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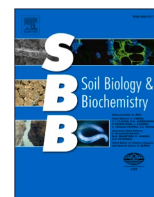

On the legacy of cover crop-specific microbial footprints

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A B S T R A C T

Apart from improving the physical and chemical condition of arable soils, cover crops have the potential to boost and activate selected soil microbiota that could contribute to improved nutrient cycling and strengthened disease suppressiveness. However, a main crop can only benefit from cover crop-induced microbial shifts if these persist until the onset of the main growing season. Here, we map the persistence of microbiome changes by cover crops over time. We performed a field experiment on a sandy soil with ten different cover crop monocultures belonging to five plant families, one cover crop mixture and a fallow control. Cover crops were grown for 4.5 months under field conditions in 70-L bottomless containers in a random block design with eight replications. We studied the total (DNA-based) and the potentially active (RNA-based) microbial fractions at the onset of the main growing season, and just after the harvest of the main crop, potato (respectively 3.5 and 10 months after cover crop termination), through MiSeq sequencing. All cover crops tested induced shifts in the soil microbiome that lasted at least until the onset of the main growing season. Cover crop treatments gave rise to species and even cultivar-specific microbial footprints, and - although roughly the same trends were observed - DNA-based microbial shifts were not necessarily paralleled by similar changes at RNA level. We conclude that cover crops have the potential to act as handles to steer the soil microbiome in a way that is supportive of sustainable crop production.

1. Introduction

Protection, restoration and promotion of sustainable use of terrestrial ecosystems belong to the targets of ‘Life on Land’, one of the United Nations Sustainable Development Goals (UN, 2015). Achieving this goal will require a wide range of adaptations of soil management practices to maintain productivity while supporting natural processes that promote and regenerate ecosystem services (Bommarco et al., 2013; Garibaldi et al., 2019; Kremen, 2020). The use of cover crops is considered a tool that could contribute to the realisation of some of these goals (Wittwer et al., 2017). Winter cover crops are non-cash crops that essentially are grown between cash crop growing seasons to protect against soil erosion and prevent nutrient leaching (Blanco-Canqui et al., 2015; Kaye and Quemada, 2017; Daryanto et al., 2018). At the end of their growing season, cover crops are mowed and incorporated into the topsoil and as such, they increase the organic matter content of soils (Blanco-Canqui et al., 2015). Furthermore, cover crops can amply affect soil microbial growth and activity (Vukicevich et al., 2016; Finney et al., 2017; Kim et al., 2020).

Rhizodeposition, the active and/or passive release of organic and inorganic components from living plant roots (Wichern et al., 2008), promotes a selection of the soil bacterial and fungal communities (Hartmann et al., 2009; Philippot et al., 2013). Plants invest heavily in the generation of a beneficial microbiome in the rhizosphere (Berendsen et al., 2012), which, in turn, supports the plant in nutrient acquisition and disease suppressiveness (Philippot et al., 2013). Together with plant exudation, predation by soil protists is the other main factor shaping the rhizosphere microbiome. By selective grazing on bacteria and fungi, protists contribute to enhancing nutrient turnover, which stimulates microbial activity (Gao et al., 2019; Xiong et al., 2020; Guo et al., 2022).

Soil microbial organisms should be metabolically active in order to have any effect on plants. In bulk soil typically 80% of the cells and 60% of the microbial taxa are dormant (Lennon and Jones, 2011). Although they can be resuscitated upon environmental changes, dormant

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microbes - as long as they are metabolically inactive - do not contribute to soil functioning. DNA-based community characterization will map both the active and the dormant fractions. To understand the effects of cover crops on the functioning of the soil microbiome it is informative to map the (RNA-based) potentially active microbial fraction in parallel (Jones and Lennon, 2010; Blagodatskaya and Kuzyakov, 2013). In some cases, ribosomal RNA (rRNA) was shown to be present in dormant bacterial cells (*e.g.,* Sukenik et al., 2012). For this reason, rRNA data are used here as an index for potential activity (Blazewicz et al., 2013), rather than for microbial activity *per se.* Contrasts between rDNA-based and rRNA-based microbial communities in an agronomic setting have been mapped previously (*e.g.,* (Duineveld et al., 2001; Ofek et al., 2014; Bay et al., 2021).

Cover crops have the potential to be used for host-mediated engineering of microbiomes (French et al., 2021; Jing et al., 2022). Cover crops might be more suitable for this purpose than main crops, as cover crops comprise a wide range of plant species belonging to multiple plant families and functional groups, providing a broad palette of options. Regulation of the soil microbiome by cover crops occurs at two distinct stages: during plant growth through rhizodeposition, and during the decomposition of plant residues, after incorporation in the topsoil (Spedding et al., 2004). Leite et al. (2021) studied the impact of cover crops on soil microbial communities during plant growth. They demonstrated that cover crops significantly affected the indigenous bacterial community, such that the impact of the individual cover crops was detectable at a high taxonomic level (phylum level). These findings were corroborated by a recent study that compared the impact of ten different cover crop species on the rhizosphere microbiome (Cazzaniga et al., 2023). Cover crops were shown to produce species-specific microbial footprints, and Brassicaceous cover crops produced more pronounced microbial signatures than most other cover crops. In the second stage, the soil microbiome is affected by the incorporation of plant residues in the topsoil after cover crop termination. Decomposition of the cover crop residues by soil microbiota regulates the microbial community in a manner that deviates from the effect during cover crop growth. The effect of crop residues *per se* was pinpointed by Liu et al. (2021). By mixing soil with dried and milled cover crop material, they observed a promotion of root-associated microbial groups such as Proteobacteria, Bacillaceae and Mortierellomycetes. Similarly, Barel et al. (2019) studied the effect of residues of cover crops on the soil microbial community. Although significant changes in bacterial and fungal biomass were reported, no effect on the overall microbial community composition was detected.

A main crop can benefit from shifts in the microbial community that are induced by prior cover crops if such a shift would last-at least - until the onset of the main growing season. Such legacies have been described for maize. Ray et al. (2022) showed that the legacy of cover crops on the soil microbiome suppressed the spreading of the pathogen *Fusarium verticillioides*, whereas Murrell et al. (2020) demonstrated that mycorrhizal cover crops resulted in increased AMF colonisation of the main crop. Although these studies focused on very specific parts of the soil microbial community only, they demonstrate that main crops can benefit from shifts in the microbial community that are previously induced by cover crops.

To study the persistence of cover crop-induced changes in the soil microbiome over time, we performed a field experiment with ten winter cover crop monocultures, one mixture of eight cover crops and one fallow control. The cover crop monocultures belonged to five different families with different above and below-ground characteristics and were shown to induce distinct changes in the rhizobiome (Cazzaniga et al., 2023). To map the impact of winter cover crops on the soil microbiome, we compared the microbial community assembly in bulk soil just before the planting of the main crop (potato) and right after the harvest of the main crop with the initial cover crop-induced microbial shifts that were brought about in the rhizosphere (Cazzaniga et al., 2023). In this study, we mapped both the total microbial community and the potentially active fractions while focusing on three organismal groups: bacteria, fungi and protists.

We addressed three research questions all relating to the persistence of changes in the soil microbiome after cover crop termination: (1) Is the impact of cover crops that were incorporated in the topsoil in late autumn on the soil microbiome still detectable just before the planting of the main crop (early spring) (T2) and/or after the harvesting of the main crop (autumn) (T3)? (2) If the impact is still detectable at T2 and/or T3, do we see differences in the total (DNA-based) or in the potentially active (RNA-based) fractions of the microbial community among individual cover crop species? (3) Does the legacy of cover crops, either directly via residues or indirectly via persistent changes in the microbial community, affect plant performance and yield of the main crop, potato? A better understanding of the persistence of cover crops-induced changes in the soil microbiome over time will be instrumental in informed cover crop selection that - via its effect on microbiota – will contribute to a reduction of the use of pesticides and mineral fertilizers in agricultural practices.

2. Materials and methods

2.1. Study sites and sampling

The field experiment was located at the Wageningen University and Research experimental farm 'Vredepeel' in the southeast of the Netherlands (51°32′27'.5N 5°50''59''.4E). This field was characterized by sandy soil (1% clay, 7% silt, 88% sand) with an organic matter content of 4.5% (4.4–4.5%) and a pH of 5.7. For full details on the experimental layout see (Cazzaniga et al., 2023). In brief, ten cover crop monocultures and one cover crop mixture (Table 1) were sown in 70 litres of bottomless containers (∅ 55 cm, height 47 cm) on August 1,

Table 1
Cover crop common name, family, species and cultivars used in the field experiment, breeding companies providing the seeds.

Common name	Family	Species	Cultivar	Company
Marigold	Asteraceae	*Tagetes patula*	Ground control	Takii Europe (NL)
Black oat	Poaceae	*Avena strigosa*	Pratex	PH Petersen (DE)
Tall fescue		*Festuca arundinacea*	Firecracker	Barenbrug (NL)
Hybrid ryegrass		*Lolium hybridum*	Daboya	Vandinter Semo (NL)
Oilseed radish	Brassicaceae	*Raphanus sativus* var. *oleiformis*	Terranova	Joordens Zaden (NL)
Oilseed radish		*Raphanus sativus* var. *oleiformis*	E1039	Joordens Zaden (NL)
Phacelia	Boraginaceae	*Phacelia tanacetifolia*	Beehappy	DSV Zaden (NL)
Borage		*Borago officinalis*	Wild type	Nebelung (DE)
Vetch	Fabaceae	*Vicia sativa*	Amelia	Joordens Zaden (NL)
Lentil		*Lens culinaris*	Eston	Joordens Zaden (NL)
Mix: 6% Alexandrian clover, 2% Ethiopian Mustard, 1% Camelina, 7% Flax, 15% Niger, 20% Black oat, 14% Oilseed radish, 35% Common vetch	Multiple families	*Trifolium resupinatum, Brassica carinata, Camelina sativa, Linum usitatissimum, Guizotia abyssinica, Avena strigosa, Raphanus sativus* var. *oleiferus, Vicia sativa*	Solarigol	DSV Zaden (NL)

2019. Thus, eleven treatments with cover crops plus an unplanted control ('fallow') were represented in each of the eight blocks. Treatments were randomized per block. In total the experiments comprised 96 containers, 14 were excluded from the experiment because of poor growth of the cover crop, and they were not concentrated on a particular treatment or replicate.

At the first sampling time point (October 3, 2019, here referred to as T1), rhizosphere soils were collected for each of the cover crop species and processed as described in (Cazzaniga et al., 2023). Cover crop mixtures were not sampled at T1 because of the impossibility to collect representative samples from the highly intertwined root systems. On December 16, 2019, cover crops were cut and manually incorporated into the soil. About 3.5 months after cover crop incorporation (April 9, 2020), bulk soil was collected from each container. This constitutes the second sampling time point hereafter referred to as T2. An auger (∅15 mm, core length 20 cm) was used to collect three soil cores per container. After thorough mixing, subsamples (6–8 g) were snap-frozen in N_2 (l) and stored at −80 °C until further processing.

On May 4, 2020, three seed potatoes (var. Agria) were planted per container. Following standard practices, plants were sprayed with fungicides to prevent the spreading of potato late blight. On June 5, plant performance (growth, health and colouration) was recorded by attributing scores on a 1–9 scale (from poor to optimal performance, following internally standardised crop assessment criteria developed and applied by Wageningen Research, Field Crops). On October 17, 2020, the potato tubers were harvested, and the tuber weight was determined per container. Just after harvesting, bulk soil was sampled again (referred to as T3) by collecting soil cores as described above for T2 sampling. Soil samples were snap frozen and stored at −80°C until further processing.

2.2. Nucleic acids extractions and sequencing library preparation

Nucleic acids (DNA and RNA) were co-isolated from subsamples of 2 g of soil following an in-house phenol-chloroform extraction protocol (Harkes et al., 2019). cDNA was synthesised from the extracted RNA using the Maxima First Strand cDNA Synthesis Kit for RT-PCR (Fermentas, Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions. Metabarcoding of DNA and cDNA was carried out in two main PCR steps following the procedure described in Cazzaniga et al. (2023). In brief, the first organismal group-specific loci were amplified: V4 region of 16S rRNA of bacteria, and V9 and V7–V8 of 18S rRNA of protozoa and fungi, respectively (Suppl. Table 2). In the second PCR, sample-specific index combinations were added to the amplicons. The resulting products were used to generate three libraries each covering 112 samples. Illumina MiSeq sequencing platform was used for paired-end sequencing (2 x 300 bp, V3 kit) at the Bioscience unit (Wageningen Research, Wageningen, The Netherlands). To guarantee sufficient sequencing depths, libraries from the three time points were sequenced in three separate MiSeq runs. Raw reads were demultiplexed by the sequencing service provider.

2.3. Pre-processing of raw sequencing data

Demultiplexed raw-sequencing data were processed following the procedure described in Cazzaniga et al. (2023). Reads were processed in QIIME2 and denoised with the DADA2 algorithm for paired-end sequences (Callahan et al., 2016). Amplicon Sequence Variants (ASVs) (Callahan et al., 2017) were assigned to taxa using the q2-feature-classifier plugin and *classify-sklearn* function (Pedregosa et al., 2011) with pre-trained reference databases for each primer-specific amplicon. Bacteria and fungi were assigned using two pre-trained databases based on the non-redundant SILVA reference database (Glöckner et al., 2017) (*silva-138-ssu-nr99-seqs-derep-uniq*, version 138, 99% identity criterion). Protists were assigned based on the pre-trained pr2 reference database (Guillou et al., 2012). QIIME2 files were imported into Rstudio (v. 1.4.1106, R version 4.0.4) and processed following the same procedure and filtering cut-offs as used in Cazzaniga et al. (2023), utilizing the R packages phyloseq (v1.34.0) (McMurdie and Holmes, 2013), metagMisc (v0.0.4) (Mikryukov, 2017), microbiome (v.1.12.0) (Lahti, 2012–2019), and metagenomeSeq v. 1.32.0) (Paulson et al., 2013). To facilitate comparison with other studies using the same reference databases, the original names assigned to the ASVs from the reference databases were left unchanged. Unassigned ASVs, ASVs assigned to chloroplasts and mitochondria, ASVs from non-target organisms as well as ASVs with a low prevalence (<10 reads per sample) were filtered out. Samples with <5,000 bacterial, <1,000 fungal and <500 protist reads were excluded from the analyses.

After processing and filtering the three resulting datasets comprised 6,487,844, 6,779,998 and 7,401,812 reads, respectively. The T1 dataset had a median sequencing depth of 50,188 reads per sample (with a standard deviation (SD) of 16,566 reads). The T2 and T3 datasets had a mean sequencing depth of 85,128 ± 17,072 (SD) and 91,103 ± 18,668 (SD) reads per sample. 4,667,586 and 4,753,973 filtered reads belonged to the total and potentially active bacterial community and were assigned to 7,528 and 7,851 ASVs respectively. The fungal DNA and RNA datasets comprised 3,135,632 and 3,371,378 reads, assigned to 828 and 1,367 ASVs. 799,511 and 2,344,919 filtered reads belonged to the protist's DNA and RNA datasets and were assigned to 2,165 and 3,870 ASVs respectively. Further information on the sequencing depth can be found in Supplementary Table 3. In the next sections, we simplified and shortened the term 'potentially active fractions of the microbiome' to 'active fractions' solely to facilitate readability.

2.4. Statistical analyses of the soil microbial communities over time

The effect of the cover crop treatments on the active and total soil microbial communities was determined at each timepoint with the PERMANOVA test subtracting the random effect of the experimental blocks (blocks and cover crop treatment terms were added sequentially in the model). The R^2 values resulting from the PERMANOVA indicate the percentage of the variation in the microbiome explained by the cover crop treatments (effects with $p < 0.05$ were considered to be statistically significant). Multiple comparisons among cover crop treatments and fallow were carried out with a Pairwise PERMANOVA test on Bray Curtis distances with Benjamini-Hochberg correction for multiple testing and 999 permutations in RVAideMemoire R package (v.0.9-81-2) (Hervé and Hervé, 2020).

2.5. Cover crops' microbial footprints over time

To pinpoint changes in the microbiome over time, differential abundance analyses were performed using ANCOM-BC (Analysis of Compositions of Microbiomes with Bias Correction (Lin and Peddada (2020). This method was employed to pinpoint the effect of cover crops on the stimulation or repression of microbial taxa i) in the cover crop rhizosphere (T1), ii) after incorporation and partial digestion of cover crop remains (T2) and, iii) after the harvest of the main crop, potato (T3). For shifts in the microbial community, fallow soil from the same sampling time point was used as a reference.

We analysed each time point separately to remove possible variation due to the sequencing batches. In the ANCOM-BC model, cover crop treatments were used as covariates of interest and the fallow soil was the reference level for each time point, while the response variable was the individual microbial groups at the family level (bacteria and fungi) and order level (protists) (ASVs from the same type were agglomerated using *tax_glom* function in phyloseq). Taxa were considered differentially abundant when they had a p-value <0.05 after correction with the Holm–Bonferroni method for multiple testing. Furthermore, ANCOM-BC allowed us to distinguish between negative beta coefficients, pointing at the suppression of a given taxon, and positive beta coefficients, indicative of stimulation of a specific taxon.

To determine the legacy of cover crop treatments on the individual

microbial taxa, we compared the differentially abundant taxa over time. To identify microbial footprints, microbial taxa differentially abundant in the cover crop rhizosphere (T1) and after the cover crop treatment throughout T2 and/or T3 were compared to the fallow at the same time point. We focused on three types of microbial footprints characterizing cover crop legacies over time: i) Type 1; a microbial taxon is significantly and consistently stimulated or repressed at T1 and T2 and T3; ii) Type 2: a microbial taxon is significantly stimulated or repressed at T1 and T2; iii) Type 3: a taxon is significantly affected with same directionality at T1 and T3 (not at T2). Patterns were represented as dot plots. The diameter of the dots represents the value of the beta coefficient, and the colour indicates stimulation (green) or repression (red) of a given microbial taxon.

2.6. Potato performance and yield

The effect of the preceding cover crop treatment on the potato plant performance and the tuber yield (kg) per container were analysed separately with mixed-models (lmerTest R package, Kuznetsova et al. (2017). In the mixed models, cover crops were the fixed effect, and blocks were treated as random effects. The pairwise comparisons were determined with Tukey HSD correction for multiple testing (emmeans R package, v. 1.7.2, Lenth, 2022).

3. Results

3.1. Legacy of cover crop-induced changes in native microbial communities

The effects of cover crops on the bacterial, fungal and protist communities lasted over time in a cover crop treatment-dependent manner (Fig. 1 A, B), but the effects gradually decreased over time both at DNA (explained variation T1: 35–43%, T2: 24–37% and T3: 16–19%, Fig. 1A) and at RNA level (T1: 36–50%, T2: 25–34%, T3: 15–17%, Fig. 1B). Cover crop legacies were detected for all three organismal groups, and the percentages of variation explained was the highest for fungi (at T2 the variation explained by cover crops was 37% and 34% for fungi vs 24% and 27% for bacteria, and 32% and 25% for protists, at DNA and RNA level respectively, Fig. 1A and B).

The PCoA ordinations show that both oilseed radish cultivars, as well as the cover crop mixture (dominated by oilseed radish), had the greatest impact on both the total and the active microbial communities at all three time points (Fig. 1). At T3, no separation between the cover crop treatments and the fallow control was observable for most treatments except for the oilseed radishes and the cover crop mixture (for all microbial groups at both DNA and RNA levels, Fig. 1 G-I, P-R). Pairwise comparisons among treatments confirmed these observations (Table 2 and Suppl. Table 4). In all pairwise analyses, the impact of each cover crop treatment was determined by comparing it with the condition of the fallow control (black circles in Fig. 1) at the corresponding time point (full pairwise comparisons presented in Supplementary Table 4). At T2, pairwise comparisons (Table 2 A, C, Suppl. Table 4 A, B) showed that oilseed radish and the mixture treatments significantly affected the bacterial, fungal and protist communities both at DNA and RNA levels. Also, marigold and hybrid ryegrass significantly affected the fungal and protist communities, although the corresponding R^2 values were considerably lower (Table 2 A, C, fungal community: 37–41% in oilseed radish and mixture vs 17 and 12% in marigold and hybrid ryegrass; in protist community 31–40% vs 14-11%). Lentil only had a significant effect on the fungal community at the DNA level, and on the protist community at RNA level. Black oat treatment only resulted in a significant effect on all organismal groups at RNA level, while tall fescue, borage and phacelia did not show a significant overall effect on the microbial communities.

At T3, just after harvesting the main crop (potato), only the impact of the two oilseed radish treatments and the cover crop mixture was still significant (Table 2 B, D, Suppl. Table 4 C, D). Focusing on the total communities, the cover crop treatment still explained 11–19% of the observed variation, whereas 11–16% of the variation was explained by the active microbial fractions (Table 2, B, D).

Hence, our data showed that both oilseed radish treatments and the cover crop mixture significantly affected each of the three organismal groups under investigation at both time points. It is noted that at T2, the onset of the main growing season, the impact of the cover crop treatments was more prominent at RNA than at DNA level.

3.2. Nature and legacy patterns of cover crop-induced microbial footprints

Differential abundance analysis (ANCOM-BC) was used to reveal the extent to which individual microbial taxa were contributing to the observed cover crop treatment effects on the different organismal groups over time. In total, we monitored 375 and 377 bacterial families at respectively DNA and RNA level, 130 and 144 fungal families, and 57 and 59 protist orders. To characterize the legacy of each of the cover crop treatments, the differentially abundant taxa at T2 (before the main growing season) and/or T3 (after the main growing season) were compared with the ones differentially abundant at T1. Based on this comparison, we defined a microbial footprint as a microbial family/order that is significantly promoted or repressed by a given cover crop treatment as compared to the corresponding fallow controls at - at least - two sampling times.

We identified 232 cover crop-affected microbial taxa at DNA level, of which 170 belonged to bacteria, 46 to fungi and 16 to protists (Suppl. Figs. 1 and 2). At RNA level, we found 268 differentially affected microbial taxa, consisting of 206 bacterial, 49 fungal and 13 protist taxa (Suppl. Figs. 3 and 4). In our analyses, we focused on footprints displaying three distinct types of consistencies in the promotion or repression of a microbial taxon over time, namely Type 1, Type 2 and Type 3, individually illustrated below. By focusing on shifts that were observed at - at least - two time points in the same direction we aimed to filter out stochastic effects.

Type 1 footprints refer to microbial taxa that are either consistently promoted or consistently repressed at all three time points for a given cover crop treatment. This type of footprint was relatively rare. It was observed only for 10 and 8 out of 232 and 268 microbial taxa at DNA and RNA level respectively. Among the ten cover crop monocultures, only six were shown to produce Type 1 microbial footprints, namely marigold, borage, phacelia, vetch and both oilseed radish cultivars (Fig. 2 A, D). Oilseed radish treatments gave rise to the highest number of Type 1 microbial footprints. These patterns were observed for nine and seven microbial taxa at DNA and RNA level respectively (Fig. 2A and D).

At the DNA level, eight bacterial families showed this footprint, five were consistently promoted and three were consistently repressed (Fig. 2A). Most notably, the bacterial families Kaistiaceae, Pseudomonadaceae, and Sanguibacteraceae were promoted by the oilseed radish treatments, whereas the Polyangiaceae were repressed. Cellulomonadaceae was increased upon borage treatment. The fungal family Plectosphaerellaceae was significantly promoted by both oilseed radish treatments. Among the protists, the order Physarales stood out as four cover crop treatments promoted the abundance of members of this order (Fig. 2 A).

At the RNA level, four bacterial families were consistently promoted by one or more cover crop treatments, namely Alcaligenaceae, Pseudomonadaceae Rhodocyclaceae, and Sanguibacteraceae (Fig. 2D). Except for Alcaligenaceae, these families were also stimulated at DNA level. It is noted that members of the family Rhodocyclaceae were promoted by four distinct cover crop treatments. One fungal family, the Plectosphaerellaceae, was consistently promoted at DNA and RNA level upon the oilseed radish treatments (Fig. 2 A, D). In contrast to our observations at DNA level, no significant effect of cover crops on the Physarales was detected at the RNA level (Fig. 2 A, D).

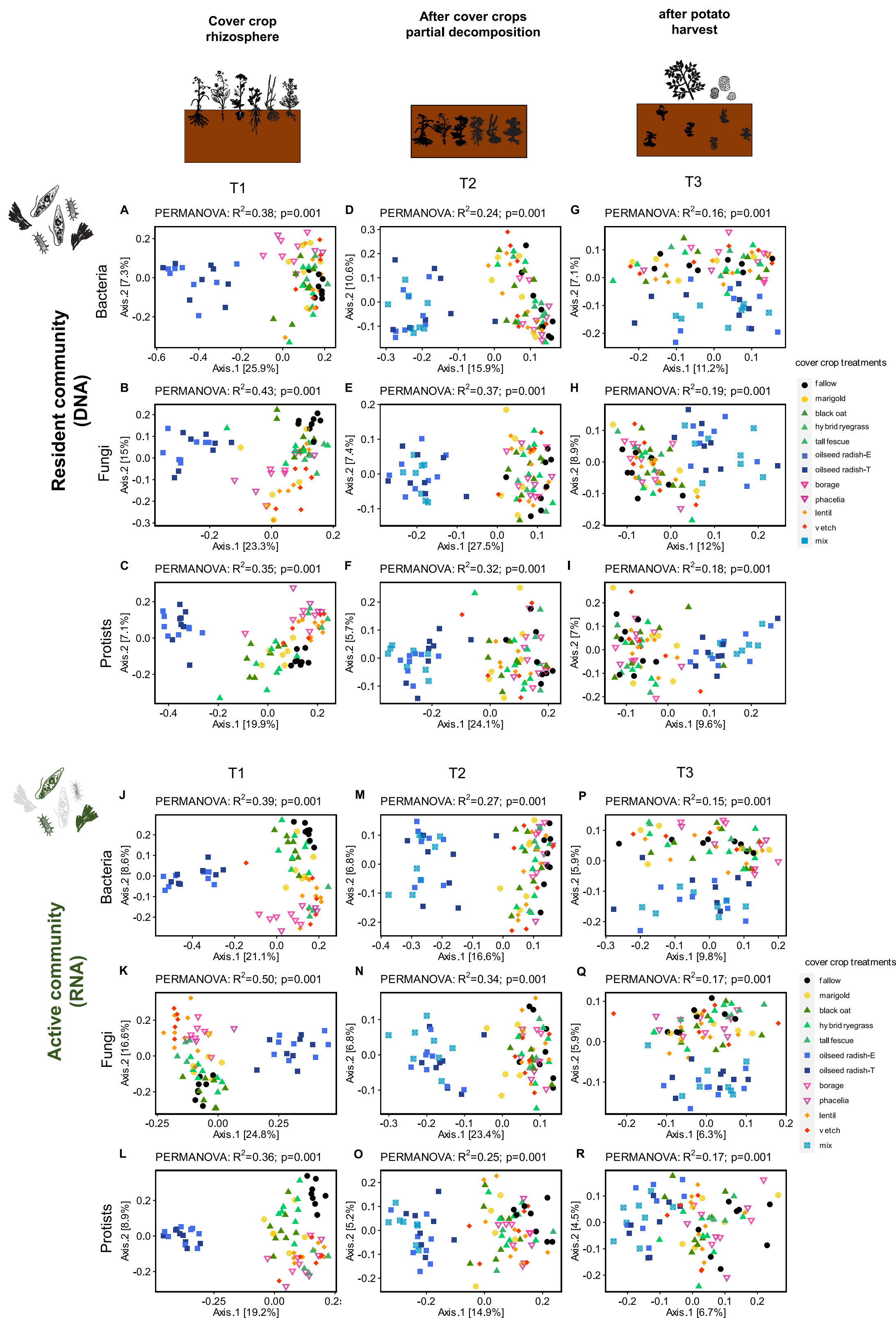


Fig. 1. Principal Coordinate Analysis (PCoA) of CSS normalised ASV data. The dissimilarity matrix was built on the Bray-Curtis metric and plotted to separate ASV based on the cover crop treatment. Different colours are used to indicate different cover crop treatments and cover crops from the same plant family share the same shape. Microbial communities are represented at DNA (A) and RNA level (B) for bacteria, fungi and protists at the three sampling time points: in the rhizosphere during cover crop growth (T1), just before planting of the main crop (potato) (T2) and right after the harvest of the main crop (T3). The microbial communities collected from the rhizosphere were mutually more distinct than communities characterized in bulk soil (PERMANOVA, $p \leq 0.001$). For the cover crop mixture (light blue closed squares), the microbial community was determined in bulk soil only at T2 and T3. Both oilseed radish cultivars, as well as the cover crop mixture (dominated by oilseed radish), had the greatest impact on both the total and the active microbial communities at all three time points as shown by the clear separation of the blue squared dots representing the oilseed radish cultivars and the mixture, from the other symbols.

Table 2
R^2 values from pairwise PERMANOVA between cover crop treatments and fallows at time points T2 and T3. Only significant treatments are shown ($p < 0.05$). For a complete overview of pairwise comparisons see Supplementary Table 4.

A) T2-DNA	Cover crop treatment	R^2	p	B) T3-DNA	Cover crop treatment	R^2	P
Bacteria	oilseed radish-E	0.28	0.004	Bacteria	mix	0.13	0.017
	Mix	0.28	0.007		oilseed radish-E	0.12	0.017
	Oilseed radish-T	0.22	0.004		oilseed radish-T	0.11	0.017
Fungi	oilseed radish-E	0.41	0.003	Fungi	oilseed radish-E	0.19	0.004
	mix	0.39	0.003		mix	0.18	0.004
	oilseed radish-T	0.37	0.005		oilseed radish-T	0.17	0.007
	marigold	0.17	0.006	Protists	mix	0.18	0.006
	hybrid ryegrass	0.12	0.020		oilseed radish-E	0.16	0.006
	lentil	0.11	0.019		oilseed radish-T	0.15	0.006
Protists	mix	0.41	0.006				
	oilseed radish-E	0.41	0.005				
	oilseed radish-T	0.30	0.005				
	marigold	0.14	0.032				
	hybrid ryegrass	0.10	0.045				
C) T2-RNA	**Cover crop treatment**	**R^2**	**p**	**D) T3-RNA**	**Cover crop treatment**	**R^2**	**P**
Bacteria	mix	0.30	0.003	**Bacteria**	oilseed radish-E	0.11	0.008
	oilseed radish-E	0.29	0.003		mix	0.11	0.008
	oilseed radish-T	0.24	0.006		oilseed radish-T	0.10	0.022
	marigold	0.11	0.041	**Fungi**	oilseed radish-E	0.13	0.007
	black oat	0.10	0.003		oilseed radish-T	0.12	0.007
	hybrid ryegrass	0.10	0.019		mix	0.11	0.008
Fungi	mix	0.38	0.003	**Protists**	mix	0.16	0.008
	oilseed radish-E	0.37	0.003		oilseed radish-E	0.14	0.008
	oilseed radish-T	0.29	0.003		oilseed radish-T	0.13	0.008
	marigold	0.17	0.003				
	hybrid ryegrass	0.13	0.008				
	black oat	0.12	0.003				
Protists	mix	0.30	0.003				
	oilseed radish-E	0.26	0.003				
	oilseed radish-T	0.25	0.003				
	marigold	0.12	0.007				
	vetch	0.12	0.015				
	black oat	0.12	0.005				
	hybrid ryegrass	0.10	0.005				
	lentil	0.10	0.017				

Type 2 footprints refer to microbial taxa that are either significantly promoted or repressed at T1 and T2 (and not at T3). This type of footprint was found among all cover crop treatments. With 48 microbial taxa showing a Type 2 footprint at the DNA level (Figs. 2 B), and 63 at the RNA level (Fig. 2 E), this was the most widespread footprint type. The oilseed radish treatments gave rise to the highest number of microbial taxa with Type 2 footprints (48 and 63 at DNA and RNA level vs 2–9 in the other cover crops). It is worth noting that at the DNA level, the two oilseed radish treatments produced distinct microbial footprints. Oilseed radish-E showed four times more repressed microbial taxa than cultivar Terranova (21 vs 5), but the same number of Type 2 promoted taxa. At RNA level, a less skewed Type 2 repression pattern was observed (19 vs 13 microbial taxa) between the two oilseed radish cultivars (Fig. 2 B, E), but oilseed radish promoted two times more microbial taxa than the cultivar Terranova (13 vs 9).

Except for oilseed radish, a considerable number of bacterial taxa was promoted or repressed at the DNA level by a single cover crop treatment only. Examples are vetch promoting Cellulomonadaceae and repressing Planctomycetes, and phacelia repressing Babeliales and Thermomicrobiales. However, most of the taxa were significantly affected simultaneously by oilseed radish and a second cover crop treatment (*e.g.*, Dehalococcoidia repressed by oilseed radish-E and tall fescue, and Cellvibrionaceae promoted by oilseed radish-E and black oat (both at DNA level). Among the fungal families, five were stimulated at DNA level (Fig. 2 B). Cladosporiaceae are exceptional as their presence was promoted by eight out of the ten cover crops tested (all but tall fescue and oilseed radish-T). Members of the Pleosporaceae were exclusively promoted upon marigold treatment. Repression Thelebolaceae was detected for three cover crop treatments (black oat, oilseed radish E, and vetch). The protist order Physarales, showing a positive Type 1 footprint upon four different cover crop treatments (both oilseed radish, phacelia and vetch), gave an additional positive Type 2 footprint upon exposure to borage.

As compared to the DNA level, more taxa were significantly promoted at the RNA level by multiple cover crops at the same time. This can be illustrated by the Armatimonadales, activated by seven cover crop treatments (not including phacelia and the two Fabaceae), and Kaistiaceae, which activity was promoted by six cover crops (not including marigold, tall fescue and phacelia). Oxalobacteraceae and Spirochaetaceae are examples of bacterial taxa that were not significantly affected at the DNA level but were shown to be activated by two Poaceous cover crop species (hybrid ryegrass and tall fescue, respectively, Fig. 2 E). The fungal family Cladosporiaceae, which presence was promoted by eight cover crops (Fig. 2B), was activated by four cover crop treatments only. On the contrary, the fungal family Pleosporaceae was promoted solely by marigold at the DNA level, whereas at the RNA level, it was shown to be activated by six distinct cover crop treatments (not including black oat, oilseed radish, and phacelia, Fig. 2B, E). Mortierellaceae and Pezizales were the only two fungal taxa which activities were repressed as compared to the fallow control. Notably, under the same treatment (oilseed radish-E) these taxa also showed a Type 2 footprint-like repression pattern at the DNA level (Fig. 2B, E). Among the protists, Sagenista was activated by hybrid ryegrass (Fig. 2 B, E), while the activity of Bacillariophyta was repressed by the oilseed radish cultivar-T (Fig. 2E).

Type 3 footprints involve microbial taxa that are significantly promoted or repressed by cover crops at T1 (during cover crop growth) and T3 (just after the main growing season), while – as compared to the corresponding fallow control - no significant change was observed at T2. Both at DNA and RNA levels, 23 families showed a Type 3 footprint,

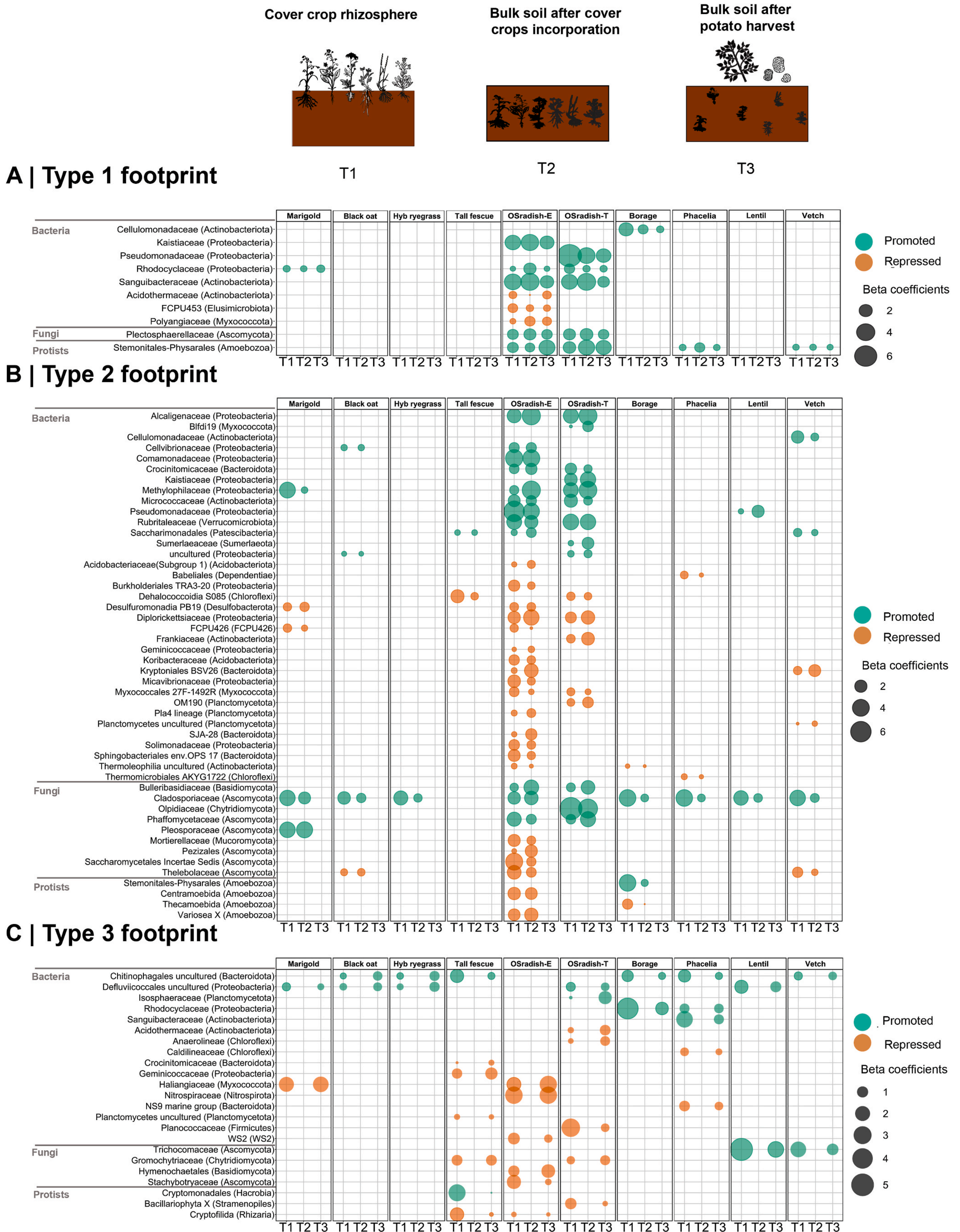


Fig. 2. Dot plots representing the cover crops' microbial footprints over time. The dots represent differentially abundant microbial features (significant at $p < 0.05$) and their size corresponds to the beta-coefficient value from the ANCOM-BC test. Differential abundances refer to microbial taxa (bacterial and fungal families and protist orders) showing a significantly different abundance after the cover crop treatment as compared to the corresponding fallow control. T1 refers to the sampling

of rhizosphere soil during cover crop growth, T2 refers to the bulk soil sampled right before the planting of the main crop (potato), and T3 refers to the bulk soil sampled right after the harvesting of the main crop. This figure is subdivided into A) taxa promoted (green) or repressed (orange) at all time points (Type 1 footprint), B) Taxa promoted or repressed at T1 and T2 and non-significant affected at T3 (Type 2), C) taxa promoted or repressed at T1 and T3, but non-significant affected at T2 (Type 3 footprint) at DNA level. C, D and E figures show Type 1, Type 2 and Type 3 footprints, respectively, at RNA level.

consisting of 16 and 16 bacterial, four and three fungal, and three and four protist taxa (Fig. 2 C, F).

At DNA level both the Chitinophagales and the Defluviicoccales were promoted by six and five different cover crop treatments. Repressive Type 3 footprints appeared to be more cover crop-specific; only the Halingiaceae were repressed by more than one cover crop species, namely marigold and oilseed radish (Fig. 2 C). DNA-based analysis of the fungal community revealed a remarkably specific Type 3 promotion of Trichocomaceae and this was observed only upon exposure to the two Fabaceae, lentil and vetch (Fig. 2C). Other fungal taxa were significantly repressed by tall fescue and oilseed radish (Gromochytriaceae, Hymenochaetales and Stachyobotryaceae). Concerning the protist community, we observed a positive Type 3 footprint for the Cryptomonadales order upon exposure to tall fescue.

RNA-based community analyses revealed that positive Type 3 footprints were induced for Reyranellaceae by all cover crop species except for tall fescue and oilseed radish-E. On the other hand, oilseed radish cultivar-E specifically gave rise to an activity reduction of the bacterial taxa Kapabacteriales, Sphingobacteriales KD3-93, Suttereliaceae and Vermiphilaceae (Fig. 2 F). Lentil induced the highest number of positive Type 3 footprints (four out of five affected taxa). Notably, none of these taxa was significantly changed at the DNA level. RNA-based fungal community analyses showed an activation of the Claroideoglomeraceae upon exposure to each of the two legumes, vetch and lentil as well as marigold (Fig. 2F). This shift was not observed at the DNA level. Concentrating on microbial activity levels, enhanced activity of members of the protist order Acanthoecida was observed upon the black oat and phacelia treatments (Fig. 2F).

Overall, our analyses of promotion or repression patterns revealed that many footprints are cover crop treatment-specific or shared between oilseed radish and another cover crop treatment. Patterns as we have seen at the DNA level for the fungal family Cladosporiaceae, and the bacterial family Reyranellaceae at the RNA level – in both cases promotion by eight of the ten cover crops – are rare. It is noted that the number of taxa showing Type 1, 2 and 3 microbial footprints is generally higher among members of the active community (at RNA level) than of the total community (at DNA level). All in all, our results show that changes at the level of individual members of the soil microbiome are mostly cover crop-specific, and often still present at the onset of the next main growing season (T2).

3.3. Effect of cover crop treatments on the main crop, potato

The cover crop treatments significantly (adjusted $p < 0.05$) affected tuber yield (kg/container). Pairwise comparisons showed that lentil and borage treatments significantly decreased potato yield in comparison to the fallow (Bonferroni adjusted p-value <0.05) (Fig. 3 A). For all other cover crop treatments, the tuber yield was not significantly different from the fallow. A non-significant trend in tuber yield was observed upon the oilseed radish and mixture treatments. This trend comprised an increase from 3.1 kg potato tuber/container in the fallow control, to 3.9 and 3.8 kg/container respectively (Fig. 3A). Potato plant performance was not significantly affected by the cover crop treatments (Fig. 3B).

4. Discussion

4.1. Persistence of cover crop-induced changes in the soil microbiome

For all ten cover crop treatments, we found significant effects on microbial taxa approximately four months after the incorporation into the topsoil (T2) just before the planting of the main crop (potato). In the case of the two oilseed radish cultivars and in a cover crop mixture that was dominated by oilseed radish, soil-borne legacies were detectable for all three organismal groups, even after the harvest of the main crop, potato (T3). Borage, phacelia, vetch and lentil did not have a significant effect on the overall community composition, but significant effects on individual microbial taxa were observed. The persistence of cover crop effects over time is also biomass-dependent (Barel et al., 2018). In our field experiment, individual cover crops produced varying amounts of biomass. This factor may have contributed to the lack of overall community composition effects for these four cover crop species. Previous studies by Walker et al. (2022) and Nevins et al. (2018) found persistent effects of cover crops on soil microbial communities under various agro-ecological conditions (*e.g.*, distinct growing periods and cover crop species). Upon exposure to brown mustard (*Brassica juncea*) and ryegrass (*Lolium multiflorum*) (Walker et al. (2022), and hairy vetch (*Vicia villosa*), cereal rye (*Secale cereale*), and a mixture of hairy vetch and cereal rye (Nevins et al., 2018), shifts in the microbial communities were still observable at the time the main crop was grown. Although the persistence of cover crop-induced shifts in the native soil microbiome at the onset of the main growing season had been demonstrated before, the effect of a prolonged crop-free post-incorporation period (as usual in the temperate climate zone) had not been assessed before. Here we demonstrate that after a crop-free period of about four months, distinct legacies could be detected for all ten cover crops tested.

4.2. Ecological understanding of observed microbial shifts

4.2.1. Cover crop-specific microbial footprints that might affect the main crop

Microbial footprints as defined here – microbial taxa that are consistently affected by a cover crop as compared to the corresponding control at, at least, two time points – were most frequently observed for bacteria. This does not come as a surprise as the number of bacterial ASVs manyfold exceeded the number of fungal and protist ASVs (see Results). Cover crops had the most persistent effect on the overall fungal community composition (Fig. 1 A, B). However, this enduring legacy was not translated into a high number of fungal footprints. Fungi are more sensitive to soil physical disturbances than bacteria (Brito et al., 2012; Orrù et al., 2021). The disturbances of the topsoil in our container experiment (*i.e.*, during cover crops termination and incorporation into the topsoil) may have caused a decrease in the number of fungal footprints. Next to this, the persistence of the induced changes in the fungal community might have been affect by a standard foliar application of fungicides during potato growth (between T2 and T3) to control the spreading of potato late blight (*Phytophthora infestans*).

Among protists, persistent shifts were relatively rare. More than their microbial counterparts, protists are highly affected by climatological conditions (Bates et al., 2013), and this may be a factor that co-explains the poor persistence of cover crop-induced changes in the protist community over different seasons.

Type 1 footprints comprise microbial taxa consistently affected in the same direction (increase or decrease) at all three time points and were relatively rare. It should be noted that taxa with a Type 1 footprint upon a given cover crop treatment, often produced Type 2 or Type 3 footprints upon other cover crop treatments. Illumina A noteworthy stimulation was observed for the bacterial family Pseudomonadaceae. A Type 1 stimulation was detected upon exposure to oilseed radish-T (both at DNA and RNA level), and a Type 2 footprint for oilseed radish-E (both at DNA and RNA level). *Pseudomonas* strains have been implicated as the main explanation for the decline of the take-all disease caused by *Gaeumannomyces graminis*. Members of the genus *Pseudomonas* have

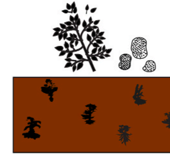
Cover crop rhizosphere

T1

Bulk soil after cover crops incorporation

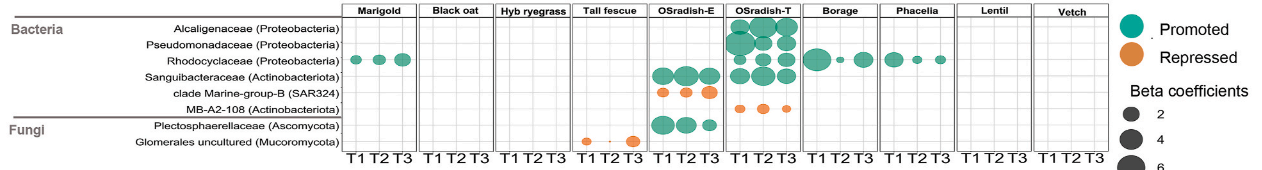
T2

Bulk soil after potato harvest

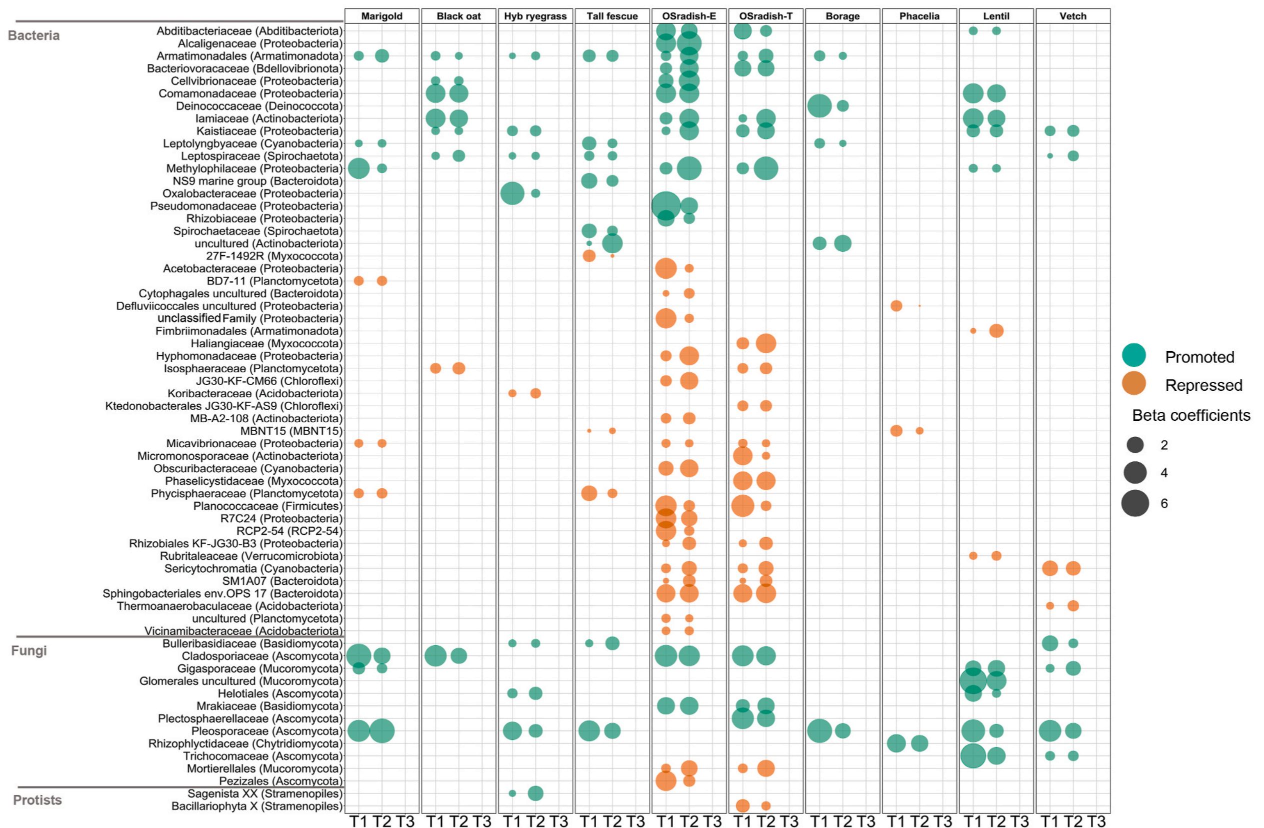


T3

D | Type 1 footprint



E | Type 2 footprint



F | Type 3 footprint

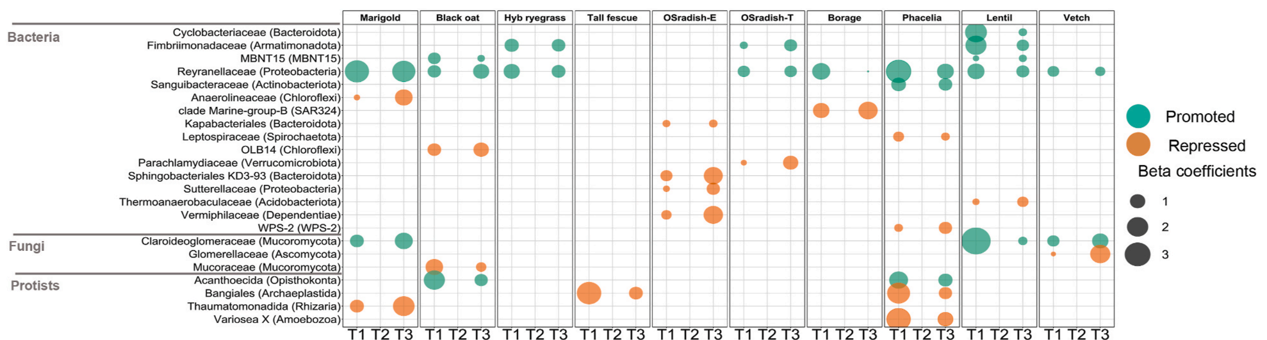


Fig. 2. (*continued*).

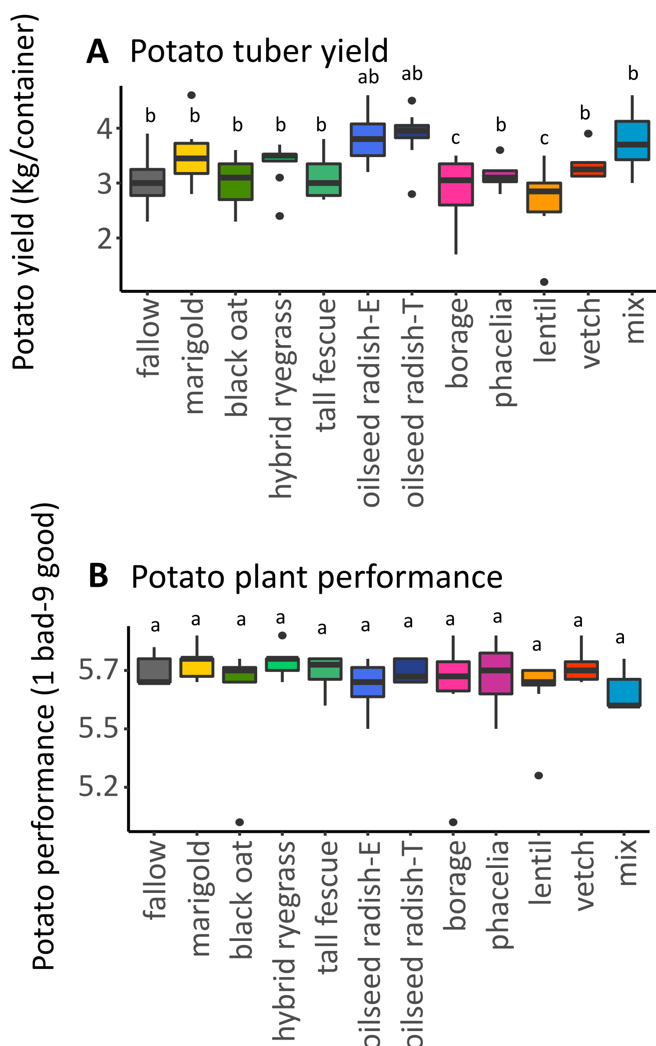


Fig. 3. Boxplots representing potato tuber yield in kg tuber per container (A) and potato plant performance (B). The potato plant performance was based on a scale between 1 and 9 which took into account plant size, growth, colouration and health. Different letters above the box indicate significant differences in tuber yield as calculated with a linear mixed model (with tuber yield and potato performance as response variables, block as a random effect and cover crop treatment as an explanatory variable) and post-hoc test with Bonferroni adjustment for multiple comparisons.

been involved in other types of disease suppressiveness, in particular towards fungal pathogens (*Fusarium oxysporum* and *Rhizoctonia solani*) and plant-parasitic nematodes (*Meloidogyne* spp.) (Kloepper et al., 1980; Mazurier et al., 2009; Adam et al., 2014). Sanguibacteraceae, a monotypic family that only comprises the genus *Sanguibacter,* showed a Type 1 stimulation for both oilseed radish cultivars, both at DNA and RNA level. *Sanguibacter* strains have been identified as potential lignocellulose degraders (de Lima Brossi et al., 2016). It is unknown, however, why this characteristic would be more boosted by oilseed radish cultivars at T2 and T3 than by other cover crop treatments.

Type 2 footprints comprise taxa that were consistently promoted or repressed during cover crop growth (T1) and just before the planting of the main crop (T2), whereas no significant change was observed after the main crop as compared to the corresponding fallow control (T3). T2 footprints were the most widespread type in our study. This was anticipated as the main crop also induced a specific shift in the soil microbiome. As a result, many cover crop induced-changes might have been nullified. The neutralisation of cover crop-induced shifts in the indigenous soil microbiome will depend on the identity of the main crop. This notion was nicely illustrated by Ulcuango et al. (2021)who showed that the differential effects of vetch, sweet clover and barley cover crops on the bacteria, fungal, AMF and archaeal communities depended on the identity of the main crop. In this case, vetch and sweet clover induced similar soil microbial responses in wheat and maize main crops, by promoting the total abundance of fungi, bacteria and archaea, while the effect of the barley cover crop was less pronounced and significantly distinct among the two main crops. Similarly, Manici et al. (2018) found that barley and hairy vetch cover crops had a more prominent effect on the bacterial community associated with the main crop tomato rather than zucchini. These studies indicate that the effect that cover crops have on the soil microbiome and its persistence co-depend on the nature of the cover crop-main crop combination.

Among the most notable taxa displaying a Type 2 footprint is the bacterial family Kaistiaceae that was shown to be activated by six cover crop treatments at RNA level. Interestingly, *Kaistia*, one of the two genera of this family, was shown to effectively suppress the fungal pathogen *F. oxysporum* (Fujiwara et al., 2016). Keeping in mind that this family was promoted at the RNA level might add to the relevance of this cover crop-specific stimulation. Also, the bacterial family Oxalobacteraceae showed a Type 2 footprint at RNA level upon exposure to hybrid ryegrass. This bacterial family has been associated with the suppressiveness of several fungal soil-borne pathogens, including *F. oxysporum, Verticillium dahliae and R. solani* (Gómez Expósito et al., 2017).

The fungal family Cladosporiaceae is exceptional as it produced Type 2 footprints at the DNA level in eight out of the ten cover crops tested (excluding tall fescue and oilseed radish-T). *Cladosporium,* the dominant and most widespread genus within this family (Bensch et al., 2015) may grow biotrophically on a wide range of plant species whereas other species can grow as a saprophyte on dead or senescing plant tissue (Bensch et al., 2012). Therefore, our results suggest that multiple and/or polyphagous representatives of the Cladosporiaceae used cover crops themselves and/or their residues as a substrate. Although not implicated in this study, it is worth mentioning that some *Cladosporium* species have been implicated in natural soil suppressiveness against cyst (Song et al., 2016) and root-knot nematodes (Giné et al., 2016).

At the RNA level, six out of the ten cover crop treatments resulted in an activation of members of the fungal family Pleosporaceae. This family includes numerous genera among which *Alternaria* and *Pleospora* are the most speciose and ecologically diverse. *Alternaria* species are mostly saprobes, but a subset has evolved into plant parasites with broad host ranges (Thomma, 2003). *Pleospora* (anamorph *Stemphylium*) harbours a wide range of saprophytic and pathogenic species (see *e.g.* McDonald et al., 2022). Hence, we attribute the widespread activation by multiple cover crop treatments to saprobic and/or polyphagous parasitic members of this family.

Type 3 footprints are here defined as microbial taxa significantly promoted or repressed in the rhizosphere at T1, and after the harvesting of the main crop (T3), but showing no significant change just before the onset of the main growing season (T2). Hence, Type 3 footprints reflect cover crop treatment effects observed in the presence of living plants only. Striking examples of relatively widespread Type 3 footprints are the bacterial orders Chitinophagales and Defluviicoccales at DNA level, and Reyranellaceae at RNA level, which were stimulated by multiple cover crop treatments from different families. Chitinophagales and Defluviicoccales have high ecological diversity which hampers the interpretation of their ecological role. Our RNA data suggested that Reyranellaceae might have been activated under eight out of the ten cover crop treatments. Within this monogenetic family, representatives of the *Reyranella* genus have been characterized as dominant denitrifier populations in dry upland soils (Pessi et al., 2022). As such, increased nitrate (NO_3^-) availability during plant growth could have triggered the generalised activation of *Reyranella* spp upon most cover crop treatments.

Interestingly, a few Type 3 footprints were associated exclusively with cover crops from the same plant family. At DNA level, the fungal family Trichocomaceae was only promoted by the fabaceous cover crops lentil and vetch. Trichocomoceae constitute an ecologically diverse family of which some members are known as endophytes of legumes (Higginbotham et al., 2013). Whether the members of this family detected here acted as endophytes of lentil and vetch remains to be established. Another example is the arbuscular mycorrhizal fungal (AMF) family Claroideoglomeraceae, which was significantly stimulated at RNA level by both fabaceous cover crops and marigold. A study by Higo et al. (2019) showed that the cover crop identity determines the establishment of the AMF community in the subsequent crop, even more than the host (main crop) identity itself. Therefore, the choice of the cover crop may also determine the success of the establishment of certain AMF taxa during the next main crop.

4.2.2. Added value of monitoring both resident and active fractions of the soil microbiome

We monitored changes over time in the resident (DNA-based) as well as the active (RNA-based) fractions of the soil microbial communities. Although similar general trends were observed for the individual cover crop treatments, DNA and RNA-based analyses revealed some remarkable contrasts. At the RNA level, we observed a higher number of significant contrasts as compared to the DNA level. This can be illustrated by the number of Type 2 footprints: 48 taxa at the DNA level, and 63 at the RNA level. This suggests that RNA-based community profiling may provide an augmented representation of how the soil microbial community composition is shaped by the cover crop treatments (Bay et al., 2021). It must be noted that the number of bacterial and fungal families and protist orders was comparable between DNA and RNA datasets. Therefore, differences in the number of families/orders *per se* are unlikely to be the cause of the contrasts between RNA and DNA-based microbial profiles.

We argue that the combined analysis of DNA and RNA community fractions represents a comprehensive approach to studying the highly dynamic soil microbial communities, especially when assessing community compositional changes over time. This approach allows for studying the active fraction of the microbiome at the time of the sampling (Blazewicz et al., 2013). In addition, the inclusion of DNA-based analyses allows us to take into account dormant and active microorganisms which can be activated in response to environmental changes or substrate input (Blagodatskaya and Kuzyakov, 2013).

The possibility to discriminate between active and dormant microbiota would be instrumental to assess the disease-suppressive as well as the plant-pathogenic potential of a given soil. The observed strong stimulation of, Pseudomonadaceae by both oilseed radish cultivars at DNA and RNA level could justify a more detailed study to investigate whether it could result in elevated levels of disease suppressiveness. On the other hand, stimulation of the pathogenic fungal family Olpidiaceae by the oilseed radish cultivar Terranova at the DNA level was not paralleled by activation at RNA level. This information could be taken along in the design of crop rotations.

4.3. Possible explanations for the limited effect of cover crop treatments on potato yield

In this study, cover crop treatments did not have a major effect on potato tuber yield. A reason for the limited effects of cover crop treatments on the potato yield could be related to the experimental field conditions. The experiment was carried out on well-fertilized soil in the absence of major soil-borne pathogens, suggesting relatively optimal soil conditions for plant growth. A study by Porter and Sisson (1991) reported a significant tuber yield increase after red clover only when nitrogen was limiting potato growth. Similarly, Sincik et al. (2008) also found that tuber yield increases following cover crops were less pronounced with increased nitrogen fertilisation rates. Therefore, the beneficial effect of the cover crop-steered microbiomes on the succeeding main crop may have been obscured by the optimal nutritional status of the experimental field (Bokhari et al., 2019; Liu et al., 2020; Trivedi et al., 2022). Future work needs to reveal how the steering effect of cover crops should be understood in the context of soil fertility, pathogen pressure and other agronomically relevant factors.

5. Conclusions

Our data showed that each of the ten cover crop treatments tested here resulted in significant microbial changes that lasted at least until the onset of the main growing season (T2). The two oilseed radish cultivars and a cover crop mixture (dominated by oilseed radish) had the most persistent effect on the microbial community as shifts were even observable after the harvesting of the main crop (potato). Furthermore, each cover crop treatment resulted in qualitatively distinct microbial footprints. Most notably oilseed radish treatments significantly and consistently boosted the presence and potential activity of members of the families Sanguibacteraceae and Pseudomonadaceae. The observed stimulation of Pseudomonadaceae by both oilseed radish cultivars over time could justify a more detailed study to see whether it resulted in higher local levels of suppressiveness against plant-parasitic fungi or nematodes. In such a study, the direct effect of isothiocyanates released from Brassicaceous crops as well as the distinct sensitivities of fungal pathogens vis-à-vis pathogenic fungi should be taken along (Sarwar et al., 1998).

Other crop treatments had a significant effect on the potential activity of other individual microbial taxa, including Glomerales and Helotiales by lentil, Deinococcaceae by borage, Claroideoglomeraceae by vetch and marigold and lentil. The data presented here demonstrate that cover crops induce species and even cultivar-specific changes in the local soil microbiome. Finally, by comparing rDNA and rRNA-based community profiles induced by cover crop treatments, we generally recognised similar trends. However, a considerable number of exceptions were observed in which the promotion or repression of a taxon at DNA level was not accompanied by similar changes at RNA level and *vice versa.* Our study demonstrates that cover crops can be used to regulate the soil microbiome in a cover crop-specific manner, and at least a part of these changes will persist in the topsoil until at least the onset of the main growing season in sandy soil. In a previous study, Harkes et al. (2019) reported that even within the same climatic zone, the soil microbiome differs among soil types, which limits the generalisation of our results beyond sandy soils with a comparable pH. For several microbial taxa, we could link induced changes to disease suppressiveness. Stimulation of microbiota that strengthen the antagonistic capacity of soil could contribute to more sustainable pest and pathogen management (Vukicevich et al., 2016). In follow-up studies, we will focus on the effects of cover crops-regulated microbiomes in the presence of soil-borne pathogens to further test the potential of cover crops in stimulating pathogen antagonists.

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Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109080.

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