bacteria and fungi in the vicinity of their roots. We showed that the effect of *A. thaliana* was stronger, especially for fungal communities. Root architecture as well as rhizodeposits are highly plant species-specific and could thus differentially act on microbial communities depending on plant species, leading to specific recruitment of root- and rhizosphere-associated microorganisms (Berg and Smalla, 2009). In the present study the presence of plants, irrespective of plant species, resulted in an increase in the dissolved soil organic carbon content and carbon in microbial biomass, likely reflecting the inflow of carbon to soil microbes through plant carbon deposition. At the same time, a depletion in mineral nitrogen was observed, which could be due to plant N uptake that occurred concomitantly with plant carbon deposition during plant growth. Tkacz et al. (2015) showed that changes in the soil microbiota following plant growth appeared quickly after only one plant generation and were plant-species dependant. However, in our study we showed a

stronger effect of *A. thaliana* than *T. aestivum* on abundances and community composition of total bacteria and fungi, while Tkacz et al. (2015) showed the opposite, probably due to its extensive root system (Tkacz et al., 2015). In our experiment the plants grew longer than in the study of Tkacz et al. (2015), allowing *A. thaliana* to reach its flowering state. This could explain the differences as it has been shown that the plant effects on soil microbiota depend on the growth stage of the plant, with the strongest effect at the flowering stage, whereas the effect is weaker during earlier growth stages (Cheng and Kuzyakov, 2005). The growth stage might thus have overtaken the effect of root density in shaping rhizospheric microbiota leading to different results than those of Tkacz et al. (2015). Another explanation could be the differences in the properties of the soils used. While we used a sandy-loam agricultural soil, Tkacz et al. (2015) used artificial soils consisting of compost or sand. They observed that plant selection on the rhizosphere microbiota depended strongly on soil composition probably due to contrasted organic nutrient contents. Nevertheless, both studies results point toward a specific plant effect on soil microbial communities that depends on the properties of the original soil.

Differences between total and active microbial community have already been demonstrated, with active microorganisms being a subset of the total community (Romanowicz et al., 2016). However, for bacteria, it is well known that the copy number of the 16S rRNA gene varies between taxa (Větrovský and Baldrian, 2013) and Blazewicz et al., (2013) pointed out that the relationship between rRNA concentration and bacterial growth rate can differ greatly among taxa. These differences could explain why we observed higher 16S rRNA copy numbers than its gene. Globally, we showed that the enriched or activated OTUs differed between plant species, indicating a plant-specific effect on both total or active bacteria and fungi. However, most of the depleted and inhibited OTUs were common to both plants and the negative interaction therefore seemed less plant-specific. The depletion or inhibition of certain OTUs was more pronounced for bacteria than for fungi. Surprisingly, both plants depleted or inhibited *Bacillus sp.* and *Bradyrhizobium sp.*, of which some are known to be plant growth-promoting bacteria (reviewed in Govindasamy et al., 2010). However, both plants also recruited plant growth-promoting bacteria, which can provide direct benefits to plant growth and health by influencing the nutrient status or affecting plant-pathogen interaction. *T. aestivum* selected more total and active bacteria, while *A. thaliana* recruited

preferentially total and active fungi and inhibited fungal pathogens like *Alternaria* sp. Some of these species, e.g. *Mycrosphaerella tassiana* and *Pyrenophora teres* (similar to OTUs 9, 7 and 2, respectively) are known to cause leaf diseases, such as net blotch (Thomma, 2003; El-said et al., 2014; Moya et al., 2018). Further, *A. thaliana* enriched *Mortierella*, *Tremellales* and *Sordariomycetes*, in agreement with both Tkacz et al., (2015) and Fabianska et al., (2019). *Mortierella* and *Tremellales* are known to be root associated saprotrophic fungi involved in litter decomposition (Keim et al., 2014; Kuramae et al., 2013). Regarding the main bacterial phyla enriched by *A. thaliana*, the *Proteobacteria* have already been detected as recruited by this plant (Micallef et al., 2009; Bulgarelli et al., 2012; Lundberg et al., 2012) and members of this phylum possess several plant growth-promoting mechanisms (see references in Naz et al., 2014) and/or are associated with diseases suppression (Mendes et al., 2011). *T. aestivum* enriched other bacteria, such as *Planctomycetes*, *Acidobacteria*, *Bacteroidetes* and *Actinobacteria*, which is in agreement with those identified by Mahoney et al. (2017). *Actinobacteria* and *Bacteroidetes* are known to be plant-growth-promoting bacteria through the synthesis of different phytohormones, siderophores and enzymes and also their ability for solubilization of mineral nutrients such as phosphorus, potassium, and zinc (reviewed in Yadav et al., 2018). The soil phosphorus contents we measured in soil planted with *T. aestivum* are in accordance with higher microbial ability for providing phosphorus from soil matrix due to enrichment of plant growth-promoting bacteria by *T. aestivum*. A renewal of the soil phosphorus pool through solubilization could explain the differences in the soil content of phosphorus between the two plants, with the phosphorus pool decreasing in *A. thaliana* but not in *T. aestivum*. Also root architecture and mycorrhizal colonization can influence the efficiency of plants phosphorus uptake (Richardson et al., 2009; Zuo et al., 2014).

4.2. No plant legacy effects on the mineralization of soil organic carbon despite higher plant-induced basal respiration

Based on the observed plant species-specific effects on the microbial community composition in the plant-soil experiment and the difference in the quantity or quality of the carbon (from rhizodeposits or litter) between plant species (Johnson et al., 2007; Gul et al., 2012), we expected different basal respiration rates during the mineralization experiment. However, there were no plant-species effects, but rather a plant effect. The quantity of

organic carbon and the respiration rates were enhanced in both soils that previously had plants compared to the bare soil when no litter was added. The previously planted soils also had higher diversity of active and total bacteria as well as total fungi compared to the previously bare soil. These results suggest functional redundancy within the communities, which could be expected for carbon respiration, which is a generalist function of central importance for soil ecosystem resilience (Bender et al., 2016). Moreover, we cannot exclude that the plant legacy might have been reduced by the soil disturbance and the homogenization that occurred during the soil sieving for conducting the carbon litter mineralization experiment.

We did not observe any plant-specific effect following the addition of *T. aestivum* litter. The seeds of *T. aestivum* used for the plant-soil experiment and the litter used for the mineralization experiment came from the same variety and batch. Hence, in the context of plant-soil feedbacks and legacy effect, we could have expected some litter specificity (Veen et al., 2015a). Due to previous plant uptake, the mineral nutrient availability in the soil was lower and could thus have limited the degradation of high C:N litter, as previously shown (Partey et al., 2014). This nutrient limitation would explain the lower mineralization rates in previously planted soil compared to the bare soil when litter was added. In agreement, we observed a pulse of emission at day 7 in the bare soil with litter and simultaneously, we identified that diazotrophic bacteria, such as *Caulobacteraceae* (*Alphaproteobacteria*), *Burkholderia* and *Cellvibrio* (*Gammaproteobacteria*) (Suarez et al., 2014; Pagé et al., 2019), were activated and positively correlated to the soil NO_3^- content. Their activity would thereby support the nitrogen demand needed for degradation of the high C:N ratio litter from *T. aestivum*. Moreover, the limitation of the litter carbon mineralization that we observed and which was linked to the inorganic nitrogen status, is in agreement with the regulatory gate theory proposed by Brookes et al. (2008). This theory suggests that the rate of the soil organic matter mineralization is directly governed by abiotic processes, such as nutrients dynamics in the soil matrix, rather than by soil microbial communities themselves (composition, abundance or activity).

A microbial legacy due to specific plant species was observed until the end of the mineralization experiment for both plants, although microbial successions with different responses to the addition of litter according to soils were demonstrated. Among the OTUs

enriched by *A. thaliana*, some bacterial OTUs were also activated in the beginning of the incubation period of the litter experiment but, globally, these OTUs did not remain activated until the end of the experiment. We previously highlighted that both plant species recruited total plant growth-promoting bacteria during their growth. Due to these intimate interactions, the disappearance of the plants could lead to the inactivation of these specific bacteria during the mineralization experiment. The peak in abundance of active bacteria observed at day 3 was potentially due to the activation of copiotrophic microorganisms (*Alphaproteobacteria* and *Ascomycota*), while at the end of the incubation, the oligotrophic *Deltaproteobacteria* were stimulated (Bastian et al., 2009; Verastegui et al. 2014; Bastida et al., 2016). Microbial abundances shared similar temporal patterns in the *T. aestivum* and *A. thaliana* soils without litter, but not when the soil was C-enriched with litter. The peak of active fungal abundances at day 21 was mainly due to unknown fungi and their decrease at day 49 was mainly due to the copiotrophic *Ascomycota*. At this timepoint, the higher abundances of active microorganisms in the soil that previously had plants were concomitant with higher daily emissions.

To conclude, the effect of plants on the soil microbiome was plant-species specific. While both plants recruited plant growth-promoting bacteria in the vicinity of their roots, *A. thaliana* had a stronger effect on fungi than *T. aestivum*. The species specific responses were also different between active and total bacterial and fungal communities. However, this was not reflected in the observed plant legacy effect on the carbon mineralization from litter as higher basal respiration rates were detected in planted soils irrespective of plant species. Our results suggest that the soil nutrient dynamics induced by the presence of plants rather than changes in soil microbial communities drive the organic carbon mineralization of added litter. Nevertheless, soils with a planting history either maintained or displayed a higher abundance of active soil microorganisms, which was concomitant with higher daily respiration rates. In addition, the present study further highlights the importance to consider both total (DNA-based) and active (RNA-based) microbial communities to be able to differentiate how plants control enrichment/depletion and activation/inhibition of specific microorganisms.

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Competing Interests

The authors declare no competing interests.

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Figure legends

Figure 1: Carbon, nitrogen and Olsen phosphorus content in the original soil and at the end of the plant-growing experiment (*A. thaliana*, *T. aestivum* and bare soil). The different letters indicate significant differences between treatments according to ANOVA and Tukey's

test ($n=4$, $p<0.05$). The red dot in each box indicates the mean value. MBC: microbial biomass carbon, MBN: microbial biomass nitrogen.

Figure 2: Microbial community abundances and diversity in the original soil and after terminating the plant-soil experiment (*A. thaliana*, *T. aestivum* and bare soil). The abundance and diversity (Shannon diversity index) of the total bacteria and fungi were obtained from DNA extracts and the abundance and diversity of active bacteria and fungi from RNA extracts. The different letters indicate significant differences between treatments according to ANOVA and Tukey's tests ($n=4$, $p<0.05$).

Figure 3: Legacy effects of previously cultivated *A. thaliana* and *T. aestivum* in soil without or with litter addition on composition of the active bacterial and fungal communities. The composition of the total bacterial and fungal communities present just after the plant-growth phase (63 days) is represented on the left part of the figure.

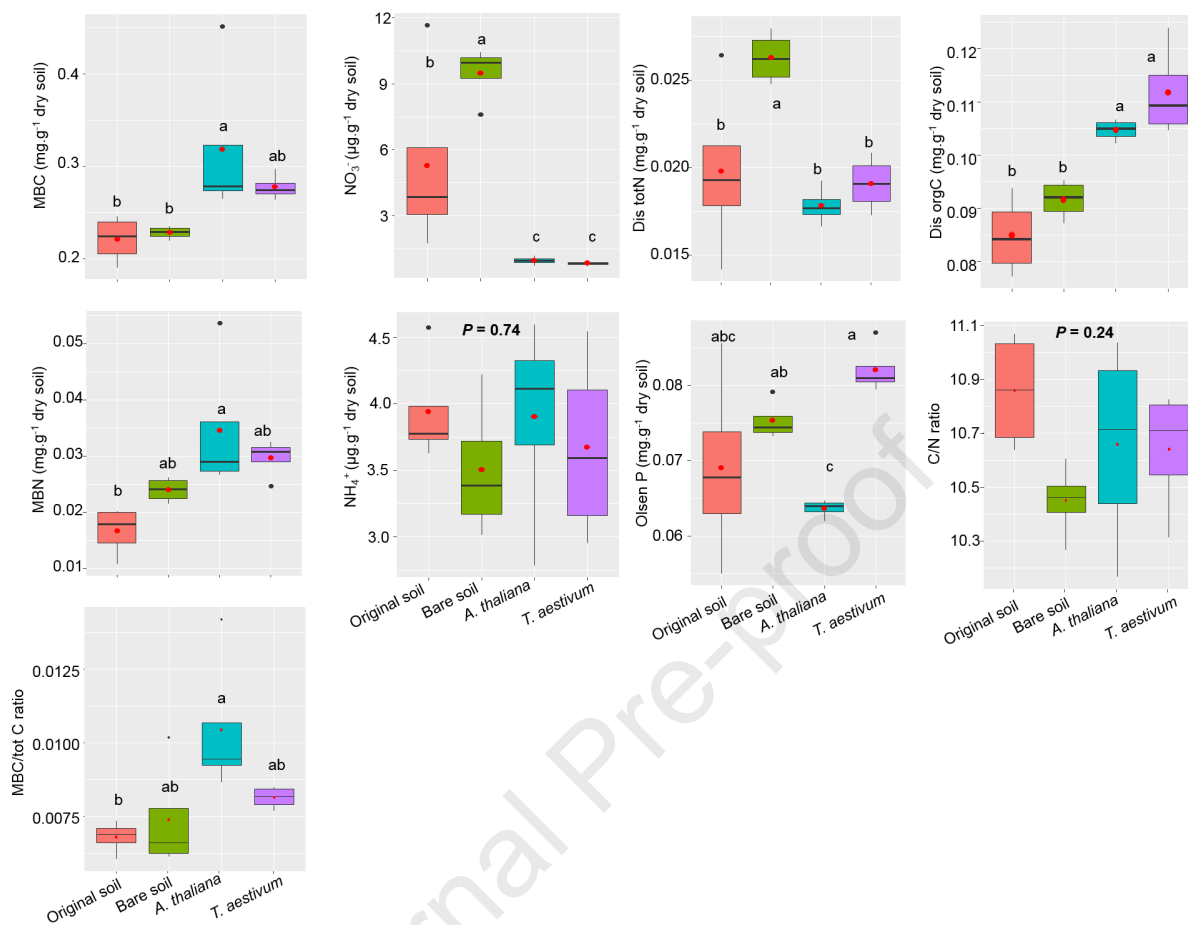
Figure 4: Structure and composition of the total and active bacterial and fungal communities in unplanted (bare soil) or planted soil with *A. thaliana* or *T. aestivum* after 63 days of plant-growth. A, C, E, G – Powered partial least squares discriminant analysis (PPLS-DA) describing the total and active bacterial and fungal community structures at the OTU level after plant-growth. B, D, F, H - Ternary plots describing the distribution of the individual bacterial and fungal DNA-based or RNA-based OTUs between the soil treatments after plant-growth (grey circles). Color code: Enriched (DNA based) or activated (RNA based) OTUs by *A. thaliana* (dark green circles) or by *T. aestivum* (blue circles), depleted (DNA based) or inhibited (RNA based) OTUs by *A. thaliana* (light green) or by *T. aestivum* (light blue), enriched or activated OTUs by both plants (orange) and depleted or inhibited by both plants (yellow). The numbers below *A. thaliana* or *T. aestivum* corresponded to the OTUs that were only present in these soils. The inserted boxes zoom on the most abundant OTUs.

Figure 5: Daily and cumulative C-CO₂ emissions at different sampling times in different soils without or with litter addition. Different letters indicate significant differences at each day between treatments according to ANOVA and Tukey's test ($n=4$, $p<0.05$). Bare soil: not planted soil; *A. thaliana*: soil previously planted with *A. thaliana*; *T. aestivum*: soil previously planted with *T. aestivum*; Bare soil-litter: not planted soil with plant litter addition; *A.thaliana*-litter: soil previously planted with *A. thaliana* and with plant litter addition; *T. aestivum*: soil previously planted with *T. aestivum* and with plant litter addition

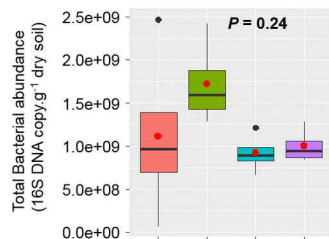
Table 1: Physical, chemical and microbiological properties of the soil used for plant-soil experiment (phase 1).
(Means and standard errors, n=4).

Physical and chemical	Data (mean, n=4)	Microbiological properties	Data (mean, n=4)
% Clay	12.9	MBC ($\mu\text{g g dry soil}^{-1}$)	221 ± 13
% silt	40.2	MBN ($\mu\text{g g dry soil}^{-1}$)	17 ± 2
% sand	46.8	Total fungi TS (copy g dry soil ⁻¹)	$2.29\text{E}+07 \pm 1.15\text{E}+07$
% organic matter	4.9	Total bacteria 16S (copy g dry soil ⁻¹)	$1.12\text{E}+09 \pm 5\text{E}+08$
Tot orgC (mg g dry soil ⁻¹)	33 ± 1	DNA (ng g dry soil ⁻¹)	21323 ± 8299
tot N (g kg dry soil ⁻¹)	3 ± 0.12	Active fungi (ITS (copy g dry soil ⁻¹))	$9.80\text{E}+07 \pm 7.81\text{E}+06$
Diss tot N ($\mu\text{g g dry soil}^{-1}$)	20 ± 23	Active bacteria 16S (copy g dry soil ⁻¹)	$1.66\text{E}+12 \pm 3.84\text{E}+10$
Diss NO ₃ ⁻ ($\mu\text{g g dry soil}^{-1}$)	5 ± 2	RNA (ng g dry soil ⁻¹)	13371 ± 1153
Diss NH ₄ ⁺ ($\mu\text{g g dry soil}^{-1}$)	4 ± 0.2		
Diss orgC ($\mu\text{g g dry soil}^{-1}$)	85 ± 4		
Olsen PO ₄ (mg g dry soil ⁻¹)	69 ± 6		
P (mg kg dry soil ⁻¹)	212		
Ca (mg kg dry soil ⁻¹)	1159		
Mg (mg kg dry soil ⁻¹)	182		
K (mg kg dry soil ⁻¹)	392		
pH _{water}	5.5		
CEC	10.8		

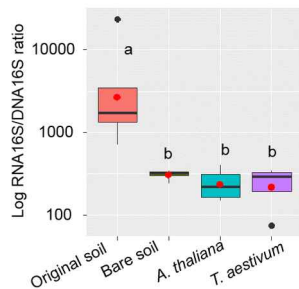
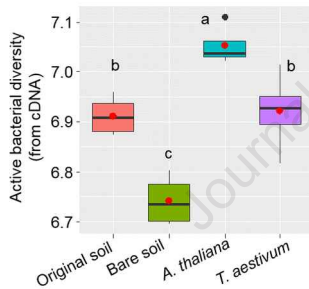
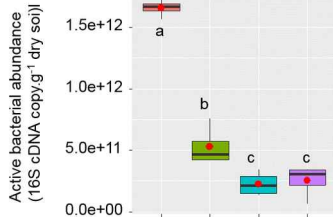
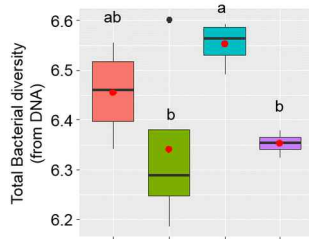
Journal Pre-proof



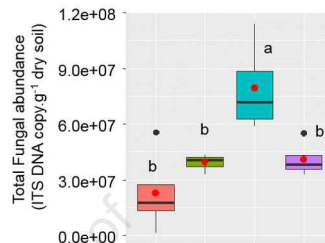
Abundances



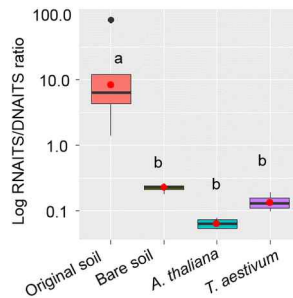
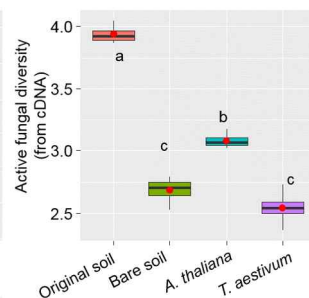
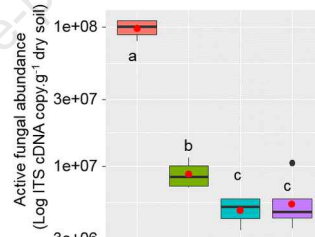
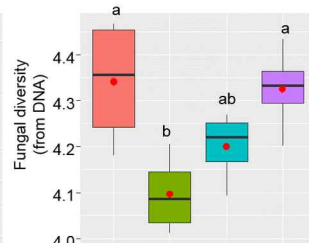
Diversity

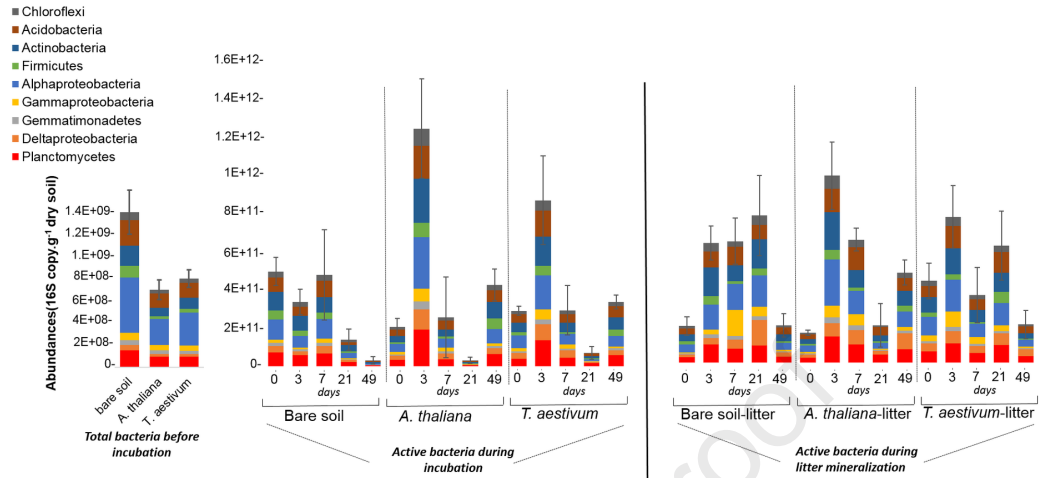
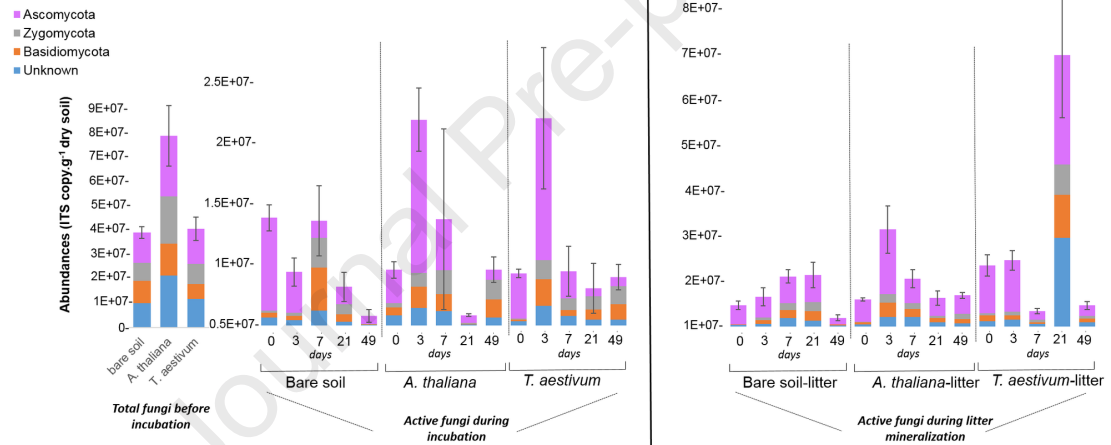


Abundances

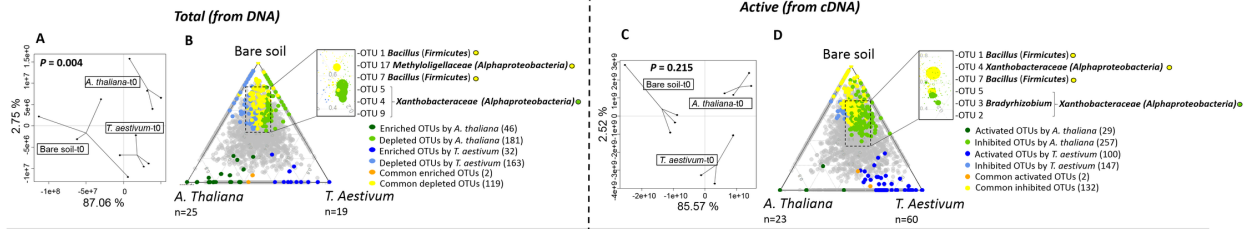


Diversity

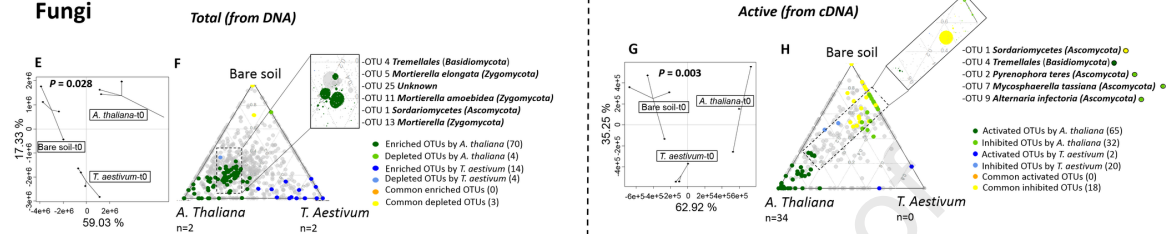


Bacterial phyla**Fungal phyla**

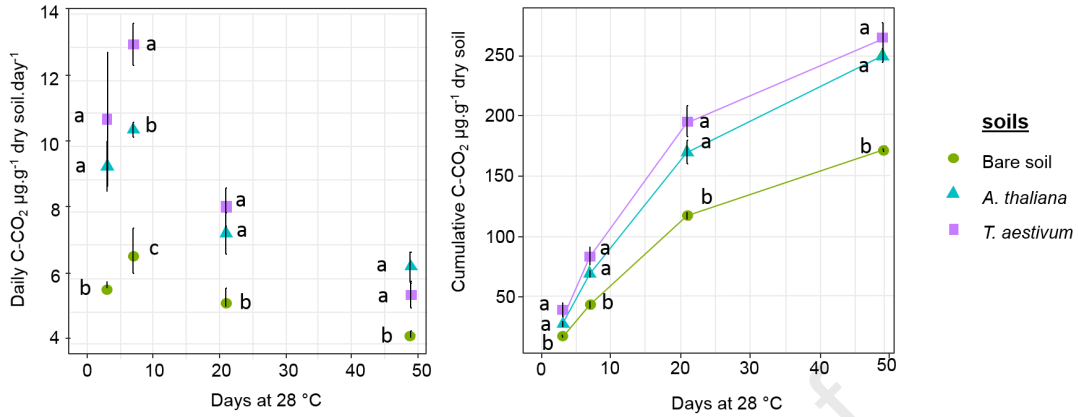
Bacteria



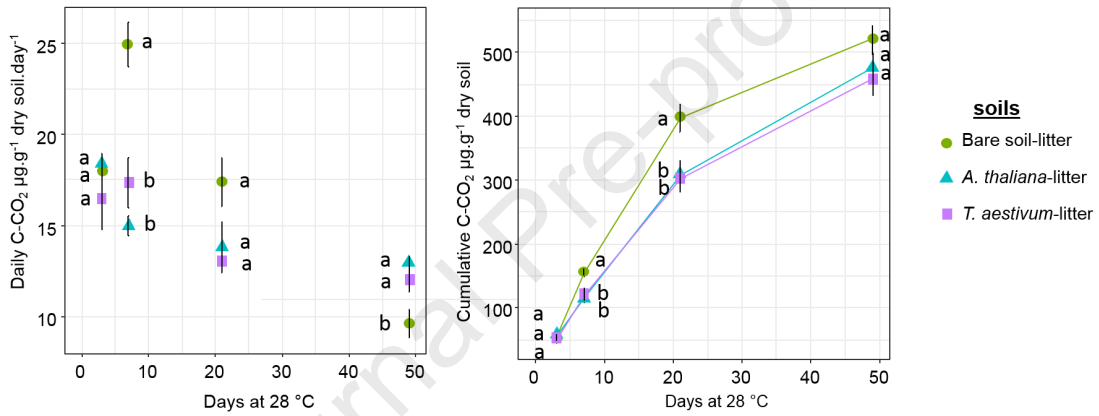
Fungi



Without litter



With litter



- *A. thaliana* and *T. aestivum* differently shaped the soil microbial communities
- *A. thaliana* had a stronger effect, especially for fungal communities
- PGPB were recruited in the vicinity of the roots of both *A. thaliana* and *T. aestivum*
- Plant legacy effects traduced in changes in soil organic carbon and nutrient dynamics.
- Plant legacy effects linked to nutrient dynamics rather than to microbial community

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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