



Legume and Non-legume Cover Crops Shape Distinct Soil Microbial Communities and C-cycling Functional Traits

Zhine Wang^{1,2} · Christopher Saski¹ · Barbara Campbell³ · Frank Oliver^{1,2} · Rongzhong Ye^{1,2}

Received: 20 August 2025 / Accepted: 31 March 2026 / Published online: 16 April 2026
© The Author(s) 2026

Abstract

Winter cover crops are commonly used as a source of nutrients in organic production. However, the impact of these crops on soil microbial functional traits at the genomic level is poorly understood. Here, we investigated the impacts of three-year inclusion of cereal rye (*Secale cereale* L), hairy vetch (*Vicia villosa*), and their mixture on soil microbial diversity and residue decomposition functional traits using 16 S rRNA gene and internal transcribed spacer (ITS) sequencing data and functional prediction tools. Integrating a mixture of rye and vetch increased bacterial richness and alpha diversity but had no impact on fungal diversity when compared to the control. The fungal community structure of the soils with either rye or vetch inclusion was significantly different from that of the mixture and the control. Compared to vetch, rye inclusion was predicted to have a significantly higher abundance of C1 compound utilization and assimilation pathways, but a lower abundance of amino acid degradation and carboxylic acid degradation pathways. Similarly, rye inclusion resulted in a significantly higher abundance of arbuscular mycorrhizae but a lower abundance of litter saprotrophs than vetch inclusion. Particularly, integrating vetch was predicted to have a significantly higher abundance of genes involved in cellulose and lignin decomposition than the rye inclusion. Five keystone cellulose and lignin bacterial decomposers were significantly more abundant with vetch inclusions than with rye inclusions. Nonlegume and legume winter cover crops imposed distinct effects on soil potential microbial C-cycling functional traits after three planting seasons. Legumes favored the growth of microbial decomposers compared to non-legumes.

Keywords Cover crops · Microbial community · Decompositional genes · C-cycling functional traits · Sandy soils

1 Introduction

Soil microbial communities provide fundamental ecosystem functions and are the key to soil biological health (Gul and Whalen 2022; Kallenbach et al. 2016). Research on microbial functional traits can accurately delineate how microbes adapt to disturbances in agricultural soils (Ranheim Sveen et al. 2024; Yang 2021). For instance, Dai et al. (2020) found that long-term phosphorus (P) input induced

microbial P immobilization by decreasing the relative abundance of P-starvation response genes and increasing that of low-affinity inorganic phosphate transporter genes. Similarly, Huang et al. (2024) revealed that grassland soil microbiomes contained more genes for cellulose and hemicellulose degradation than forest microbiomes, due to the priming effect of high N organic inputs on microbial function. Moreover, certain microbial species exhibit outstanding ecosystem functionality. For instance, Dasgupta et al. (2024) identified *Piskurozymaceae* and *Sphingomonadaceae* as keystone bacterial families mineralizing wheat straw nutrients in soil, while Zheng et al. (2021) reported *Chryseobacterium* as a keystone bacterium for litter decomposition in rice farmland. Collectively, these studies demonstrate that both functional traits and keystone taxa, which exert a disproportionate influence on community composition and processes, are crucial for linking microbial communities to ecosystem functioning (Banerjee et al. 2016; Vick-Majors et al. 2014).

✉ Rongzhong Ye
rongzho@clemson.edu

¹ Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA

² Pee Dee Research & Education Center, Clemson University, Florence, SC 29506, USA

³ Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA

Recognizing the central roles of microbial communities in soil health, researchers and farmers continue to explore practical ways to manipulate soil microbial communities in organic agriculture (Pandey et al. 2018; van Rijssel et al., 2022). To better harness their ecosystem services, further research on microbial functional profiles is necessary. Recent advances in microbial functional prediction algorithms have enabled the characterization of microbial communities based on their ecological functions (Douglas et al. 2020; Zeng et al. 2024). For instance, PICRUSt has been used to predict that crown vetch upregulated soil cellulolytic and hemicellulolytic functional genes in an apple orchard field (Zheng et al. 2018), whereas PICRUSt2 predicted that Siratro increased the microbial functional potential for fatty acid and lipid biosynthesis in a banana plantation field (S. Xu et al. 2024a, b). However, these predictions remain approximations because the amplicon sequences and reference databases might underrepresent the genetic microdiversity (Toole et al. 2021). Yet, despite the growing evidence of the importance of microbial functional traits and keystone taxa, the specific ways in which management practices, such as integrating different types of cover crops, modify microbial C-cycling functions in agroecosystems remain insufficiently characterized.

Cover crops are significant components of soil health management and are commonly used as nutrient sources in organic agriculture (Novara et al. 2020; Olson et al. 2010). They are often planted during the fallow season and terminated before planting cash crops. Empirical studies indicated that cover crops can increase soil organic C (SOC) content and nutrient availability by supplying significant organic inputs to soils, either as root exudates (Dhungana et al. 2023; Zhalnina et al. 2018) or through dead residues (Thapa et al. 2021). The impacts of cover crops on microbial communities have therefore been increasingly explored (Kim et al. 2020). Cover crops are frequently reported to select rhizospheric microbes through diverse root exudates (Chavarria et al. 2016; Mbuthia et al. 2015). Additionally, the impacts of cover crops on microbial communities are often species- or site-specific (Hu et al. 2024; Saleem et al. 2020; Wang et al. 2025). For instance, cereal rye (a nonlegume cover crop) has a relatively higher biomass C production potential, which favors the growth of oligotrophic fungi and Acidobacteria (Muhammad et al. 2021; Ullah et al. 2023). In contrast, hairy vetch (a legume cover crop) has a relatively higher N content, which attracts copiotrophic microbes, such as certain members of the Pseudomonadota and Bacteroidetes (Brennan and Acosta-Martinez 2017; Griffith et al. 2017). Our recent study also suggested that integrating legume cover crops resulted in a higher abundance of ammonium-oxidizing bacteria and archaea compared to non-legume cover crops (Wang et al. 2024). Such a

difference in residue quality of the legume and non-legume cover crops may influence microbial C-use strategies and C-cycling functions in distinct ways. However, these effects remain insufficiently characterized and poorly defined in agroecosystems, particularly under sandy Coastal Plain soils.

In the present study, we investigated the effects of continual organic inputs from cover crop residues on microbial phylogenetic diversity, functional traits, and soil nutrient availability. Two widely used winter cover crops in the southeastern Coastal Plains of the USA, cereal rye and hairy vetch, were investigated on an established organic field with typical sandy Ultisols, where both SOC and nutrient availability are intrinsically low. The objectives were to determine (1) whether continual short-term organic inputs from two cover crops with distinct functional traits modify soil microbial community composition and structure, and (2) whether distinctive organic inputs shape microbial functional traits relevant to cover crop residue decomposition. We hypothesized that cereal rye and hairy vetch would express fundamental differences in biomass C and N production potential, and would differentially influence microbial community composition, richness, and C-cycling functional traits.

2 Materials and Methods

2.1 Descriptions of the Experimental Site and Design

This study was conducted in a certified organic field in Florence, SC, United States (Fig. S1), which was established in 2020 to investigate the interactive impacts of cover cropping, compost application, and tillage on soil functions, microbial community, and vegetable performance. Detailed site descriptions and experimental design can be found in Wang et al. (2024). The soil was a loamy sand (Loamy, kaolinitic, thermic Arenic Kandiodults) consisting of 79% sand and 7% clay with a pH of 7.2, 5,000 mg kg⁻¹ of total C, and 500 mg kg⁻¹ of total N at 0–15 cm depth.

This field study was established with four cover crop treatments replicated eight times, i.e., cereal rye (RY), hairy vetch (VE), the mixture of the two (MX), and a no cover crop control (CO). They were drill-seeded each year in the fall at a rate of 200 kg ha⁻¹ for cereal rye, 36 kg ha⁻¹ for the vetch, and half of each monoculture rate for the mixture. In the spring of the next year, cover crops were terminated by a roller-crimper, followed by planting vegetables. The rotation was tomato-cucumber-sweet corn-tomato from 2021 to 2023, and they were managed according to the Southeastern U.S. 2020 Vegetable Crop Handbook (Reiter 2020).

No-tillage was applied across all treatments. In April 2023, before cover crop termination, ten soil cores (0–15 cm depth) were randomly collected from each experimental plot (5.5 m by 6.1 m), composited, passed through a 2-mm sieve, and stored at 4 °C. A subsample was stored at -80 °C for DNA extraction.

2.2 Cumulative Organic Inputs from Cover Crop Residues

Cover crop aboveground biomass was collected with a 0.25 m² quadrant yearly before the termination and oven-dried at 60 °C. Their C and N contents were quantified by the Macro Carbon and Nitrogen Analyzer (LECO, St. Joseph, MI, USA). Cumulative organic C and N inputs from cover crop residue return were quantified by adding the yearly production from 2021 to 2023.

2.3 Soil Properties

Soil pH and electrical conductivity (EC) were determined using an Orion Star A325 pH/conductivity meter (ThermoFisher Scientific, Waltham, MA, USA) in deionized (DI) water (1:1 ratio) (Ye et al. 2021). The concentrations of dissolved organic C (DOC) were quantified by a TOC-L organic C analyzer (Shimadzu, Kyoto, Japan) after extracting the soils with 0.5M K₂SO₄ (Sparling et al. 1990). Soil ammonium (NH₄⁺) and nitrate (NO₃⁻) were extracted with 1M KCl, followed by colorimetric analyses (Wolf and Baker 1985).

2.4 Soil DNA Extraction and Sequencing

Approximately 0.5 g of soil was extracted with a DNeasy PowerSoil Pro kit (Qiagen, Venlo, Netherlands). The DNA quality and quantity were checked using the NanoDrop One UV spectrophotometer and the Invitrogen Quant-iT PicoGreen dsDNA Assay kit (ThermoFisher Scientific, Waltham, MA, USA), respectively. PCR amplifications and high-throughput DNA sequencing were conducted by the University of Michigan Microbiome Core (Ann Arbor, MI, USA). The PCR reaction took place in a 20 µl volume consisting of 2 µl of 2X Accuprime PCR buffer (ThermoFisher Scientific, Waltham, MA, USA), 10.85 µl of molecular biology grade pure water (Cytiva, Marlborough, MA, USA), 1.5 µl of AccuPrime HiFi Taq Polymerase, 2 µl of DNA template, 2.5 µl of forward primers and reverse primers. The following thermocycling conditions were applied: initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 15 s, and extension at 72 °C for 10 s, then final extension at 72 °C for 10 min. Sequence libraries were prepared using

a SequelPrep Normalization Plate Kit (ThermoFisher Scientific, Waltham, MA, USA) following the manufacturer's protocols. Paired-end 2 × 250 bp dual-index sequencing was performed on the Illumina MiSeq platform using a standard MiSeq Reagent Kit V2 (500 cycles; Illumina, San Diego, CA, USA). The DNA primers and PCR conditions of 16 S and fungal ITS1 are listed in Table S1.

2.5 Bioinformatic Processing

The DNA sequences of the 16 S rRNA gene and ITS1 amplicons are deposited in the National Center for Biotechnology Information (NCBI) under the project PRJNA1276541. The 16 S rRNA gene V4 region and ITS1 region raw sequencing data were merged, trimmed, and analyzed with the Quantitative Insights into Microbial Ecology 2 (QIIME 2), version 2022.8 (Bolyen et al. 2019), followed by being denoised with the DADA2 plugin (Callahan et al. 2016). Bacterial sequences were trimmed to a 240 bp forward read length and a 150 bp reverse read length, and we trimmed the ITS1 sequences to a 220 bp forward and 220 bp reverse read length. Approximately 70% of reads were analyzed after merging and denoising. The taxonomy assignment of bacterial and fungal amplicon sequence variants (ASV) was conducted using the SILVA database v138 (Glöckner et al. 2017) and the UNITE database v9 (Nilsson et al. 2019), respectively. For diversity analysis, the 16 S rRNA gene and ITS1 region sequences from each sample were rarefied to 17,211 and 8,959, respectively. Species with the cumulative ASV count less than 10 were discarded, and the relative ASV abundances of the remaining microbial communities were clustered by principal coordinate analysis based on Bray-Curtis dissimilarity. Bacterial functional traits were predicted by PICRUSt2 (Ye and Doak 2009; Barbera et al. 2019; Douglas et al. 2020). The default cutoff of the nearest sequenced taxon index (NSTI) of PICRUSt2 was set at 2.0. Bacterial metabolic pathways and fungal lifestyles were identified in the MetaCyc (Caspi et al. 2020) and the FungalTraits (Pöhlme et al. 2020) databases, respectively. Cellulose, hemicellulose, and lignin decomposition genes were identified in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2016).

2.6 Statistical Analysis

Levene's and Shapiro–Wilk tests (Shapiro and Wilk 1965) were performed to check the data's homogeneity of variance and normality. Cumulative organic C and N input was cube root transformed before being used for the statistical analyses, while the NH₄⁺ concentration was log_(x+1) transformed. One-way ANOVA (Fisher 1992) was performed using JMP Pro 16 (SAS Institute, NC, USA) to test the main

Table 1 Annual and cumulative organic C and N inputs from cover crop residue returns (kg ha⁻¹) from the 2021–2022 to 2023–2024 seasons

Biomass	Treat- ment	2021–2022	2022–2023	2023–2024	Cumula- tive
	MX	1638±315	1951±294	2038±252	6008±461
		a		a	
C	RY	1050±228	1803±328	1495±240	4349±665
		b		b	
	VE	1291±215	1388±222	1898±216	4725±552
		ab		ab	
	MX	137±32	112±21	170±31	461±86
		a		a	
N	RY	78±25	112±27	107±30	297±80
		b		b	
	VE	128±30	107±23	169±27	431±77
		a		a	

MX, the mixture of rye and vetch; RY, cereal rye; VE, hairy vetch. Numbers are means and standard errors ($n=8$). Different letters suggest significant differences between the cover crop treatments within a season at $p<0.05$

treatment effects on these data. Microbial Shannon diversity index and ASV richness were calculated by QIIME2. One-way ANOVA was then performed to test the main treatment effects on microbial alpha diversity. Microbial community structure dissimilarities were tested by permutational multivariate analysis (PERMANOVA) using the function “adonis2” in the R package “vegan” (Core Team 2014). Linear and Mantel correlation tests were conducted to test correlations between the measured variables and microbial community structures, at $p<0.05$ using the R package “linkET” in R4.2.1 (devtools::install_github (“Hy4m/linkET”, force=TRUE) (Huang 2021). Treatment effects on microbial abundances were tested using the R package “ANCOM-BC2” (Huang et al. 2020). Discriminating bacterial families harboring decomposition genes were tested by the similarity percentage test using the R package “vegan” (Core Team 2014). The dissimilarity matrices of functional gene KO and bacterial community were built by the function “vegdist”, and matrix correlation analyses were performed by the function “mantel” in the R package “vegan”.

3 Results

3.1 Cumulative Organic C and N Inputs from Cover Crop Residues

In the 2021–2022 and 2023–2024 seasons, the MX treatment produced higher biomass C than the RY treatment, while the MX and VE treatments generated higher biomass N than the RY treatment (Table 1 and S2). However, no difference was observed between the treatments in the 2022–2023 season. Additionally, the MX and VE treatments introduced higher cumulative organic.

Table 2 Selected soil properties in 2023 after three years of cover crop inclusion

	CO	MX	RY	VE
pH	7.1±0.0 ab	6.9±0.0 b	7.1±0.1 a	6.9±0.0 b
EC	1.1±0.1 b	1.3±0.1 ab	1.1±0.7 b	1.5±0.1 a
NH ₄ ⁺	0.9±0.2 a	0.9±0.3 a	0.9±0.1 a	1.0±0.2 a
NO ₃ ⁻	7.4±0.7 b	9.7±1.0 a	7.4±1.2 b	10.3±1.1 a
DOC	54±4 a	52±7 a	51±2 a	56±4 a

CO, control; MX, the mixture of rye and vetch; RY, cereal rye; VE, hairy vetch. EC (dS m⁻¹), electrical conductivity; NO₃⁻ and NH₄⁺, extractable NO₃⁻ and NH₄⁺ concentrations (mg kg⁻¹); DOC, dissolved organic C (mg kg⁻¹). Numbers are means and standard errors ($n=8$). Different letters indicate a significant effect of cover crops at $p<0.05$

N inputs than the RY treatment. No difference was found for the cumulative organic C inputs between the treatments.

3.2 Soil Properties

Continual organic inputs for three seasons did not affect soil NH₄⁺ and DOC concentrations compared to the CO treatment (Table 2 and S2). The VE and MX treatments increased soil NO₃⁻ concentrations compared to the CO and RY treatments. The VE treatment increased EC compared to the CO and RY treatments. The MX and VE treatments decreased soil pH compared to the RY treatment.

3.3 Soil Microbial Diversity

Bacterial Shannon index (Fig. 1A) and ASV richness (Fig. 1B) ranged from 8.9 to 9.7 and 848 to 1462, respectively. The MX treatment had a higher bacterial Shannon index than the CO and VE treatments, and a higher ASV richness than the VE treatment (Table S2). The fungal Shannon index (Fig. 1C) and ASV richness (Fig. 1D) ranged from 5.6 to 6.4 and 92 to 234, respectively. No treatment effect was found (Table S2).

3.4 Microbial Community Composition and Structure

Bacterial community structure remained unchanged after three years of cover crop inclusion (Fig. 2A; Table S3), which was dominated by *Alphaproteobacteria*, *Gamma-proteobacteria*, *Bacilli*, *Bacteroidia*, and *Actinobacteria*, accounting for more than 50% of the bacterial population (Fig. S2A; Table S4). In contrast, the fungal community structure of the CO treatment differed from that of the RY and VE treatments, and that of the VE treatment also differed from that of the RY and MX treatments (Fig. 2B; Table S3). Soil fungi were dominated by classes of *Sordariomycetes*, *Mortierellomycetes*, *Dothideomycetes*, and *Tremellomycetes*, accounting for more than 60% of the fungal population. (Fig. S2B; Table S4).

Fig. 1 Impacts of cover crop inclusion on bacterial Shannon index (A) and amplicon sequence variant (ASV) richness (B), and fungal Shannon index (C) and ASV richness (D). The whiskers of boxplots extend to the maximum and minimum data points, and the horizontal lines and cross marks in the plots represent the median and the mean ($n=8$), respectively. CO, no cover crop control; RY, cereal rye; VE, hairy vetch; MX, the mixture of rye and vetch. Different letters suggest significant differences between cover crop treatments at $p < 0.05$

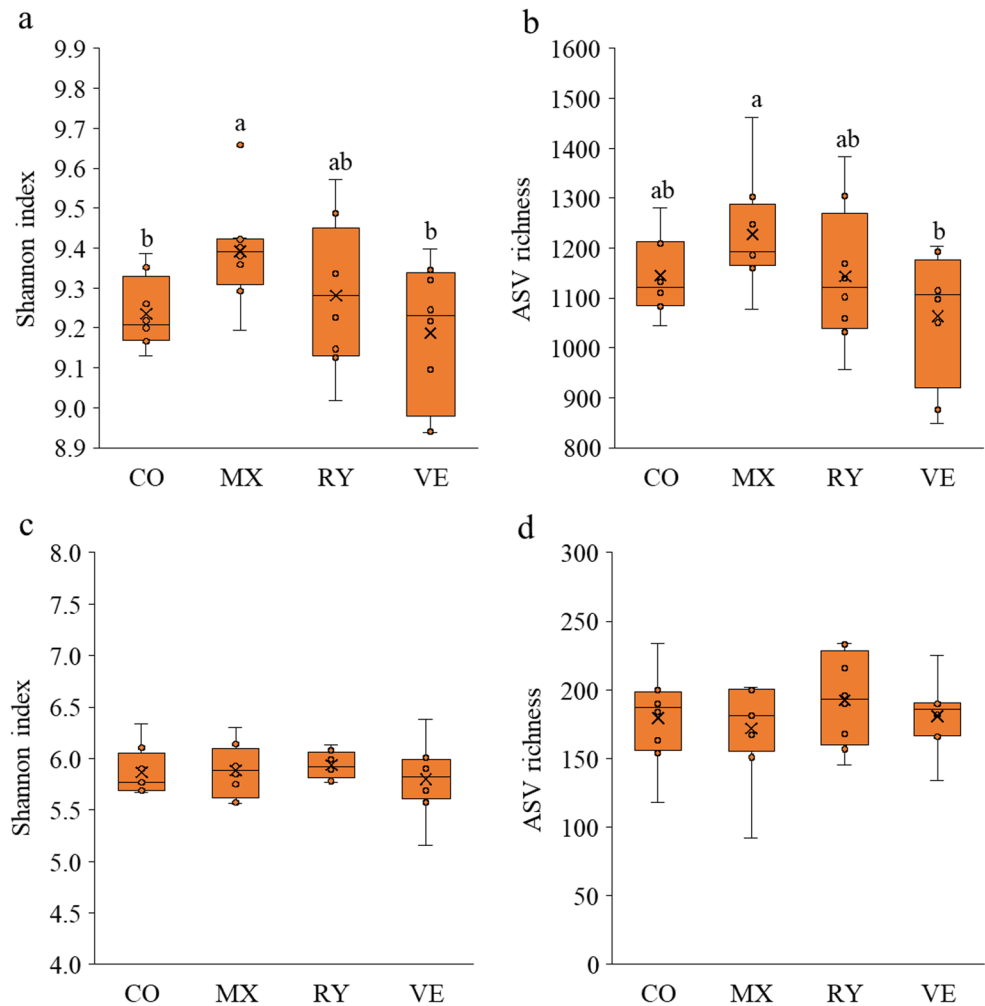
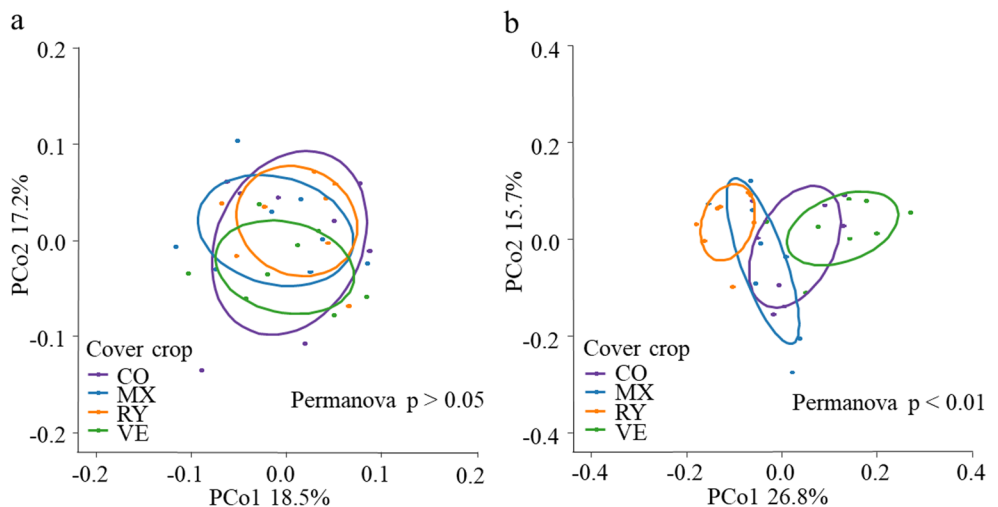


Fig. 2 Principal coordinate analysis of bacterial (A) and fungal (B) community structures performed on the Bray-Curtis dissimilarity matrix. Ellipses suggest 95% confidence regions around group centroids. CO, no cover crop control; RY, cereal rye; VE, hairy vetch; MX, the mixture of rye and vetch

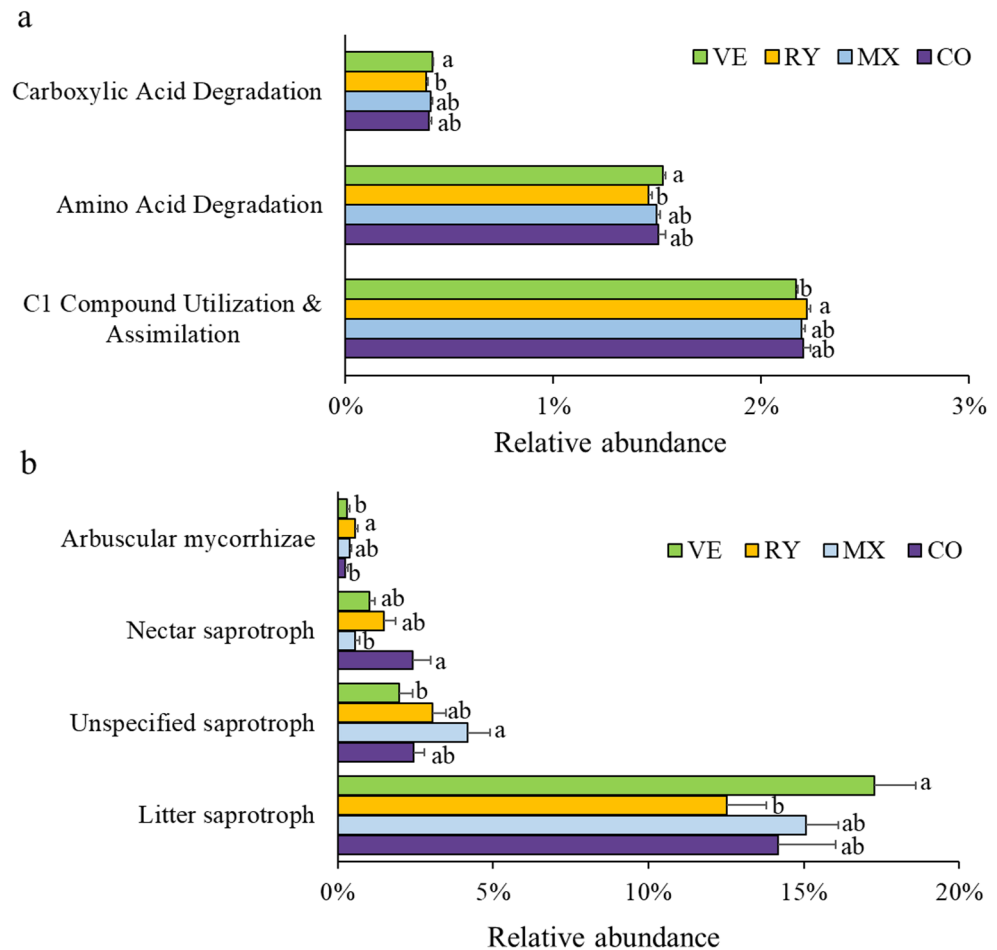


3.5 Soil Microbial Functional Traits

Approximately 95% of the total bacterial sequencing reads (16420 out of 17211) were included in PICRUST2 functional prediction analysis. Twenty dissimilation, assimilation, and

degradation pathways directly related to soil C-cycling were predicted from the MetaCyc database (Figs. 3 A and S3A). No significant treatment effects were observed, except for the pathways of carboxylic acid degradation, amino acid degradation, and C1 compound utilization and assimilation,

Fig. 3 Impacts of cover crop inclusion on the relative abundances of Metacyc level-3 C assimilation, dissimilation, and degradation pathways (A) and FungalTraits secondary lifestyles (B). Bars represent the mean $\pm$ standard error of the mean ($n=8$). Different letters suggest significant differences between cover crop treatments at $p<0.05$. CO, no cover crop control; RY, cereal rye; VE, hairy vetch; MX, the mixture of rye and vetch



in which differences were only observed between the RY and VE treatments. The RY treatment was predicted to have a higher relative abundance of the C1 Compound Utilization and Assimilation pathways than the VE treatment, but a lower relative abundance of the amino acid degradation and carboxylic acid degradation pathways.

FungalTraits identified six lifestyles from 161 fungal genera (Figs. 3B and S3B). The VE treatment was predicted to have a higher relative abundance of litter saprotrophs than the RY treatment. In contrast, the RY treatment was predicted to have a higher relative abundance of arbuscular mycorrhizal (AM) fungi than the VE and CO treatments. In addition, the MX treatment was predicted to reduce the relative abundance of nectar saprotrophs compared to the CO treatment, which was not observed in other cover crop treatments.

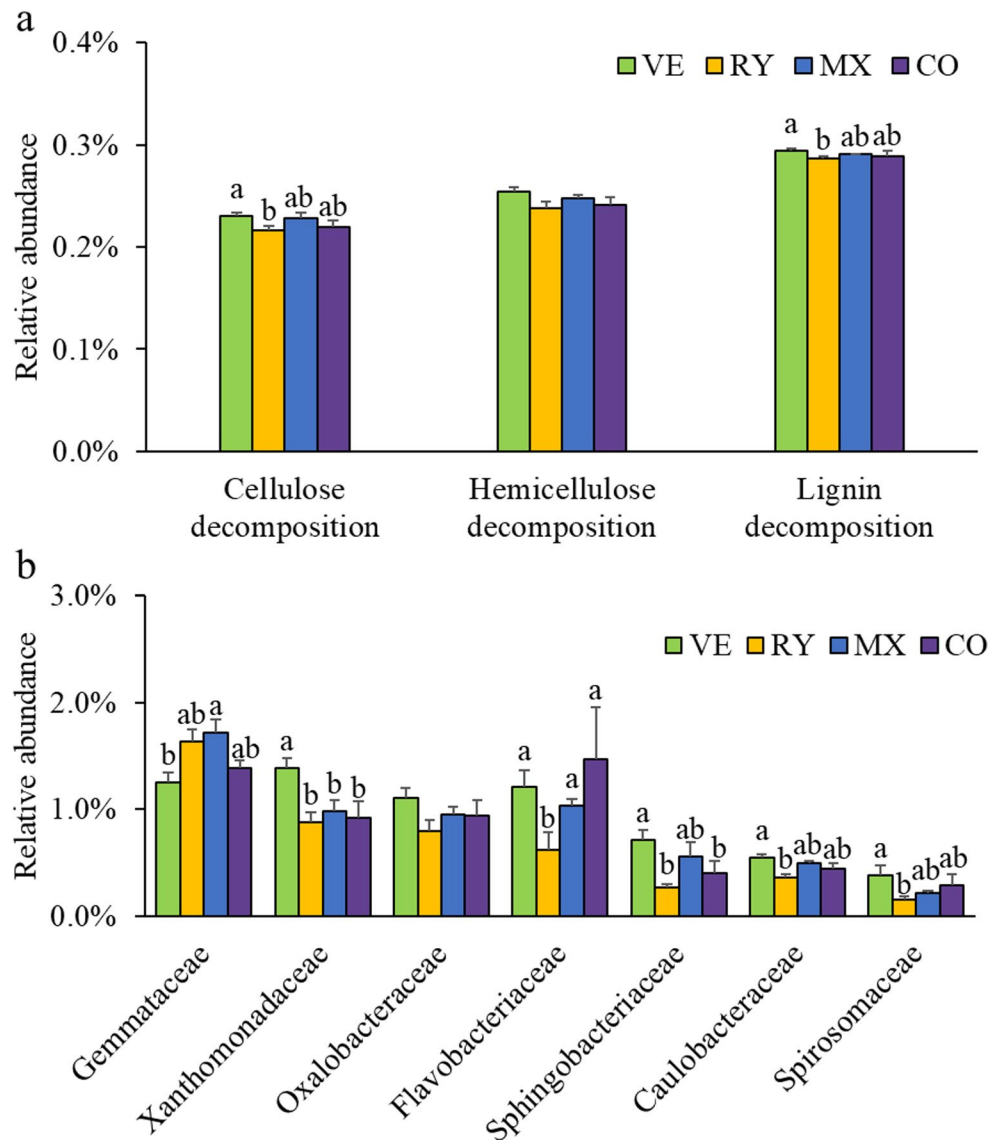
PICRUSt2 analysis predicted 10, 19, and 10 individual KEGG orthologs (KOs) directly involved in decomposing major soil organic polymers, i.e., cellulose, hemicellulose, and lignin, respectively, from the bacterial 16 S rRNA sequencing data. The VE treatment was predicted to increase the abundances of cellulose and lignin decomposition KOs compared to the RY treatment (Fig. 4A). To further

distinguish the bacteria between VE and RY treatments, we filtered the top 40 bacterial families that harbor more than 0.5% of total abundances of cellulose and lignin decomposing KOs (Figs. S4A and S4B). SIMPER analysis identified eight distinct bacterial families associated with cellulose and lignin decomposition, among which *Xanthomonadaceae*, *Flavobacteriaceae*, *Sphingobacteriaceae*, *Caulobacteriaceae*, and *Spirosomaceae* were more abundant under the VE treatment than under the RY treatment (Fig. 4B).

3.6 Correlations between Measured Variables

EC was positively correlated with cumulative organic C and N inputs and the concentrations of NO_3^- and DOC, but negatively correlated with fungal Shannon index and the abundance of arbuscular mycorrhizal fungi (Fig. 6). NO_3^- concentrations were positively correlated with cumulative organic C and N inputs and DOC concentrations but negatively correlated with fungal Shannon index. Soil bacterial community structure was correlated with NO_3^- and DOC concentrations (Fig. 6; Table S5), while fungal community structure was correlated with cumulative organic N inputs, EC, and the concentrations of NO_3^- and DOC.

Fig. 4 Relative abundances of KEGG cellulose, hemicellulose, and lignin decomposition orthologs (**A**), and relative abundances of discriminating cellulose and lignin decomposition bacterial families (**B**). Bars represent the mean $\pm$ standard error of the mean ($n=8$). Different letters suggest significant differences between cover crop treatments at $p<0.05$. CO, no cover crop control; RY, cereal rye; VE, hairy vetch; MX, the mixture of rye and vetch



Fungal Shannon diversity was positively correlated with the abundance of arbuscular mycorrhizal fungi but negatively correlated with EC, NO_3^- , and DOC concentrations. The structures of cellulose and lignin KO dissimilatory matrices were positively correlated with the bacterial community structures of these selected families (Fig. 5A and B).

4 Discussion

Soil microbial diversity underpins soil nutrient cycling (Bastida et al. 2021; Gupta et al. 2022). It also maintains microbial functional redundancy among different taxa that share metabolic functions, allowing soils to deliver necessary ecological services when encountering environmental disturbances (Louca et al. 2018). In the present study, integrating cereal rye or hairy vetch as single-species cover

crops for three consecutive seasons did not change bacterial diversity, contrary to our hypothesis. Similar studies have also reported such neutral impacts of individual cover crops on bacterial diversity (Chamberlain et al. 2020; Wipf et al. 2021). By contrast, when the two cover crops were introduced as a mixture, the bacterial diversity and richness increased, aligning with previous reports on cover crop mixtures (Eisenhauer et al. 2010; Gao et al. 2022). This response is likely because the mixture increased the diversity of C and N sources, which supported bacterial population growth more effectively than the fallow or vetch residue alone (Eisenhauer et al. 2011; Purahong et al. 2016). Similarly, Drost et al. (2020) suggested that mixture residues produced more substrate niches than individual cover crops, addressing the need for composition analysis on cover crop residues to confirm whether increased organic substrate diversity is

Fig. 5 Spearman's rank correlation between the decomposition KOs structures of cellulose (a) and lignin (b) and bacterial community structures. EC (dS m^{-1}), electrical conductivity; DOC, dissolved organic C (mg kg^{-1}); ASV, amplicon sequence variant

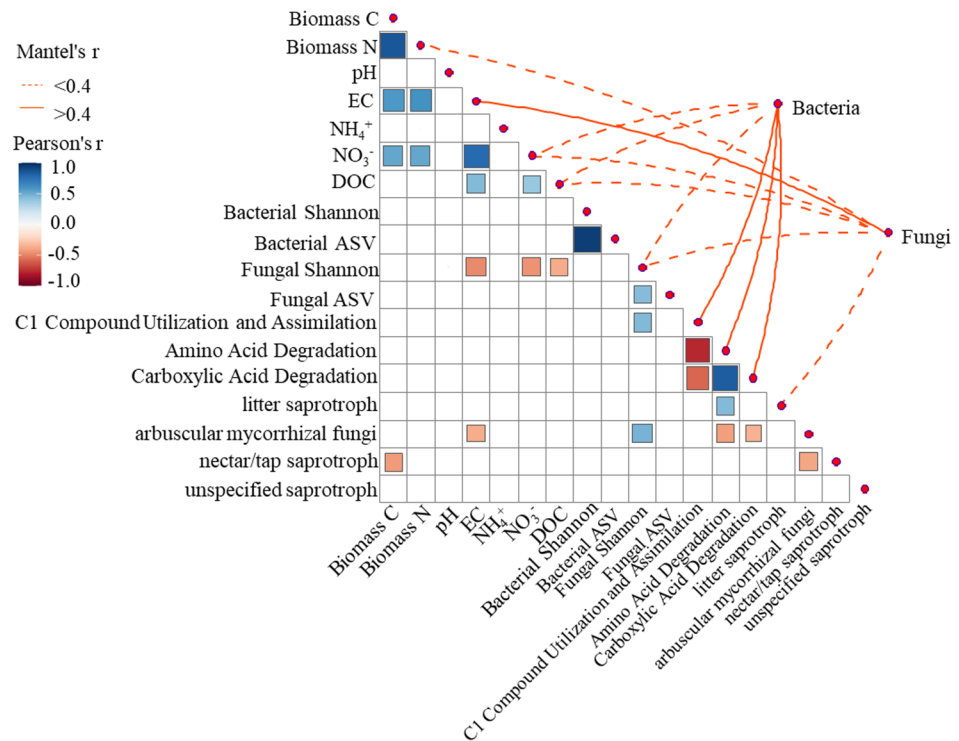
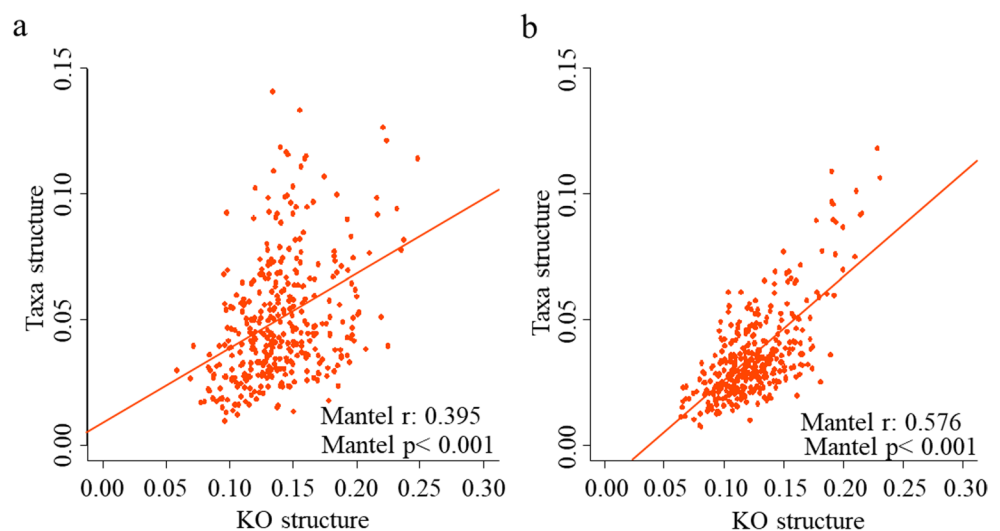


Fig. 6 Linear correlations between microbial traits and soil biogeochemical properties. Orange lines and colored squares indicate significance at $p < 0.05$. KO, KEGG ortholog



the primary mechanism driving shifts in bacterial community composition.

In contrast to the bacterial community, no changes in soil fungal diversity were observed after three years of cover crop inclusion, a finding consistent with previous studies (Cloutier et al. 2020). Xu et al. (2024a, b) suggested that soil fungal diversity is highly correlated with soil C availability. In the present study, no treatment effects on the DOC concentrations were observed, which may partly explain the unchanged fungal diversity. In addition, fungal growth rates are up to an order of magnitude slower than bacterial growth rates (Dopheide et al. 2023; Gao et al. 2022), which may

also explain why fungal diversity remained unchanged after three years of cover cropping.

Both bacterial and fungal community structures showed significant correlations with soil NO_3^- and DOC concentrations, consistent with their recognized role as driving factors of microbial community structure (Hu et al. 2023; Vukicevich et al. 2016). Zhong et al. (2020) further suggested that C sources can be more important than N availability in shaping microbial community structure during the early stages of microbial assembly. In the present study, the inclusion of cover crops for three years changed soil NO_3^- availability, but not DOC concentrations. While a number

of copiotrophic bacteria within certain classes, such as Alphaproteobacteria and Gammaproteobacteria, prefer labile C substrates for growth (Wang et al. 2021; Zhang et al. 2022), their abundances were not affected by the cover crop treatments. Therefore, the unchanged DOC concentrations and stable copiotroph abundances likely explain the unshifted bacterial community structure, supporting the notion that C substrates may be more influential than N availability in regulating bacterial community composition under these conditions (Fu et al. 2022).

Unlike the bacterial community, the fungal community structure was more sensitive to the three-year cover crop inclusion, likely due to the changed N availability, as indicated by its correlation with cumulative organic N inputs, NO_3^- concentration, and EC. A few studies have suggested that the bacterial community may be more responsive to the quantity of organic inputs, whereas the fungal community is more responsive to the quality of the inputs (i.e., the C-to-N ratio of cover crop residue) (Maul et al. 2014; Schmidt et al. 2018). Mohamed and Martiny (2011) suggested that the varying adaptation strategies of fungi to salinity may lead to community structure variation along salinity gradients, which is consistent with the observed relationships between fungal community structure and EC. In addition, Tosi et al. (2022) suggested that minor compositional changes in the fungal community may be more pronounced than in the bacterial community, due to the relatively low fungal diversity, as observed in the present study.

The microbial processes of C sequestration through cover crop residue decomposition are critical for climate-smart agricultural practices (Hu et al. 2024). Soil bacteria utilize C sources through various mechanisms (Maron et al. 2018; Schimel and Schaeffer 2012), including dissimilation, assimilation, and degradation pathways, which are influenced by environmental changes (Wu et al. 2024). We estimated that VE inclusion led to higher abundances of amino acid and carboxylic acid degradation pathways in bacterial communities than RY, thereby supporting the hypothesis. Similarly, Mardani-Korrani et al. (2021) also found that VE enriched the metabolic pathway of the amino acid L-canavanine. This result suggests that VE inclusion may promote organic N mineralization by adding N-rich residues (Aliashkevich et al. 2018; Canarini et al. 2019). Carboxylic acids are commonly found in plant root exudates (Seitz et al. 2024). Leguminous roots secrete more carboxylic acids than gramineous roots (Seitz et al. 2023; Wink 2013), which could partly trigger a higher carboxylic acid degradation activity, as observed in the present study. In contrast, RY inclusion enhanced the C1 compound utilization and assimilation pathway, allowing bacteria to utilize one-carbon substrates for growth (Le and Lee 2022). Cereal rye has a larger root biomass than VE, which might

attract more microbial methanol consumers (Sievers and Cook 2018). Another group of major C1 compound consumers, methanotrophs, prefer complex C sources (Tiwari et al. 2018). This could also explain why the C1 compound utilization pathway was estimated to be higher in the RY treatment than in the VE treatment. Moreover, most methylotrophic microbes are obligate aerobes (Kolb 2009). Compared to the taproot of VE, the fibrous root of RY may create better aeration in the rhizosphere, increasing the activity of methylotrophic microbes (Griffiths et al. 2022). To the best of our knowledge, this is the first attempt to estimate the differences between VE and RY in the carboxylic acid degradation pathway and the C1 compound utilization and assimilation pathway.

Similarly, differences in fungal C-cycling traits were found mainly between the VE and RY treatments, again supporting the hypothesis. Higher abundances of litter saprotrophic fungi in the VE than RY treatments were also reported by Yu et al. (2022). This is likely due to the relatively higher soil N availability (Marañón-Jiménez et al. 2021; Sterkenburg et al. 2018). In contrast, we estimated more abundant AM fungi in RY than in the VE treatments (Fig. 3B), likely because AM fungi are associated with the acquisition and transformation of more complex C compounds derived from RY residues (Hodge et al. 2001).

Cellulose, hemicellulose, and lignin are the primary components of plant cell walls, serving as food sources for soil microbes (Huang et al. 2024; Richard 1996). Three years of VE inclusion were estimated to lead to a higher relative abundance of microbial cellulose and lignin decomposition genes than integrating RY, a finding also reported by similar studies (Li et al. 2024; Xie et al. 2022). It is possible that the relatively higher N content of VE residues may have favored the proliferation of bacteria carrying genes for lignin and cellulose decomposition (He et al. 2019; Thapa et al. 2023). The positive correlation between bacterial community structure and the structures of cellulose and lignin decomposition pathways also supports the notion that bacterial communities strongly mediate the production of genes involved in lignin and cellulose decomposition. Secondly, VE residues are often decomposed more rapidly than RY residues due to their higher biomass N content. The rapid decomposition of VE residues may, in turn, positively prime the decomposition of cellulose and lignin (Kuzakov et al. 2000; Talbot and Treseder 2012).

Microbial keystone taxa are microbes that make the most significant contributions to the community's functional profile, exerting non-redundant impacts on microbial ecosystem services (Paine 1969). Emerging studies suggest that certain microbial species possess a strong capacity for C acquisition, playing leading roles in organic matter decomposition (Dasgupta et al. 2024; Zheng et al. 2021). Therefore, identifying

these keystone taxa will give us a deeper understanding of C decomposition function under disturbance (Banerjee et al. 2016; Vick-Majors et al. 2014). Supporting this hypothesis, we found that five keystone bacterial cellulose and lignin decomposers were more abundant under the VE treatment than under the RY treatment. *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Spirosomaceae* are saprotrophic bacteria that belong to the phylum Bacteroidota. These three bacterial families possess a strong decomposition ability, which can be stimulated by adding N-rich VE residues (Chiba et al. 2021; Kraut-Cohen et al. 2021; Olakanye and Ralebitso-Senior 2018). *Xanthomonadaceae* and *Caulobacteraceae* belong to the phylum Pseudomonadota (Abraham et al. 2014), which are also capable of decomposing cellulose and lignin, and can be induced by VE treatment (Dasgupta et al. 2024; Doronina and Trotsenko 2003; Wilhelm et al. 2019). In addition, numerous species of these two families are symbiotic N-fixers (Montañez et al. 2009; Oren 2014), which could explain their higher abundances in the VE treatment compared to the RY treatment. Our results highlight the importance of N availability in enhancing the soil microbial potential for decomposing cellulose and lignin in sandy soils under our experimental conditions.

5 Conclusion

Integrating legume and nonlegume cover crops for three years provided continual organic inputs that differed in quantity and quality, resulting in their distinctive impacts on soil microbial communities. Overall, the inclusion of cover crops had limited impacts on microbial richness. Integrating hairy vetch and winter rye did not change the composition and structure of the bacterial community, but modified those of the fungal community. The vetch and rye also demonstrated distinct C-cycling pathways. Specifically, the vetch increased the predicted gene abundances for cellulose and lignin decomposition compared to the rye treatment, predominantly driven by five copiotrophic keystone taxa. These species-specific impacts of legume versus non-legume cover crops likely derive from their contrasting functional traits, such as biomass production potential and N provision capacity, resulting in differences in N availability and organic substrate concentrations. Microbial functional prediction and the identification of keystone taxa can provide critical insights into soil nutrient and fertility management in organic systems. However, the functional prediction pipelines applied here are based on amplicon sequences and may not accurately capture genetic microdiversity. Future studies should investigate the long-term impacts of these differences on nutrient cycling, residue decomposition,

and C sequestration, and further improve the robustness of microbial functional prediction tools.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42729-026-03241-5>.

Acknowledgments We thank Binaya Parajuli, Pratima Poudel, Allen Dewitt, Chuck Parker, Matt Lennon, and Shawn Chandler for their lab and field assistance. Any reference to the mentioned commercials stands solely for research purposes and should not be interpreted as an endorsement or recommendation.

Funding Open access funding provided by the Carolinas Consortium. This study was funded by the USDA National Institute of Food and Agriculture (USDA-NIFA) Grant 2020-51106-32363.

Data Availability All data are available upon request to the corresponding authors.

Declarations

Disclosure The authors have no relevant financial or non-financial interests to disclose.

Competing 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.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abraham W-R, Rohde M, Bennisar A (2014) The Family Caulobacteraceae. In *The Prokaryotes* (pp. 179–205). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-30197-1_259
- Aliashkevich A, Alvarez L, Cava F (2018) New Insights Into the Mechanisms and Biological Roles of D-Amino Acids in Complex Eco-Systems. *Front Microbio* 9. <https://doi.org/10.3389/fmicb.2018.00683>
- Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, Richardson AE (2016) Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biol Biochem* 97:188–198. <https://doi.org/10.1016/j.soilbio.2016.03.017>
- Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, Stamatakis A (2019) EPA-ng: Massively Parallel Evolutionary

- Placement of Genetic Sequences. *Syst Biol* 68:365–369. <https://doi.org/10.1093/sysbio/syy054>
- Bastida F, Eldridge DJ, García C, Kenny Png G, Bardgett RD, Delgado-Baquerizo M (2021) Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *ISME J* 15:2081–2091. <https://doi.org/10.1038/s41396-021-00906-0>
- Bolyen, E., Rideout, J.R., Dillon, M.R. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37, 852–857 (2019). <https://doi.org/10.1038/s41587-019-0209-9>
- Brennan EB, Acosta-Martinez V (2017) Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biol Biochem* 109:188–204. <https://doi.org/10.1016/j.soilbio.2017.01.014>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583. <https://doi.org/10.1038/nmeth.3869>
- Canarini A, Kaiser C, Merchant A, Richter A, Wanek W (2019) Root Exudation of Primary Metabolites: Mechanisms and Their Roles in Plant Responses to Environmental Stimuli. *Front Plant Sci* 10. <https://doi.org/10.3389/fpls.2019.00157>
- Caspi R, Billington R, Keseler IM, Kothari A, Krummenacker M, Midford PE, Ong WK, Paley S, Subhraveti P, Karp PD (2020) The MetaCyc database of metabolic pathways and enzymes - a 2019 update. *Nucleic Acids Res* 48:D445–D453. <https://doi.org/10.1093/nar/gkz862>
- Chamberlain LA, Bolton ML, Cox MS, Suen G, Conley SP, Ané J-M (2020) Crop rotation, but not cover crops, influenced soil bacterial community composition in a corn-soybean system in southern Wisconsin. *Appl Soil Ecol* 154:103603. <https://doi.org/10.1016/j.apsoil.2020.103603>
- Chavarría DN, Verdenelli RA, Serri DL, Restovich SB, Andriulo AE, Meriles JM, Vargas-Gil S (2016) Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. *Eur J Soil Biol* 76:74–82. <https://doi.org/10.1016/j.ejsobi.2016.07.002>
- Chiba A, Uchida Y, Kublik S, Vestergaard G, Buegger F, Schlöter M, Schulz S (2021) Soil Bacterial Diversity Is Positively Correlated with Decomposition Rates during Early Phases of Maize Litter Decomposition. *Microorganisms* 9:357. <https://doi.org/10.3390/microorganisms9020357>
- Cloutier ML, Murrell E, Barbercheck M, Kaye J, Finney D, García-González I, Bruns MA (2020) Fungal community shifts in soils with varied cover crop treatments and edaphic properties. *Sci Rep* 10:6198. <https://doi.org/10.1038/s41598-020-63173-7>
- Core Team R, R (2014) R: A language and environment for statistical computing. <http://www.R-project.org/>
- Dai Z, Liu G, Chen H, Chen C, Wang J, Ai S, Wei D, Li D, Ma B, Tang C, Brookes PC, Xu J (2020) Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. *ISME J* 14:757–770. <https://doi.org/10.1038/s41396-019-0567-9>
- Dasgupta D, Richardson AE, Camuy-Vélez LA, Kirkby C, Kirkegaard JA, Banerjee S (2024) Microbial dynamics during in-situ organic matter decomposition reveals the importance of keystone taxa in the core microbiome. *Appl Soil Ecol* 199. <https://doi.org/10.1016/j.apsoil.2024.105396>
- Dhungana I, Kantar MB, Nguyen NH (2023) Root exudate composition from different plant species influences the growth of rhizosphere bacteria. *Rhizosphere* 25:100645. <https://doi.org/10.1016/j.rhisph.2022.100645>
- Dopheide A, Davis C, Wakelin SA, Whitehead D, Grelet G-A (2023) Labile carbon inputs support the recovery of bacterial communities, but not fungal communities, from a simulated bovine urine event. *Biol Fertil Soils* 59:333–349. <https://doi.org/10.1007/s00374-023-01710-y>
- Doronina NV, Trotsenko YA (2003) Reclassification of ‘*Blastobacter viscosus*’ 7d and ‘*Blastobacter aminooxidans*’ 14a as *Xanthobacter viscosus* sp. nov. and *Xanthobacter aminooxidans* sp. nov. *Int J Syst Evol Microbiol* 53:179–182. <https://doi.org/10.1099/ij.s.0.02231-0>
- Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUST2 for prediction of metagenome functions. *Nat Biotechnol* 38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>
- Drost SM, Rutgers M, Wouterse M, de Boer W, Bodelier PLE (2020) Decomposition of mixtures of cover crop residues increases microbial functional diversity. *Geoderma* 361:114060. <https://doi.org/10.1016/j.geoderma.2019.114060>
- Eisenhauer N, Beßler H, Engels C, Gleixner G, Habekost M, Milcu A, Partsch S, Sabais ACW, Scherber C, Steinbeiss S, Weigelt A, Weisser WW, Scheu S (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91:485–496. <https://doi.org/10.1890/08-2338.1>
- Eisenhauer N, Milcu A, Sabais ACW, Bessler H, Brenner J, Engels C, Klarner B, Maraun M, Partsch S, Roscher C, Schonert F, Temper-ton VM, Thomisch K, Weigelt A, Weisser WW, Scheu S (2011) Plant Diversity Surpasses Plant Functional Groups and Plant Productivity as Driver of Soil Biota in the Long Term. *PLoS ONE* 6:e16055. <https://doi.org/10.1371/journal.pone.0016055>
- Fisher RA (1992) Statistical Methods for Research Workers (pp. 66–70). https://doi.org/10.1007/978-1-4612-4380-9_6
- Fu X, Song Q, Li S, Shen Y, Yue S (2022) Dynamic changes in bacterial community structure are associated with distinct priming effect patterns. *Soil Biol Biochem* 169:108671. <https://doi.org/10.1016/j.soilbio.2022.108671>
- Gao H, Tian G, Khashi u Rahman M, Wu F (2022) Cover Crop Species Composition Alters the Soil Bacterial Community in a Continuous Pepper Cropping System. *Front Microbiol* 12. <https://doi.org/10.3389/fmicb.2021.789034>
- Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, Bruns G, Yarza P, Peplies J, Westram R, Ludwig W (2017) 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J Biotechnol* 261:169–176. <https://doi.org/10.1016/j.jbiotec.2017.06.1198>
- Griffith JC, Lee WG, Orlovich DA, Summerfield TC (2017) Contrasting bacterial communities in two indigenous *Chionochloa* (Poaceae) grassland soils in New Zealand. *PLoS ONE* 12:e0179652. <https://doi.org/10.1371/journal.pone.0179652>
- Griffiths M, Delory BM, Jawahir V, Wong KM, Bagnall GC, Dowd TG, Nusinow DA, Miller AJ, Topp CN (2022) Optimisation of root traits to provide enhanced ecosystem services in agricultural systems: A focus on cover crops. *Plant Cell Environ* 45:751–770. <https://doi.org/10.1111/pce.14247>
- Gul S, Whalen JK (2022) Perspectives and strategies to increase the microbial-derived soil organic matter that persists in agroecosystems. In *Advances in Agronomy* (Vol. 175, pp. 347–401). Academic Press Inc. <https://doi.org/10.1016/bs.agron.2022.04.004>
- Gupta A, Singh UB, Sahu PK, Paul S, Kumar A, Malviya D, Singh S, Kuppasamy P, Singh P, Paul D, Rai JP, Singh HV, Manna MC, Crusberg TC, Kumar A, Saxena AK (2022) Linking Soil Microbial Diversity to Modern Agriculture Practices: A Review. *Int J Environ Res Public Health* 19:3141. <https://doi.org/10.3390/ijerph19053141>
- He M, Zhao R, Tian Q, Huang L, Wang X, Liu F (2019) Predominant effects of litter chemistry on lignin degradation in the early stage of leaf litter decomposition. *Plant Soil* 442:453–469. <https://doi.org/10.1007/s11104-019-04207-6>
- Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly

- from organic material. *Nature* 413:297–299. <https://doi.org/10.1038/35095041>
- Hu Y, Fair H, Liu Q, Wang Z, Duan B, Lu X (2023) Diversity and co-occurrence networks of bacterial and fungal communities on two typical debris-covered glaciers, southeastern Tibetan Plateau. *Microbiol Res* 273:127409. <https://doi.org/10.1016/j.micres.2023.127409>
- Hu Q, Zhang Y, Cao W, Yang Y, Hu Y, He T, Li Z, Wang P, Chen X, Chen J, Shi X (2024) Legume cover crops sequester more soil organic carbon than non-legume cover crops by stimulating microbial transformations. *Geoderma* 450. <https://doi.org/10.1016/j.geoderma.2024.117024>
- Huang H (2021) linkET: Everything is Linkable. R package version 0.0.3.
- Huang L, Peddada S, Das (2020) Analysis of compositions of microbiomes with bias correction. *Nat Commun* 11. <https://doi.org/10.1038/s41467-020-17041-7>
- Huang Q, Wang B, Shen J, Xu F, Li N, Jia P, Jia Y, An S, Amoah ID, Huang Y (2024) Shifts in C-degradation genes and microbial metabolic activity with vegetation types affected the surface soil organic carbon pool. *Soil Biol Biochem* 192:109371. <https://doi.org/10.1016/j.soilbio.2024.109371>
- Kallenbach CM, Frey SD, Grandy AS (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nat Commun* 7. <https://doi.org/10.1038/ncomms13630>
- Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* 44:D457–D462. <https://doi.org/10.1093/nar/gkv1070>
- Kim N, Zabaloy MC, Guan K, Villamil MB (2020) Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biol Biochem* 142. <https://doi.org/10.1016/j.soilbio.2019.107701>
- Kolb S (2009) Aerobic methanol-oxidizing Bacteria in soil. *FEMS Microbiol Lett* 300:1–10. <https://doi.org/10.1111/j.1574-6968.2009.01681.x>
- Kraut-Cohen J, Shapiro OH, Dror B, Cytryn E (2021) Pectin Induced Colony Expansion of Soil-Derived Flavobacterium Strains. *Front Microbiol* 12. <https://doi.org/10.3389/fmicb.2021.651891>
- Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* 32:1485–1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)
- Le HTQ, Lee EY (2022) Insights into C1 and C3 assimilation pathways in type I methanotrophic bacterium from co-production of 1,2-propanediol and lactate. *Bioresour Technol* 365:128172. <https://doi.org/10.1016/j.biortech.2022.128172>
- Li G, Leng K, Qin W, Chen X, Lin Y, Liu M, Wu M, Fan J, Wang X, Jiang Y, Liu J (2024) Litter quality regulates cover crop litter decay alongside altered microbial facets. *Appl Soil Ecol* 204:105761. <https://doi.org/10.1016/j.apsoil.2024.105761>
- Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW (2018) Function and functional redundancy in microbial systems. In *Nature Ecology and Evolution*. Nat Publishing Group 2:936–943. <https://doi.org/10.1038/s41559-018-0519-1>
- Marañón-Jiménez S, Radujković D, Verbruggen E, Grau O, Cuntz M, Peñuelas J, Richter A, Schimpf M, Rebmann C (2021) Shifts in the Abundances of Saprotrophic and Ectomycorrhizal Fungi With Altered Leaf Litter Inputs. *Front Plant Sci* 12. <https://doi.org/10.3389/fpls.2021.682142>
- Mardani-Korani H, Nakayasu M, Yamazaki S, Aoki Y, Kaida R, Motobayashi T, Kobayashi M, Ohkama-Ohtsu N, Oikawa Y, Sugiyama A, Fujii Y (2021) L-Canavanine, a Root Exudate From Hairy Vetch (*Vicia villosa*) Drastically Affecting the Soil Microbial Community and Metabolite Pathways. *Front Microbiol* 12. <https://doi.org/10.3389/fmicb.2021.701796>
- Maron P-A, Sarr A, Kaisermann A, Lévêque J, Mathieu O, Guigue J, Karimi B, Bernard L, Dequiedt S, Terrat S, Chabbi A, Ranjard L (2018) High Microbial Diversity Promotes Soil Ecosystem Functioning. *Appl Environ Microbiol* 84. <https://doi.org/10.1128/AEM.02738-17>
- Maul JE, Buyer JS, Lehman RM, Culman S, Blackwood CB, Roberts DP, Zasada IA, Teasdale JR (2014) Microbial community structure and abundance in the rhizosphere and bulk soil of a tomato cropping system that includes cover crops. *Appl Soil Ecol* 77:42–50. <https://doi.org/10.1016/j.apsoil.2014.01.002>
- Mbuthia LW, Acosta-Martínez V, DeBruyn J, Schaeffer S, Tyler D, Odoi E, Mphesha M, Walker F, Eash N (2015) Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biol Biochem* 89:24–34. <https://doi.org/10.1016/j.soilbio.2015.06.016>
- Mohamed DJ, Martiny JBH (2011) Patterns of fungal diversity and composition along a salinity gradient. *ISME J* 5:379–388. <https://doi.org/10.1038/ismej.2010.137>
- Montañez A, Abreu C, Gill PR, Hardarson G, Sicardi M (2009) Biological nitrogen fixation in maize (*Zea mays* L.) by ¹⁵N isotope dilution and identification of associated culturable diazotrophs. *Biol Fertil Soils* 45:253–263. <https://doi.org/10.1007/s00374-008-0322-2>
- Muhammad I, Wang J, Sainju UM, Zhang S, Zhao F, Khan A (2021) Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma* 381. <https://doi.org/10.1016/j.geoderma.2020.114696>
- Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res* 47:D259–D264. <https://doi.org/10.1093/nar/gky1022>
- Novara A, Catania V, Tolone M, Gristina L, Laudicina VA, Quatrini P (2020) Cover Crop Impact on Soil Organic Carbon, Nitrogen Dynamics and Microbial Diversity in a Mediterranean Semiarid Vineyard. *Sustainability* 12:3256. <https://doi.org/10.3390/su12083256>
- Olakanye AO, Ralebitso-Senior TK (2018) Soil metabarcoding identifies season indicators and differentiators of pig and *Agrostis/Festuca* spp. decomposition. *Forensic Sci Int* 288:53–58. <https://doi.org/10.1016/j.forsciint.2018.04.015>
- Olson KR, Ebelhar SA, Lang JM (2010) Cover Crop Effects on Crop Yields and Soil Organic Carbon Content. *Soil Sci* 175:89–98. <https://doi.org/10.1097/SS.0b013e3181cf7959>
- Oren A (2014) The Family Xanthobacteraceae. In *The Prokaryotes* (pp. 709–726). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-30197-1_258
- Paine RT (1969) A Note on Trophic Complexity and Community Stability. *Am Nat* 103:91–93. <https://doi.org/10.1086/282586>
- Pandey A, Li F, Askegaard M, Rasmussen IA, Olesen JE (2018) Nitrogen balances in organic and conventional arable crop rotations and their relations to nitrogen yield and nitrate leaching losses. *Agric Ecosyst Environ* 265:350–362. <https://doi.org/10.1016/j.agee.2018.05.032>
- Pöhlme S, Abarenkov K, Henrik Nilsson R, Lindahl BD, Clemmensen KE, Kauserud H, Nguyen N, Kjeller R, Bates ST, Baldrian P, Frøslev TG, Adojaan K, Vizzini A, Suija A, Pfister D, Baral H-O, Järv H, Madrid H, Nordén J, Tedersoo L (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers* 105:1–16. <https://doi.org/10.1007/s13225-020-00466-2>
- Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F (2016) Life in leaf litter:

- novel insights into community dynamics of bacteria and fungi during litter decomposition. *Mol Ecol* 25:4059–4074. <https://doi.org/10.1111/mec.13739>
- Ranheim Sveen T, Hannula SE, Bahram M (2024) Microbial regulation of feedbacks to ecosystem change. *Trends Microbiol* 32:68–78. <https://doi.org/10.1016/j.tim.2023.06.006>
- Reiter MS (2020) In: Kemble JM, Meadows IM, Jennings KM, Walgenbach JF (eds) *Southeastern us 2020 vegetable crop handbook* 1–100. Virginia Cooperative Extension
- Richard T (1996) The effect of lignin on biodegradability. *Cornell Composting*
- Saleem M, Pervaiz ZH, Contreras J, Lindenberger JH, Hupp BM, Chen D, Zhang Q, Wang C, Iqbal J, Twigg P (2020) Cover crop diversity improves multiple soil properties via altering root architectural traits. *Rhizosphere* 16:100248. <https://doi.org/10.1016/j.rhisph.2020.100248>
- Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. In *Frontiers in Microbiology* (Vol. 3). Frontiers Research Foundation. <https://doi.org/10.3389/fmicb.2012.00348>
- Schmidt R, Gravuer K, Bossange AV, Mitchell J, Scow K (2018) Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS ONE* 13:e0192953. <https://doi.org/10.1371/journal.pone.0192953>
- Seitz VA, Chaparro JM, Schipanski ME, Wrighton KC, Prenni JE (2023) Cover crop cultivar, species, and functional diversity is reflected in variable root exudation composition. *J Agric Food Chem* 71:11373–11385
- Seitz VA, McGivern BB, Borton MA, Chaparro JM, Schipanski ME, Prenni JE, Wrighton KC (2024) Cover Crop Root Exudates Impact Soil Microbiome Functional Trajectories in Agricultural Soils. <https://doi.org/10.21203/rs.3.rs-3956430/v1>
- Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrika* 52:591–611. <https://doi.org/10.1093/biomet/52.3-4.591>
- Sievers T, Cook RL (2018) Aboveground and Root Decomposition of Cereal Rye and Hairy Vetch Cover Crops. *Soil Sci Soc Am J* 82:147–155. <https://doi.org/10.2136/sssaj2017.05.0139>
- Sparling GP, Feltham CW, Reynolds J, West AW, Singleton P (1990) Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the kec-factor. *Soil Biol Biochem* 22:301–307. [https://doi.org/10.1016/0038-0717\(90\)90104-8](https://doi.org/10.1016/0038-0717(90)90104-8)
- Sterkenburg E, Clemmensen KE, Ekblad A, Finlay RD, Lindahl BD (2018) Contrasting effects of ectomycorrhizal fungi on early and late stage decomposition in a boreal forest. *ISME J* 12:2187–2197. <https://doi.org/10.1038/s41396-018-0181-2>
- Talbot JM, Treseder KK (2012) Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. *Ecology* 93:345–354. <https://doi.org/10.1890/11-0843.1>
- Thapa VR, Ghimire R, Acosta-Martínez V, Marsalis MA, Schipanski ME (2021) Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. *Appl Soil Ecol* 157:103735. <https://doi.org/10.1016/j.apsoil.2020.103735>
- Thapa R, Cabrera M, Schomberg HH, Reberg-Horton C, Poffenbarger H, Mirsky SB (2023) Chemical differences in cover crop residue quality are maintained through litter decay. *PLoS ONE* 18:e0289352. <https://doi.org/10.1371/journal.pone.0289352>
- Tiwari S, Singh C, Singh JS (2018) Land use changes: a key ecological driver regulating methanotrophs abundance in upland soils. *Energy Ecol Environ* 3:355–371. <https://doi.org/10.1007/s40974-018-0103-1>
- Toole DR, Zhao J, Martens-Habbena W, Strauss SL (2021) Bacterial functional prediction tools detect but underestimate metabolic diversity compared to shotgun metagenomics in southwest Florida soils. *Appl Soil Ecol* 168. <https://doi.org/10.1016/j.apsoil.2021.104129>
- Tosi M, Drummelsmith J, Obregón D, Chahal I, Van Eerd LL, Dunfield KE (2022) Cover crop-driven shifts in soil microbial communities could modulate early tomato biomass via plant-soil feedbacks. *Sci Rep* 12:9140. <https://doi.org/10.1038/s41598-022-11845-x>
- Ullah MR, Carrillo Y, Dijkstra FA (2023) Relative contributions of fungi and bacteria to litter decomposition under low and high soil moisture in an Australian grassland. *Appl Soil Ecol* 182:104737. <https://doi.org/10.1016/j.apsoil.2022.104737>
- van Rijssel SQ, Veen GF (Ciska), Koorneef GJ, Bakx-Schotman JMT, ten Tanja FC, Geisen S, van der Putten WH (eds) (2022) Soil microbial diversity and community composition during conversion from conventional to organic agriculture. *Mol Ecol* 31:4017–4030. <https://doi.org/10.1111/mec.16571>
- Vick-Majors TJ, Priscu JC, Amaral-Zettler LA (2014) Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *ISME J* 8:778–789. <https://doi.org/10.1038/ismej.2013.190>
- Vukicevich E, Lowery T, Bowen P, Urbez-Torres JR, Hart M (2016) Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. *Agron Sustain Dev* 36:48. <https://doi.org/10.1007/s13593-016-0385-7>
- Wang X, Zhang W, Liu Y, Jia Z, Li H, Yang Y, Wang D, He H, Zhang X (2021) Identification of microbial strategies for labile substrate utilization at phylogenetic classification using a microcosm approach. *Soil Biol Biochem* 153:107970. <https://doi.org/10.1016/j.soilbio.2020.107970>
- Wang Z, Saski C, Williamson C, Campbell B, Ye R (2024) Crop cover and manure compost: Their varied effects on nitrogen availability and nitrogen cycling functional gene abundances in sandy soils for organic farming. *Appl Soil Ecol* 200:105446. <https://doi.org/10.1016/j.apsoil.2024.105446>
- Wang Z, Saski C, Campbell B, Huang L, Ye R (2025) Cover crops and manure compost shift microbial functions and diversity in Southeastern sandy soils. *Soil Sci Soc Am J* 89. <https://doi.org/10.1002/saj2.70149>
- Wilhelm RC, Singh R, Eltis LD, Mohn WW (2019) Bacterial contributions to delignification and lignocellulose degradation in forest soils with metagenomic and quantitative stable isotope probing. *ISME J* 13:413–429. <https://doi.org/10.1038/s41396-018-0279-6>
- Wink M (2013) Evolution of secondary metabolites in legumes (Fabaceae). *South Afr J Bot* 89:164–175. <https://doi.org/10.1016/j.sajb.2013.06.006>
- Wipf HM-L, Xu L, Gao C, Spinner HB, Taylor J, Lemaux P, Mitchell J, Coleman-Derr D (2021) Agricultural Soil Management Practices Differentially Shape the Bacterial and Fungal Microbiomes of Sorghum bicolor. *Appl Environ Microbiol* 87. <https://doi.org/10.1128/AEM.02345-20>
- Wolf AM, Baker DE (1985) Comparisons of soil test phosphorus by Olsen, Bray P1, Mehlich I and Mehlich III methods. *Commun Soil Sci Plant Anal* 16:467–484. <https://doi.org/10.1080/00103628509367620>
- Wu H, Cui H, Fu C, Li R, Qi F, Liu Z, Yang G, Xiao K, Qiao M (2024) Unveiling the crucial role of soil microorganisms in carbon cycling: A review. In *Science of the Total Environment* (Vol. 909). Elsevier B.V. <https://doi.org/10.1016/j.scitotenv.2023.168627>
- Xie Z, Yu Z, Li Y, Wang G, Liu X, Tang C, Lian T, Adams J, Liu J, Liu J, Herbert SJ, Jin J (2022) Soil microbial metabolism on carbon and nitrogen transformation links the crop-residue contribution to soil organic carbon. *Npj Biofilms Microbiomes* 8:14. <https://doi.org/10.1038/s41522-022-00277-0>
- Xu F, Li C, Chen Y, Wu J, Bai H, Fan S, Yang Y, Zhang Y, Li S, Su J (2024a) Soil microbial community structure and soil fertility jointly regulate soil microbial residue carbon during the

- conversion from subtropical primary forest to plantations. *Geoderma* 441:116767. <https://doi.org/10.1016/j.geoderma.2023.116767>
- Xu S, Zhang W, Goodwin PH, Wang Y, Zheng SJ, Li X (2024b) Effect of cover crop on soil fertility and bacterial diversity in a banana plantation in southwestern China. *Soil Tillage Res* 240. <https://doi.org/10.1016/j.still.2024.106092>
- Yang Y (2021) Emerging Patterns of Microbial Functional Traits. *Trends Microbiol* 29:874–882. <https://doi.org/10.1016/j.tim.2021.04.004>
- Ye Y, Doak TG (2009) A Parsimony Approach to Biological Pathway Reconstruction/Inference for Genomes and Metagenomes. *PLoS Comput Biol* 5:e1000465. <https://doi.org/10.1371/journal.pcbi.1000465>
- Ye R, Parajuli B, Szogi AA, Sigua GC, Ducey TF (2021) Soil health assessment after 40 years of conservation and conventional tillage management in Southeastern Coastal Plain soils. *Soil Sci Soc Am J* 85:1214–1225. <https://doi.org/10.1002/saj2.20246>
- Yu S, Wang T, Meng Y, Yao S, Wang L, Zheng H, Zhou Y, Song Z, Zhang B (2022) Leguminous cover crops and soya increased soil fungal diversity and suppressed pathotrophs caused by continuous cereal cropping. *Front Microbiol* 13. <https://doi.org/10.3389/fmicb.2022.993214>
- Zeng Q, Peñuelas J, Sardans J, Zhang Q, Zhou J, Yue K, Chen Y, Yang Y, Fan Y (2024) Keystone bacterial functional module activates P-mineralizing genes to enhance enzymatic hydrolysis of organic P in a subtropical forest soil with 5-year N addition. *Soil Biol Biochem* 192:109383. <https://doi.org/10.1016/j.soilbio.2024.109383>
- Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP, Firestone MK, Northen TR, Brodie EL (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat Microbiol* 3:470–480. <https://doi.org/10.1038/s41564-018-0129-3>
- Zhang H, Fang Y, Zhang B, Luo Y, Yi X, Wu J, Chen Y, Sarker TC, Cai Y, Chang SX (2022) Land-use-driven change in soil labile carbon affects microbial community composition and function. *Geoderma* 426:116056. <https://doi.org/10.1016/j.geoderma.2022.116056>
- Zheng W, Zhao Z, Gong Q, Zhai B, Li Z (2018) Effects of cover crop in an apple orchard on microbial community composition, networks, and potential genes involved with degradation of crop residues in soil. *Biol Fertil Soils* 54:743–759. <https://doi.org/10.1007/s00374-018-1298-1>
- Zheng H, Yang T, Bao Y, He P, Yang K, Mei X, Wei Z, Xu Y, Shen Q, Banerjee S (2021) Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics. *Soil Biol Biochem* 157:108230. <https://doi.org/10.1016/j.soilbio.2021.108230>
- Zhong Y, Liu J, Jia X, Shangguan Z, Wang R, Yan W (2020) Microbial community assembly and metabolic function during wheat straw decomposition under different nitrogen fertilization treatments. *Biol Fertil Soils* 56:697–710. <https://doi.org/10.1007/s00374-020-01438-z>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.