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Differential Response of Soil Microbial Diversity and Community Composition Influenced by Cover Crops and Fertilizer Treatments in a Dryland Soybean Production System

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Abstract: The response of soil microbial communities to management practices is composite, as it depends on the various environmental factors which contribute to a shift in the microbial communities. In this study we explored the impact of combinations of soil management practices on microbial diversity and community composition in a dryland soybean production system. Soil samples were collected from the experimental field maintained under no till, cover crops, and fertility treatments, at Pontotoc Ridge-Flatwoods Branch Experiment Station, MS, USA. Targeted amplicon sequencing of 16S rRNA and ITS2 genes was used to study the bacterial and fungal community composition. Poultry litter amendment and cover crops significantly influenced soil bacterial diversity. Fertilizer sources had significantly different bacterial communities, as specific microbial taxa were strongly influenced by the changes in the nutrient availability, while cover crops influenced the soil fungal community differences. Differential enrichment of advantageous bacterial (*Proteobacteria*, *Actinobacteria* and *Acidobacteria*) and fungal (*Mortierellomycota*) phyla was observed across the treatments. Soil pH and easily extractable glomalin-related soil proteins (EE-GRSP) were correlated with bacterial communities and aggregate stability (WSA) was influenced by the poultry litter amendment, thus driving the differences in bacterial and fungal communities. These findings suggest that a long-term study would provide more inferences on soil microbial community response to management changes in these dryland soybean production systems.

Keywords: soil microbial community; bacterial diversity; fungal diversity; soil management practices; soil health; poultry litter; cover crops; dryland soybean

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1. Introduction

Dryland environmental condition poses a wide range of challenges in crop production in the southern US, mainly Mississippi. Dryland soybeans make up more than half of the acres in Mississippi. However, lower productivity is observed due to the inconsistent rainfall at all the growth stages [1,2]. The U.S. state of Mississippi resides in the lower Mississippi River alluvial valley (LMRAV). LMRAV precipitation patterns show high variability between the January–March cool season and the July–September warm season. The cool season has more overall precipitation which is more predictable and is less spatially variable than the warm season [3]. Consequently, the area depends on irrigation from groundwater sources for crop production, which is costly to farmers and challenges the sustainability of agricultural water resources [4]. The vast majority of irrigation water used in Mississippi is supplied by the Mississippi River Valley alluvial aquifer, which is one of

the primary aquifers in an area known as the Mississippi Embayment [4,5]. Pumping of groundwater has made the Mississippi Embayment one of the most rapid groundwater depletion regions in the world [4,6,7]. Therefore, development and implementation of conservation farming practices that conserve soil water are critically important to Mississippi and the surrounding region.

Conservation of crop-available soil water by more efficient rainfall replenishment of the soil profile and reduced evapotranspiration through the use of conservation tillage, including no-till, has been widely documented [8,9]. Cover crops, belonging to a major conservation farming practice, improve soil moisture storage by adding to soil organic matter from residues and root exudates, and affecting water conductivity through root channels [10,11]. In addition, the use of no-till and cover crops can reduce production costs [12]. The use of conservation practices cannot, however, ignore yield goals. Yield decline for many crops has been seen in the first years following the initiation of no-till [13]. Also, yields under no-till differ significantly with soil type and climatic conditions [11,14]. For instance, no-till crop production is more promising than conventional tillage and has higher corn and soybean yields in well-drained soils compared to poorly drained soils [15]. Cover crop effects on cash crop yields can vary. Where available water is limited, cover crops can reduce the water available for the cash crop [16].

No-till and cover crops are now widely accepted practices for the promotion of soil health [11,17,18]. They contribute to soil health by improving aggregate size and stability and moderating soil temperature [19], which are related to the soil moisture benefits referred to above. Conservation farming practices can change aggregate stability over relatively short periods of time [20,21]. They also contribute to higher levels of soil organic matter [22,23]. Importantly, no-till and cover crops enhance soil microbial growth and activity [24,25]. Organic soil amendments, such as poultry litter, a soil health practice which when combined with tillage and cover crop conservation practices are agronomically beneficial and promote soil health [26–28]. Even in soybeans, which fix their own nitrogen, poultry litter improves organic carbon, which influence the soil health and increases soybean yields [28,29], where application time and rate are critical.

Soil health is closely linked to soil microbial characteristics such as soil microbial community structure, soil microbial biomass, and soil enzymatic activity [30,31]. These characteristics are strongly correlated with soil organic carbon, which serves as a key substrate [30]. Several studies have demonstrated the effects on microbial properties of tillage, cover crops and organic amendments, i.e., soil health practices [24,32,33]. The long-term impacts of soil conservation practices (no-till, cover crops and poultry litter) with crop rotations (Soybean, corn, and cotton) showed higher microbial diversity in the no-till and poultry litter treatment than the cover crops [34,35]. Research by Brooks et al. [36] indicated that the residual effect of poultry litter application suggested increased bacterial diversity and enrichment, where the litter effects persisted 4 years after application, evidenced by residual library community structures. A meta-analysis of 60 studies reported that the cover cropping system significantly increase the soil microbial abundance (27%), activity (22%), and diversity (2.5%) [33]. In addition, the chemically terminated cover crops showed less marked effects. Therefore, cover cropping with proper agriculture management practices aids in the enrichment of soil microbial communities. Microbial community structure, or diversity, which links to microbial activity, is therefore central to soil health which is influenced by conservation farming practices [37].

As there is a potential for conservation farming practices to alter yield expectations in the early phase of implementation, it is critical to determine the initiation and extent of soil health indicators, in particular the soil microbial characteristics. Because of the previously observed relevance of soil microbial diversity to soil health, its assessment is a key component to conservation management of agricultural soils in the LMRAV. There are few studies of soils in this region in which a combination of no-till, cover crops, and poultry litter amendment have been evaluated in soybean production systems. The objectives

of this study were to determine the effects from combinations of these practices on soil bacterial and fungal communities on a study site located in Mississippi.

2. Materials and Methods

2.1. Experimental Site, Design, and Treatments

The study was conducted at the Pontotoc Ridge-Flatwoods Branch Experiment Station in Pontotoc County, MS, USA (34°07′ N, 88°59′ W). The soil was classified at the site as a fine-silty, mixed, semi-active, thermic Typic Paleudalf (Atwood silt loam series) on a 3% slope. The experiment was carried out under no-tillage, rainfed conditions, and preliminary soil characterization of the experimental site indicated a pH of 6.67 and 1.57% organic matter. Winter cover crops were planted in 2016 and continued through 2019. Soybeans were planted from 2017–2019.

The experimental plots were arranged as a randomized complete block with three replications. Cover crops were the main plot factor with fertilizer treatments as split-plot factors. Four drill-seeded cover crop treatments consisting of wheat (Triticum aestivum, seeding rate of 94 kg ha^{-1}), vetch (Vicia villosa, 22 kg ha^{-1}), cereal rye (Secale cereal, 92 kg ha^{-1}), and native vegetation as a control (naturally seeded weeds) were used. Each year cover crops were planted in late October and terminated mid-April using N-(phosphonomethyl) glycine (glyphosate). The herbicide was applied twice at 10-day intervals before planting herbicide-resistant soybean. Two fertilizer treatments, namely poultry litter and inorganic fertilizer (phosphorus (P), potassium (K), and sulfur (S) were applied on the second of April. Fertilizer application rates were based on soil test results from Southern Soil & Plant Lab, LLC (Yazoo City, MS, USA) using the Lancaster method (Oldham, 2014) with a yield goal of 2690 kg ha^{-1}. In 2019 the fertilizer application rates were 135 kg P_2O_5 ha^{-1} as triple superphosphate, 34 kg K_2O ha^{-1} as muriate of potash, and 22 kg S ha^{-1} as 90% elemental S. The application rate of poultry litter was based on the inorganic P content of the litter equivalent to the soil test results for mineral fertilizer. This led to an application rate of 4483 kg ha^{-1} in 2019.

2.2. Soil Sampling and Analyses

Soil samples, 0–10 cm depth, were collected on 30 April 2019 from three locations within each plot, composited and placed on ice. Soil samples used for measurement of physical and chemical properties were stored at −20 °C and subsamples used for DNA analysis were stored at −80 °C (in 50 mL tubes). Detailed protocols were described in one of our previous studies [28]. Particle size [38], total C and N [39], soil pH [40], water-stable aggregates (WSA) [41], easily extractable glomalin-related soil proteins (EE-GRSP) [42], and permanganate oxidizable C (POXC) [43] were measured on air-dried samples, which were ground to pass a 2-mm sieve.

2.3. DNA Extraction and Amplicon Sequencing

Total soil DNA was extracted from 400 mg of soil using the DNeasy PowerSoil® Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol with minor modification in the lysis and elution steps. The extracted genomic DNA concentration and purity were determined using the Nanodrop® ND-1000 spectrophotometer and quality was assessed by agarose gel electrophoresis. The extracted DNA was submitted for sequencing to the Novogene, advancing genomic services (Sacramento, CA, USA), who amplified the bacterial and fungal community composition using 16S rRNA and ITS2, respectively. The amplification of the V4 hypervariable region of the bacterial 16S rRNA gene was carried out using the 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) primers [44]. The primer set ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) was used to amplify the fungal ITS2 region [45]. Pooled amplicon libraries were sequenced on the Illumina MiSeq platform (250-bp paired-end sequencing).

2.4. Sequence Processing and Data Analysis

Amplicon sequencing data were processed and analyzed using the QIIME 2 (version 2021.11) (Quantitative Insights Into Microbial Ecology) software [46]. Raw sequences were demultiplexed and allied metadata were imported into QIIME via q2-import. Quality filtration of sequences was done using the q2-demux plugin followed by denoising with DADA2. Further, Amplicon Sequence Variants (ASV's) were generated using the DADA2 version (2021.11.0) [47] and each ASV was taxonomically annotated using the q2-feature-classifier, with SILVA SSU rel. 132 database [48], trimmed for theV4 region for bacterial community analysis and untrimmed UNITE database v7.2 dynamic for fungal community analysis.

Statistical analysis was performed using the MicrobiomeAnalyst pipeline (https://www.microbiomeanalyst.ca/ (accessed on 21 February 2022)) [49,50]. Taxonomic diversity profiling of bacterial (16S rRNA) and fungal (ITS2) marker genes was carried out using the Marker Data Profiling module of MicrobiomeAnalyst. First, BIOM file was uploaded to the Marker Data Profiling, and data filtering was performed using the set default parameters by applying the mean abundance value and standard deviation. Data was rarefied to the minimum library size with total sum scaling (TSS), but not transformed. Alpha diversity was derived from the Chao1 (richness) and Shannon index (richness and evenness). The significance between the indices was calculated using a t-test. Beta diversity was plotted as Principal Coordinate Analysis (PCoA), using the Bray-Curtis distance matrix; the significance test was achieved by Permutational ANOVA (PERMANOVA).

Canonical Correspondence analysis was performed to examine the relationship between microbial communities (bacteria and fungus) and soil physicochemical properties (pH, total C and N, WSA, EE-GRSP, and POXC) using the PAST software (version 4.08). The significant effects of soil characteristics on bacterial and fungal communities were determined by Mantle test using PC-ORD software (version 6.22; MJM Software, Gleneden Beach, OR, USA).

3. Results and Discussion

The study aimed to inspect the effect of cover crops and fertilizer source treatment on soil microbial community composition and soil characteristics of dryland soybean production system in the LMRAV.

3.1. Alpha Diversity of Microbial Communities

Using Illumina MiSeq sequencing, a total of 1,307,969 16S and 1,469,437 ITS, high-quality sequences were obtained from 24 soil samples. Analyses of the high-quality sequences revealed the presence of 27,930 and 4898, bacterial and fungal OTUs, respectively.

3.1.1. Impact of Cover Crops and Fertilizer Treatments on Bacterial Diversity

Shannon diversity index and richness (Chao1) showed a significant difference ($p < 0.05$) for the cover crop and fertilizer treatments, respectively (Figure 1) in the soils sampled after 20 days of cover crop termination. Higher alpha diversity was observed in cereal rye and native vegetation (Figure 1a,b) than other cover crops, whereas inorganic fertilizer had significantly lower alpha-diversity (Shannon diversity index, $p = 0.002$) and richness (Chao1, $p = 0.012$) than poultry litter treatment (Figure 1c,d). The response of microbial communities to soil conservation practices differs and is complex [51], as they are sensitive to differential soil properties, moisture, temperature, nutrient variability, and climate variability. Poultry litter showed significantly higher bacterial diversity and species richness than inorganic fertilizer. However, bacterial Shannon diversity of wheat was lower compared to other cover crops. This indicates the early phase of cover crop decomposition at the time of soil sampling. Similar greater microbial diversity for the nutrient-rich bio-covers with poultry litter was observed in the previous study [34]. Thus, interactive factors of cover crop residue decomposition and nutrient enrichment under no-tillage aids in attaining higher bacterial diversity [24], however, it mainly depends on the soil moisture and the temperature [52]. Additionally, we assumed that the previous year's effect of soil management practices

may have contributed to increased bacterial diversity for the poultry litter and cover crop treatments. Thus, soil conservation practices and characteristics play a major role in the variability of microbial communities [53–56].

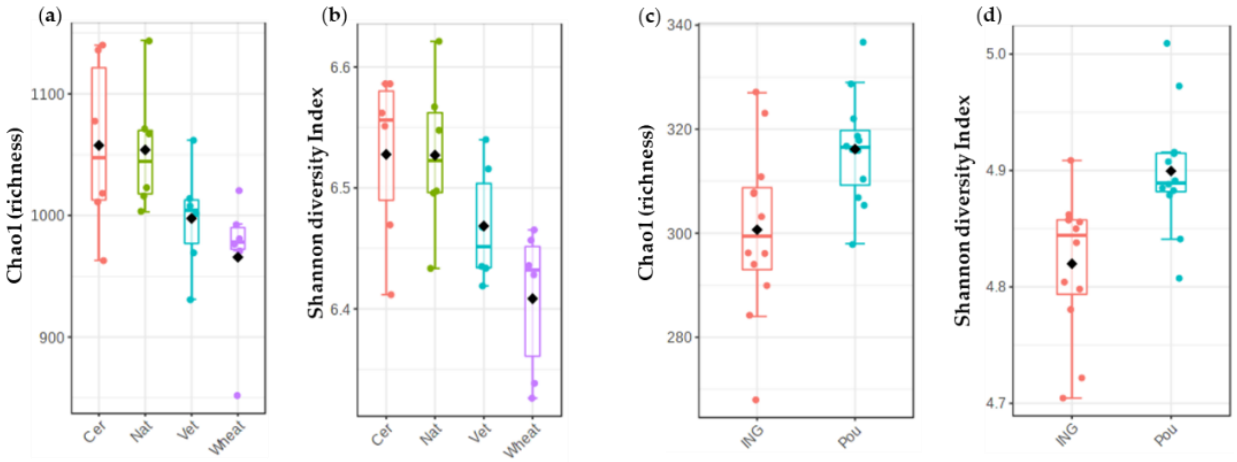

Figure 1. Impact of cover crops ((**a**), Chao1 and (**b**), Shannon diversity index) and fertilizer treatments ((**c**), Chao1 and (**d**), Shannon diversity index) on bacterial alpha diversity indices in soils sampled after 20 days of cover crop termination. (Cer-Cereal Rye; Nat-Native Vegetation; Vet-Vetch and Wheat; ING-Inorganic fertilizer; Pou-Poultry). Significance level at $p < 0.05$.

3.1.2. Impact of Cover Crops and Fertilizer Treatments on Fungal Diversity

Cover crops exhibited the significant difference ($p = 0.012$) for the fungal alpha-diversity (richness), but the Shannon diversity index was not affected by the cover crop treatment (Figure 2a). Wheat showed higher richness level compared to the other cover crops. However, there was a marginal difference between the cover crops. This explains that cover crop species, function or diversity is not the major factor that affects the diversity of fungal communities. Yet, some fungal communities at the genus level are influenced by the sampling season and time [57]. Higher fungal diversity was observed for the inorganic fertilizer compared to the poultry litter treatment (Figure 2b). Constantly, Fungal communities are greatly influenced by microhabitats. A recent study by Delgado-Baquerizo et al. [58] documented that soil fungal diversity increases with the soil fertility level. The Shannon diversity index showed significant differences ($p = 0.030$) for the fertilizer treatment (Figure 2b). Similarly, fertility treatments showed significantly higher Shannon diversity index compared to cover crops treatment. Surprisingly, in the current study, poultry litter showed lower alpha diversity index. On the contrary, many studies have shown an increase in the soil bacterial and fungal communities. In the research by Celestina et al. [59], the addition of poultry litter to soil reduced the diversity, richness, and evenness of microbial communities in the different layers of soil. However, there is a solid association between soil fertility and fungal biodiversity, which may be deteriorated by weather factors, soil moisture, or possibly the effect of the poultry litter at the time of sampling no longer being active. Fertilizer sources did not influence the species richness, this may be due to lesser ASV numbers, spatial factors, and differential soil properties influencing the fungal community structure.

3.2. Beta Diversity of Microbial Communities

The bacterial community compositions were significantly different ($p < 0.013$) for the fertilizer sources, which accounts for 34.9% of the variation (Figure 3a), but there were no significant differences observed for cover crops treatment (data not shown). Thus, fertilizer source impacts the bacterial community structure. It is evident that [60] fertilization source (organic and poultry litter) influences the soil microbial community structure. Hence, it

represents a potential source of beneficial bacterial taxa for the decomposition and carbon fixation, ultimately improving soil health.

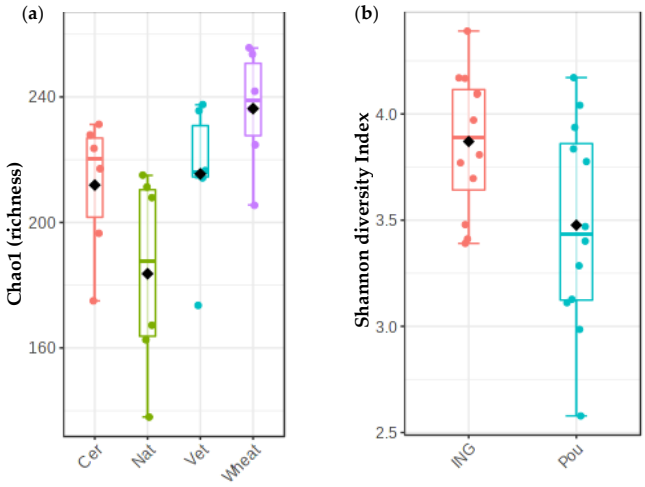

Figure 2. Fungal alpha diversity indices. Impact of cover crops and fertilizer treatment on (**a**) species richness (Chao1 index) and (**b**) Shannon diversity index, respectively in soils sampled after 20 days of cover crop termination. (Cer-Cereal Rye; Nat-Native Vegetation; Vet-Vetch and Wheat; ING-Inorganic fertilizer; Pou-Poultry). Significance level at $p < 0.05$.

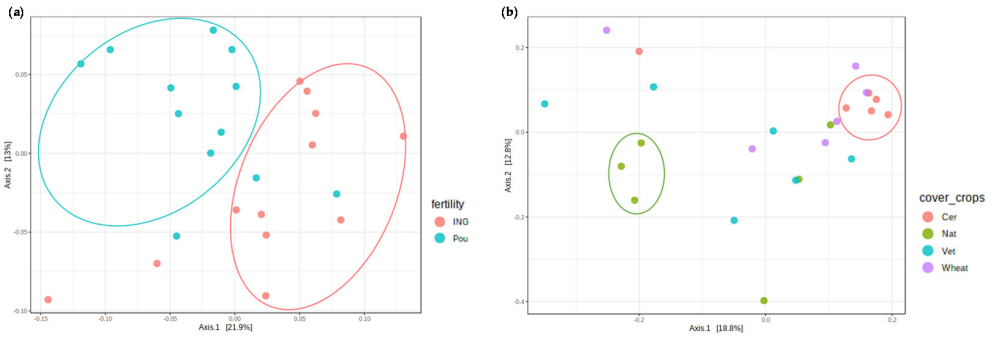

Figure 3. Effect of fertilizer and cover crop treatments on soil (**a**) bacterial and (**b**) fungal communities, respectively using Principal coordinate analysis (PCoA), represented by the beta diversity metric-Bray Curtis dissimilarity matrix. Significant influence of treatments at $p < 0.05$.

PERMONOVA revealed significant differences of fungal communities (p-value < 0.014) with a distinct pattern for the cereal rye and native vegetation, with 31.6% of total variation (Figure 3b). Similarly, in the findings of Longley et al. [61] fungal communities differed strongly for the soil management practices like no-till, cover crops, as well as organic and conventional farming systems. However, there was no clear pattern of fungal communities for other cover crops observed. Fertilizer sources showed no significant differences for fungal communities (data not shown). On the contrary, many studies have reported the positive effect of fertilizer sources in relation to microbial diversity and community structure [62–64]. Yet, fertilizer sources in combination with reduced tillage and cover crops greatly benefit the soil by increasing the nutrient content and microbial diversity.

3.3. Impact of Management Practices on the Bacterial Community Abundance

Microbial relative abundance analyses are effective in revealing taxonomic changes under the differential sample environment. In the current study, the relative proportion of bacteria was analyzed at the phylum level. Twenty-one phyla were identified across the sample set and the top 10 dominant bacterial phyla were, Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Verrucomicrobia, Gemmatimonadetes, Chloroflexi,

Bacteroidetes, Crenarchaeota, and Planctomycetes. Proteobacteria was the most abundant phylum and the relative abundance ranged from 38–39% for both cover crop and fertilizer treatments (Figure 4a,b). Our results are consistent with previous studies, where the abundance of Proteobacteria in farmland and tallgrass prairie soil was reported [65–67]. Proteobacteria phyla are distributed over different soil layers and are involved in the biogeocycling of the important soil nutrients (carbon, nitrogen, and sulfur). After Proteobacteria, the phylum Actinobacteria displayed higher abundance (23%) for both the treatments, which are recognized for their action of disease suppression, growth promotion, and plant health by the production of secondary metabolites [68,69]. Acidobacteria were relatively abundant compared to the remaining top 10 bacterial phylum, while these three bacterial phyla are common in farmland soils globally [37]. Higher abundance of Proteobacteria in the soil samples may aid in the availability of soil organic matter to soybean plants, which improves plant growth and development. Further, classical univariate statistical comparisons analysis was carried out to study how the top taxa differ for the treatments. However, similar relative abundance of bacterial phylum across the treatments was observed. The phylum Bacteroidetes ($p = 0.027$) and Cyanobacteria ($p = 0.005$) showed significant difference for the fertilizer and cover crop treatments, respectively (Figure 4c,d). In the study, Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes were the most prevalent phyla for both the treatments, which could be found in the organic matter abundant soil. This explains the decomposition of the cover crops at the sampling time.

Figure 4. *Cont.*

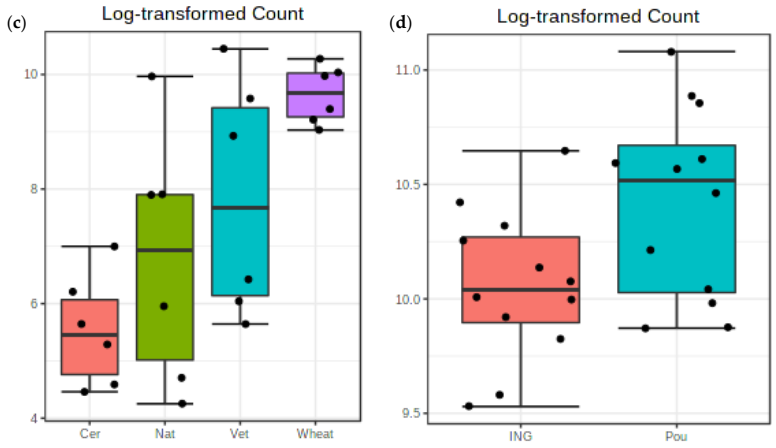

Figure 4. Differential bacterial community profiles at the phylum level. Relative abundance of bacterial communities across the (**a**) cover crops and (**b**) fertilizer treatments. Univariate statistical comparisons analysis of (**c**) *Cyanobacteria* abundance between cover crops and (**d**) *Bacteroidetes* abundance between fertilizer source treatment. Significance differences were tested using *t*-test/ANOVA (significance cutoff at $p = 0.05$).

To identify the specific functions of the bacterial groups, the relative abundance of soil bacteria at genus level was studied. The abundant genera were Pseudomonas, Rhodoplanes, DA101, Bacillus, Kaistobactor, Bradyrhizobium, Nitrososphaera, Pseudonocardia and Solibacter. The genera Rhodoplanes, Kaistobactor, and Bradyrhizobium belongs to the phylum Proteobacteria, that shows the Proteobacteria predomination in our soil samples. Among the phylum Proteobacteria, Pseudomonas (55–57%) is the dominant genus across and within the treatments (Figure S1a,b). Cover crops showed a similar level of Pseudomonas abundance, however inorganic fertilizer had higher relative abundance (57.1%) than the poultry litter (55%) treatment (Figure S1b). It has been reported that the genus Pseudomonas promotes phosphate solubilization, nitrogen fixation, induced systemic resistance, and siderophore production, thus favoring plant growth and phytopathogen control, hence acting as a bioinoculant [70–72]. The genus, Rhodoplanes depicted higher abundance in cereal rye and native vegetation (4.9%) compared to wheat and vetch (Figure S1a). In the genera DA101 (2–2.7%) and Bacillus (1.5–2.8%; belongs to phylum, Firmicutes), relatively comparable abundance level was observed (Figure S1a,b) for both cover crop and fertilizer treatments. The phylum Verrucomicrobia comprising the genus DA101, particularly promotes soil health as it plays a role in facilitating nitrogen use efficiency (available N content) in plants and promotes plant growth [73,74]. Consequently, these genera promote soil health thus showing differential and beneficial bacterial abundance across the soil management factors.

3.4. Impact of Management Practices on the Fungal Community Abundance

Fungal relative abundance obtained from the phylum level classification is depicted in Figure **??**. Soil fungal community in this study was dominated by Ascomycota, which accounts for 85–90% and 86–91% of the cover crops and fertilizer sources, respectively (Figure **??**a,b). Phylum Ascomycota are known to be dominant in dry land soils [75]. The other fungal communities are, Basidiomycota, Mortierellomycota, Chytridiomycota, Rozellomycota, Mucoromycota, and Glomeromycota. Among them, Basidiomycota and Mortierellomycota account for 5–12% of relative abundance across the treatments. The top three phyla were consistent with previous studies [75,76]. Basidiomycota mainly belongs to the saprophytic group, vital for decaying dead organic matter (dead leaves, wood). Hence, these play a substantial role in carbon cycling [77]. Cereal rye showed higher relative abundance (92%) of Ascomycota compared to other cover crops and fertilizer treatment (Figure **??**a), whereas Ascomycota abundance level (85%) was reduced in the native vegetation. Relative abundance of Basidiomycota (13%) was higher in the native

vegetation than other cover crops. For fertilizer treatment, marginal differences were observed for the relative abundance of phyla Ascomycota and Basidiomycota (Figure **??**b). These results suggest that fungal communities and abundance are strongly affected by the plant community compared to the fertilizer treatment. Classical univariate statistical comparisons analysis revealed significant difference ($p = 0.04$) of Mortierellomycota across the cover crops (Figure **??**c). N-rich cover crops stimulate the abundance of Mortierellomycota [78], which indicates the higher availability of nitrogen in the soil. Some species of Mortierellamycota phyla play a role in plant growth and disease suppression by releasing phytohormones [79–81]. These fungal taxa clearly explain the implications of soil management practices.

At the genus level, the most abundant genera were Fusarium, Plectosphaerella, Gibellulopsis, Roussoella, Epicoccum, Fusicolla, Stropharia, and Gibberella. Unclassified taxa at the genus level were highest in both the treatments (Figure S2). All the obtained genera belong to the phylum Ascomycota except Stropharia, which belongs to the phylum Basidiomycota. Fusarium was the most abundant phylum compared to others with an average relative abundance of 10–18% (Figure S2), where native vegetation showed lower abundance (10%) than the cereal rye (18.5%) and other cover crops (15%) (Figure S2a). Fusarium genus is complex and includes both pathogenic and nonpathogenic forms [82]. This result explains the cover crop decomposition, as these saprophytes are able to survive on the dead organic material for an extended period. A higher level of Plectosphaerella (15%) was observed in the poultry litter treatment than with the inorganic fertilizer (6%) and cover crops (7–13%) (Figure S2a,b). A similar result was observed in the previous study where genera belonging to the Ascomycota, including Plectosphaerella showed higher abundance in the poultry litter treatment and were beneficial for the soil [83]. The differences in the relative abundance of the genus Stropharia varied largely in both cover crops and fertilizer treatments. Inorganic fertilizer (4.7%), native vegetation (5.6%), and vetch (3.7%) showed relatively high Stropharia abundance, however poultry litter and other cover crops depicted reduced abundance level (Figure S2a,b). Stropharia are macrofungi and studies have shown that it balances soil nutrient, improves soil aeration, increases biological activity and maintains soil structure [84–87]. In the study, higher abundance of Ascomycota, Basidomycotaand Mortierellomycota can be related to the early phase of cover crop degradation, as these phyla comprise the fast-growing fungal species which are abundant in organic matter rich soils in the agriculture field [78,88]. Thus, these fungal communities help in degrading the complex carbon sources that endorse soil health which complements plant growth and productivity.

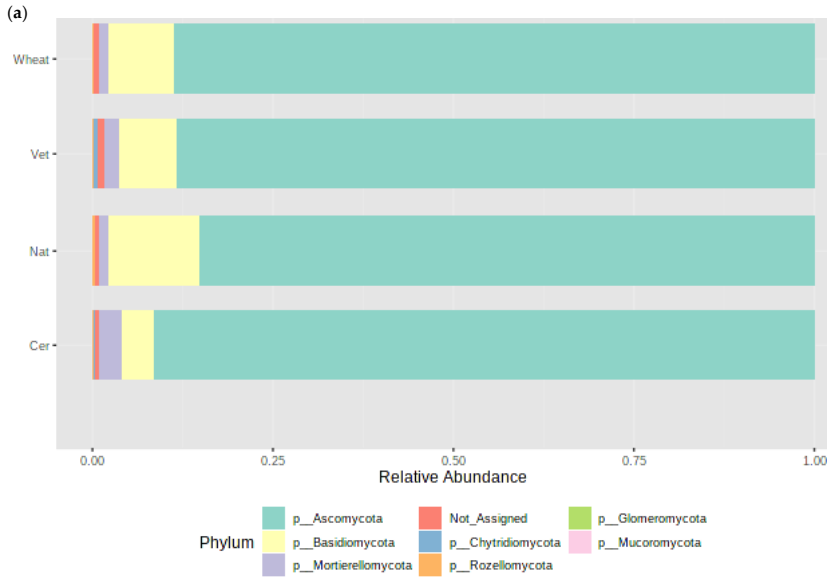

Figure 5. *Cont.*

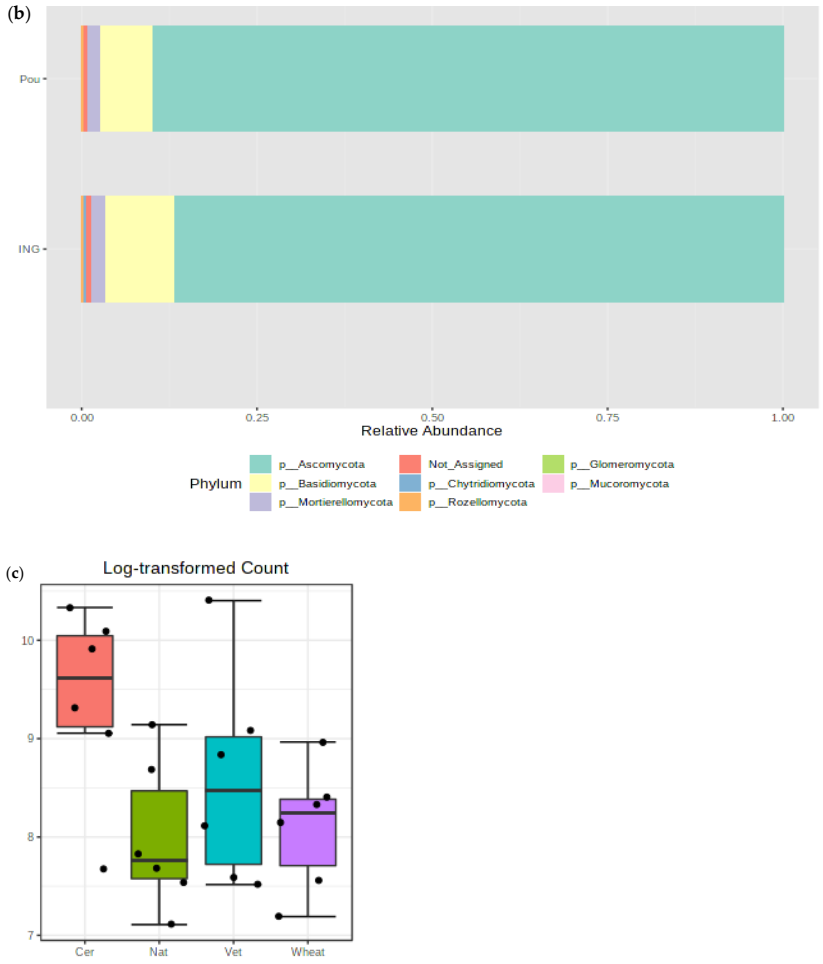

Figure 5. Differential fungal community profiles at the phylum level. Relative abundance of fungal communities across the (**a**) cover crops and (**b**) fertilizer treatments. (**c**) Univariate statistical comparisons analysis of phylum *Mortierellomycota* abundance between the cover crops. Significance differences were tested using *t*-test/ANOVA (significance cutoff at $p = 0.05$).

Further, we observed fewer fungal taxa compared to the bacterial taxa groups. Many environmental factors drive microbial diversity and taxa abundance. Any shift would create a major impact on the microbial communities. For example, in the dryland environment due to aridity, soil microbial diversity and abundance reduce, thus affecting the soil fertility. Maestre et al. [75] found that the relative abundance of Chloroflexi and α-Proteobacteria increases in the dryland atmospheric condition, while at the same time decreasing the Acidobacteria and Verrucomicrobia. Additionally, we assume that chemical termination of cover crops might have an influence on the marked reduction in fungal taxa.

3.5. Changes in Soil Physicochemical Properties

Soil management practices, reduced tillage, mulching, and organic manures influence soil properties, which play a role in shaping the composition of soil bacterial and fungal communities [89–91]. In the study, after 3 years of continuous no-tillage, cover cropping and poultry litter treatment, a decrease in soil pH from 6.67 to ~5.6 over the years, was observed. It is known from studies that, long-term use of the fertilizers (urea and ammonia) leads to decrease in soil pH [92,93]. Many studies have revealed that neutral soil pH is the major factor for predicting the microbial community structure. It increases the soil microbial community composition and taxonomic resolution, relatively [94–97]. However, no significant changes in the soil characteristics were noticed across the treatments (Table 1).

Table 1. Soil physical and chemical properties, which were collected after 20 days of cover crop termination at a depth of 0–10 cm for all the treatments (cover crop + fertilizer). (no significant differences were observed).

Fertilizer Source	Cover Crops	pH	Total C (%)	Total N (%)	WSA%	EE-GRSP (mg g^{-1})	POXC (mg kg^{-1})	Clay (%)	Sand (%)	Silt (%)
Poultry Litter	Wheat	5.8 ± 0.1	1.6 ± 0.3	0.2 ± 0	52.0 ± 7.2	86.5 ± 11.4	509.7 ± 126.7	14.2 ± 0.6	17.9 ± 1.9	67.9 ± 1.4
	Vetch	5.6 ± 0.5	1.9 ± 0.3	0.2 ± 0	56.0 ± 7	88.8 ± 15.1	579.4 ± 117.2	14.9 ± 1.7	17.4 ± 1	67.7 ± 1.5
	Cereal Rye	5.7 ± 0.2	1.8 ± 0.3	0.2 ± 0	54.4 ± 3.7	87.7 ± 4.7	543.6 ± 60.7	13.5 ± 1.5	17.8 ± 1.4	68.6 ± 1.8
	Native veg.	5.8 ± 0.1	1.6 ± 0.2	0.2 ± 0	60.4 ± 3.8	86.1 ± 1.9	527.1 ± 6.3	14.2 ± 0.6	16.9 ± 3.7	68.9 ± 4.3
Inorganic Fertilizer	Wheat	5.8 ± 0.2	1.5 ± 0.2	0.1 ± 0	47.1 ± 5.4	73.5 ± 15.6	509.7 ± 72.2	13.9 ± 1.0	17.6 ± 0.9	68.5 ± 0.9
	Vetch	5.4 ± 0.2	1.7 ± 0.3	0.2 ± 0	57.1 ± 7.7	80.1 ± 8.3	573.2 ± 112.5	15.2 ± 1.2	17.8 ± 1.6	67.0 ± 1.6
	Cereal Rye	5.8 ± 0.2	1.7 ± 0.2	0.2 ± 0	48.0 ± 12.7	78.3 ± 7.9	563.5 ± 80.3	14.5 ± 0.6	15.9 ± 0.5	69.5 ± 0.9
	Native veg.	5.6 ± 0.1	1.5 ± 0.4	0.2 ± 0	50.1 ± 11.2	90.6 ± 8.5	502.8 ± 103.4	13.5 ± 1.5	16.5 ± 2.4	70.0 ± 3.9

Abbreviations: C = total carbon; N = total nitrogen; WSA = water stable aggregate; EE-GRSP = easily extractable-glomalin-related soil protein; POXC = permanganate oxidizable carbon.

3.6. Association of Soil Physicochemical Properties with the Bacterial and Fungal Communities

A distance-based canonical correspondence analysis (CCA) was performed to evaluate the effect of soil properties on the bacterial communities and ASVs (300) were used for the analysis. Samples were clustered for the inorganic fertilizer treatment. The first two axes, 38.62% and 32.69% respectively, showed a strong correlation between the soil pH and bacterial communities (Figure 6a). However, CCA results did not group the samples according to poultry litter treatment, therefore no association of poultry litter on the soil properties was observed. As, implications of nutrient treatments are spatially limited, this could be due to soil moisture level, sampling time, and may be the slow degradation of the poultry litter compared to inorganic fertilizer. For cover crops, cereal rye showed association with the soil pH (Figure 6b). Further, Mantle test results exhibited that the soil pH and EE-GRSP were significantly correlated ($p < 0.001$) with bacterial communities (Table 2). Several reasons and mechanisms contribute to this relationship of pH and bacterial community composition as pH levels may create stress on the bacterial cell walls, which influence the differential bacterial community composition and abundance of diverse bacteria [98]. In addition, continental scale pH can be a good predictor of soil bacterial community composition [97,99,100]. Fierer and Jackson [99] also showed that neutral pH (pH = 6.8) had a strong correlation with bacterial OTU richness compared to acidic pH (pH = 5.1). Glomalin enhances soil aggregation thus protecting the rapid degradation of carbonaceous material from the soil [101,102]. There was a positive relationship for WSA and fungal communities were observed for the poultry litter (Figure 7).

Cover crops did not show a significant relationship between the soil properties and fungal communities. WSA is a measure of soil structure and is based on the soil resistance to dispersion and compaction [27]. In the study poultry litter is associated with increasing the WSA in soils and maintaining soil aggregate stability, which can be related to preserving the soil organic matter. Further, organic fertilization (compost and poultry manure) in combination with reduced tillage and the use of cover crops as mulch (RTOF) promote higher soil aggregation and nutrient availability [60]. Therefore, these results depict that soil pH and aggregate stability play a key role in determining the microbial community composition and abundance, thus maintaining the soil structure and arrangement.

Table 2. Correlation analysis between soil characteristics and bacterial communities (significance level at $p = 0.05$) using the Mantle test.

Mantle Test	Bacterial Communities					
	pH	C	N	WSA	EE-GRSP	POXC
R	0.654	−0.120	−0.158	−0.01	0.362	−0.067
p-Value	<0.001	0.287	0.178	0.931	<0.001	0.609

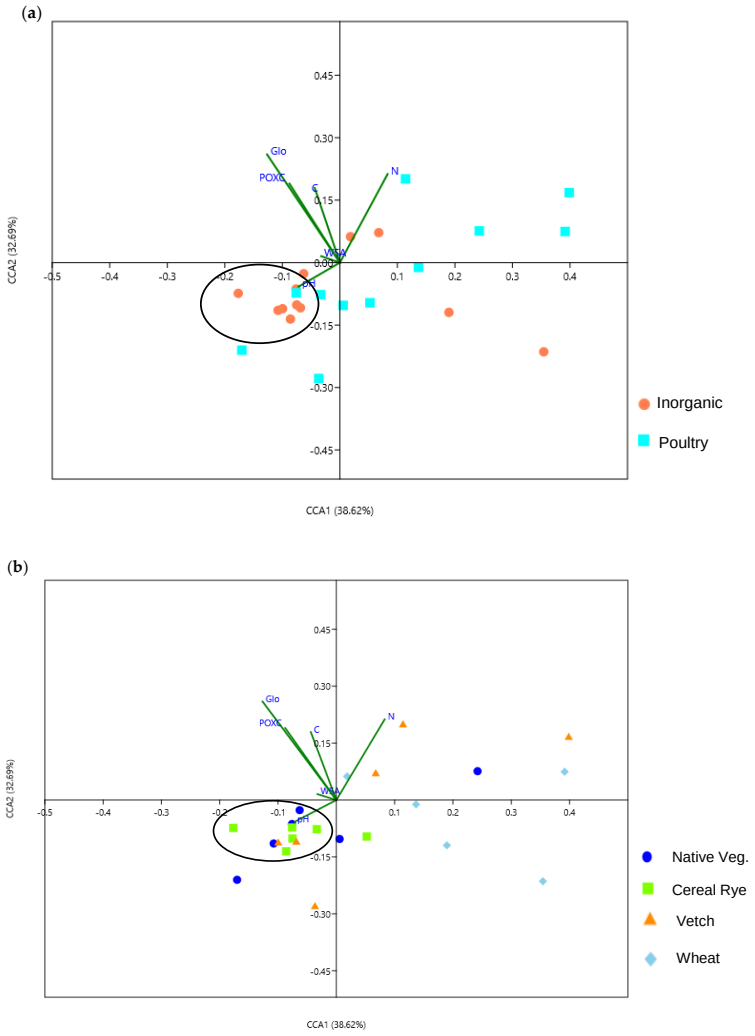

Figure 6. Effect of soil properties on bacterial communities evaluated using distance-based canonical correspondence analysis (CCA). Influence of soil factors for the (**a**) fertilizer and (**b**) cover crop treatments.

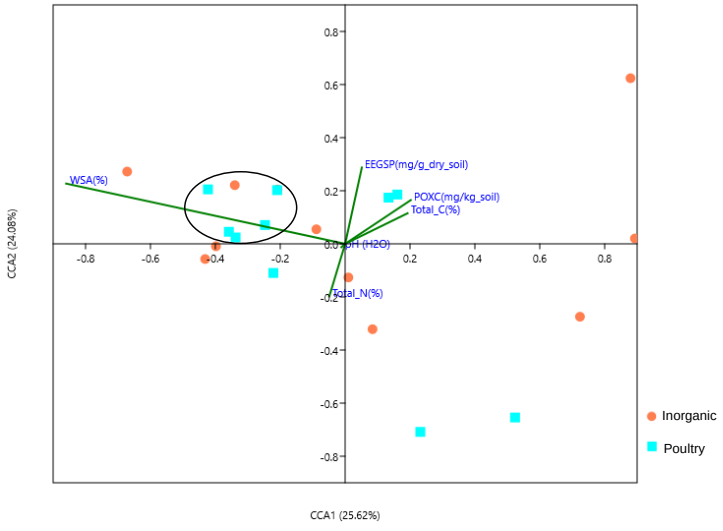

Figure 7. Influence of soil properties on fungal communities for the fertilizer treatment evaluated using distance-based canonical correspondence analysis (CCA).

To understand the relationship between bacterial/fungal abundance and soil characteristics, which are managed under cover crops and fertilizer treatment, CCA was performed. This revealed that most of the soil characteristics were identified as significant contributors to soil bacterial and fungal abundance across the samples (Figure 8a,b). The abundance of Proteobacteria and Actinobacteria was explained by POXC, WSA, C content, and total N to a lesser extent. Many studies have proved a significantly positive relationship between POXC and microbial biomass [43,103], and soil organic carbon content [104]. Thus, POXC is the suggested method for C food sources of microbes. Additionally, no-tillage also increases the soil active C content. This explains the higher abundance of the carbon decomposing bacteria, Proteobacteria and Actinobacteria in the soil samples and it is reflected by the influence of soil C content and decomposition of cover crops during the sampling, while Chloroflexi and Bacteriodetes were influenced by soil pH. For instance, at continental scale, bacterial community composition is chiefly driven by the relative abundance of Acidobacteria, Actinobacteria, and Bacteriodetes, that are influenced by the different range of soil pH. However, Proteobacteria (Alpha- and Beta/GammaProteobacteria) abundance showed no relationship with the soil pH [97]. In the fungal study, Ascomycota abundance was influenced by the soil pH and Mortierellomycota was influenced by the significant increase in the POXC, nitrogen, and carbon content of the soil (Figure 8b). The study supports the idea that soil is rich in nitrogen, as Mortierellomycota generally grows on nitrogen-rich organic materials [78]. However, the initial decomposition of carbon-rich materials causes immobilization of nitrogen, as it operates in the reverse direction as metabolism of suitable C substrates causes transformation of inorganic N to organic constituents [105]. Hence, these findings strongly suggest that spatial and temporal factors and soil properties are the major drivers of microbial diversity and community structure.

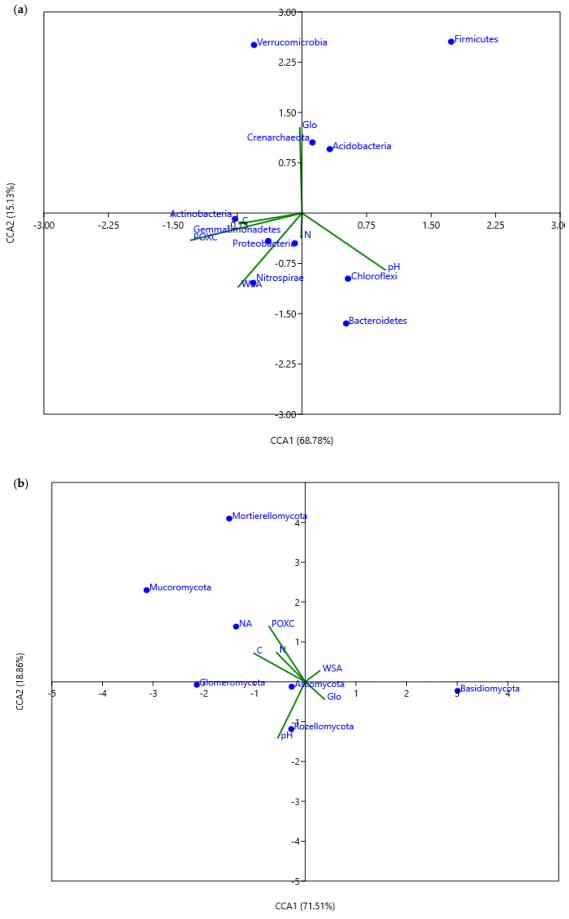

Figure 8. Effect of soil characteristics on the top 10 (**a**) bacterial and (**b**) fungal taxa abundance, evaluated using distance-based canonical correspondence analysis (CCA).

4. Conclusions

Microbial diversity and abundance are critical for maintaining the soil environment and health. In the study, the combination of cover crops and poultry litter application improved the bacterial diversity but enhanced fungal diversity was observed in the inorganic fertilizer treatment. Fertilizer treatments recorded significant impacts on the bacterial community structure. On the other hand, the fungal community structure was influenced by the cover crops, unlike the fertilizer treatment. Yet we observed similar abundance of beneficial bacterial and fungal phyla which play a major role in organic matter decomposition and nutrient cycling. Our study showed that poultry litter in combination with cover crops would promote soil health in this production system, in the long run. In addition, the study delivers an approach to use poultry litter treatment in the dryland environment for enriching soil microbes (bacteria), which is crucial for improving soil properties, soil health and ultimately encourages the plant growth and productivity of soybean plants. Though, no significant differences were observed for soil characteristics, bacterial and fungal communities depended more strongly on the fertilizer treatment and soil properties (pH, EE-GRSP, and WSA) compared to cover crops treatment. It is evident from the study that soil microbial communities exhibit temporal and spatial variability. Therefore, this research indicates that studying the long-term impact of soil management practices can give further insight on the impacts of cover crops and the combination of cover crops and fertilizer treatments on soil bacterial and fungal communities in dryland soybean production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12030618/s1, Figure S1: Stacked histograms showing the relative abundance of major bacteria in (a) cover crops and (b) fertilizer treatments at the genus level. (Cer-Cereal Rye; Nat-Native Vegetation; Vet-Vetch and Wheat; ING-Inorganic fertilizer; Pou-Poultry); Figure S2: Stacked histograms showing the relative abundance of major fungi in (**a**) cover crops and (**b**) fertilizer treatments at the genus level. (Cer-Cereal Rye; Nat-Native Vegetation; Vet-Vetch and Wheat; ING-Inorganic fertilizer; Pou-Poultry).

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