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Post-termination Effects of Cover Crop Monocultures and Mixtures on Soil Inorganic Nitrogen and Microbial Communities on Two Organic Farms in Illinois

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Cover crops can continue to affect agricultural systems even after they have been terminated by influencing nitrogen dynamics and by altering soil microbial communities. These post-termination effects can influence soil fertility, weed pressure, and the dynamics of potential plant pathogens in the narrow window of time between cover crop termination and cash crop emergence. We evaluated the post-termination effects of 12 different spring-sown cover crop mixtures and monocultures on soil nitrogen and microbial communities on two different organic farms in Central Illinois (on Lawson silt loam soil) and Northern Illinois (on Virgil silt loam soil). In comparison to control plots with no cover crops, all cover crop treatments significantly reduced soil nitrate levels but increased the potentially mineralizable nitrogen pool following termination. Nitrate levels of cover crop plots approached those of controls after 2 and 4 weeks, respectively, but potentially mineralizable nitrogen levels in cover plots remained elevated for at least 4 weeks following termination. Monocultures of Brassica cover crops showed the greatest decrease in soil nitrate, while Brassicas and unplanted control plots containing high biomass of weeds showed the greatest increase in potentially mineralizable nitrogen in comparison to plant-free control plots. In contrast to their effect on soil nitrogen, cover crops had very limited impact on the composition of soil microbial communities. Overall microbial community composition varied across sites and years, and only soil fungi significantly responded to cover cropping treatments. Nevertheless, we found that some highly correlated groups of soil microbes showed significant responses to soil nitrate and to high plant biomass. Key members of these correlated groups included ammonia-oxidizing organisms and saprotrophic fungi. Our results suggest that cover crops may reduce the potential for springtime nitrogen leaching losses by retaining nitrogen in the soil organic pool, and they may also have impacts on the soil microbial community that are particularly relevant for nitrogen cycling and decomposition of plant residues.

Keywords: cover crops, nitrogen, microbial community, organic agriculture, early season pulse, nitrate, organic N, agricultural management

INTRODUCTION

Cover crops are important tools employed in organic agriculture to improve soil quality and fertility (1–4). Living cover crops can prevent soil erosion during fallow periods and compete directly with weeds for sunlight and nutrients (5, 6). However, cover crops can continue to effect the soil system after they have been terminated, and these impacts can persist for hours, days, or weeks (7). For example, red clover cover crop residues suppressed weed seed germination for 30 days after termination through a combination of allelochemical release and stimulation of weed-suppressive microbial activity (8). These post-termination effects of cover crops can influence the soil microbial environment by altering nutrient pools, rates of residue decomposition, and relative abundance of plant mutualists and pathogens (9–18). Even if post-termination cover crop effects are short-lived, they can have important impacts on agriculture because they occur in a critical window of time when agroecosystems are subject to pressure from early season weeds and increased risk of soil nitrogen leaching losses (19, 20).

Living cover crops, their decaying "green manure" residues, and the soil microbial community can all influence the amount, timing, and the form of plant-available nitrogen (1, 21). These effects can vary widely across different combinations of plant and microbial species. Winter and spring cover crops can be used to take up excess or residual nitrates in the soil over the fallow season (22), reducing nitrate leaching and deprive early season weeds of nitrogen (5, 6). Grasses like oat (*Avena sativa*) and spring wheat (*Triticum aestivum*) are particularly good at this (2, 22–25). Legume species such as field pea (*Pisum sativum*) and fava bean (*Vicia faba*) can provide significant nitrogen contributions to agricultural systems via nitrogen fixation (26, 27). Plants of the family *Brassicaceae* can rapidly accumulate biomass to choke out weeds and sequester nitrogen (28), but they also produce allelopathic chemicals that can reduce plant growth and microbial activity (29, 30).

Upon termination, cover crop residues release nitrogen back to the soil, where the processes of decomposition, nitrogen mineralization, and nitrogen immobilization are governed by soil microorganisms interacting with plant tissues of varying qualities and composition. Soil microorganisms have a threshold carbon to nitrogen ratio (C:N) of 26:1. Plants with lower C:N ratios result in net nitrogen mineralization, while residues with higher C:N result in net nitrogen immobilization (31, 32). Legumes, which have low C:N ratios of 10–15 (33, 34), decompose rapidly as organic nitrogen is mineralized into plant-available forms (nitrate and ammonium) once microbial nitrogen demand is satisfied (35, 36). Grasses have high C:N ratios, ranging from 33 to 94 for oat and wheat, respectively (13, 31), and this can result in slower residue decomposition and net immobilization of nitrogen (37). The combination of high grass biomass and high C:N ratio results in less inorganic nitrogen made available for the crops that follow. Though there is variation among Brassicas, they vary between 10 and 31 C:N ratios of their plant tissues (38–40), so they are generally below the 26:1 microbial threshold. However, Brassica allelopathic secondary metabolites can suppress microbial decomposition and nitrogen mineralization (6, 21, 41, 42), which can result in slower overall conversion of organic nitrogen to plant-available forms (28).

In addition to their effect on soil nitrogen pools, cover crops can also affect soil-borne pathogen prevalence and promote plant-beneficial microbes. Cover cropping with canola (*Brassica napus* L.) was shown to reduce the incidence of disease caused by *Rhizoctonia solani* in potato (10) and apple (43). Many beneficial, pathogen-antagonistic, soil bacteria and fungi have also been identified to respond positively cover cropping (10, 15, 16). Wheat has also been found to enrich fungal diversity and reduce pathogen populations compared to oat (44). In general, more diverse microbial communities have been shown to experience a greater degree of resilience and are better equipped to suppress potentially pathogenic taxa (16, 45–47). However, fungi tend to cause more damage to agricultural crops than bacteria (46), so is not always the case that increased fungal diversity is a net benefit for crops. In order to fully understand how cover crops influence the soil in ways that can promote or hinder future crop growth, we need to identify specific microbial taxa that respond to cover cropping with different plant species.

Legumes, grasses, and simple mixtures of the two are most commonly used cover crops (5, 6, 48). Mustards and other plants from the *Brassicaceae* family can be used as short-season cover crops in the cooler climates of the upper Midwest (49), and they are sometimes included with other cover crop species as part of diverse mixtures (50). While there is considerable research showing that cover crops shape the soil microbial community (5, 6, 10, 15, 16), it is not well-understood if more diverse mixtures of grass, legume, and Brassica species yield increased benefits to soil quality and microbiology that may, in turn, improve subsequent crop growth. Planting diverse cover crop mixtures may allow us to take advantage of the myriad effects of different plant types on soil fertility and microbiology, and there is a growing popularity in the use of diverse cover crop mixtures, or "cocktails," in the organic farming community (3, 5, 24, 51–54). In this study, we aimed to investigate whether different multi-species cover crop mixtures had differential effects on soil nitrogen pools and soil microbial composition in the weeks following cover crop termination. By looking at six species of cover crops grown in monocultures and diverse, five-species mixtures, we sought to answer the following questions: (1) do the dynamics of soil nitrogen pools (nitrate, ammonium, and potentially mineralizable nitrogen) vary among different cover crop combinations; (2) how do soil microbial communities change as a result of cover cropping with different plant types (grasses, weeds, Brassicas, legumes, or mixtures); and (3) what are the most important drivers in determining the dynamics of microbial communities following cover crop termination?

MATERIALS AND METHODS

Field Study Design and Sample Collection

Our research took place as part of the same field experiment that has been previously described by Holmes and colleagues (55). Two organic vegetable farms participated in the experiment in 2015 and 2016: PrairiErth Farm in Atlanta, IL (40°13'N 89°13'W) and Kinnikinnick Farm in Caledonia, IL (42°27'N 88°52'W).

The soil type at PrairiErth farm was Lawson silt loam (fine-silty, mixed, superactive, mesic Aquic Cumulic Hapludoll), and the dominant soil type at Kinnikinnick farm was Virgil silt loam (fine-silty, mixed, superactive, mesic Udollic Endoaqualf). Cropping history at both sites was highly varied, including both vegetable and grain crops. Both farms were certified organic under the United States Department of Agriculture National Organic Program guidelines; Kinnikinnick Farm since 1994 and PrariErth Farm since 2004.

Spring-sown cover crops were planted in a randomized complete block design with four replicates of 12 treatments and two controls. Blocks were 4 m by 56 m in size, with each block accommodating fourteen 4 m by 4 m plots for the treatments (twelve cover crop plantings and two controls). Six cover crops were included in the study: two grasses (oat, *Avena sativa*, and spring wheat, *Triticum aestivum*), two legumes (field pea, *Pisum sativum*, and fava bean, *Vicia faba*) and two Brassicas (Yellow mustard, *Sinapis alba*, and purple top turnip, *Brassica campestris*). A "weedy" control treatment was included that received no cover crop seed but allowed volunteer weed growth, and the experiment also included a plant-free control maintained by hand-pulling. Cover crops were planted in monocultures and all possible five-species mixtures for a total of six monocultures and six mixture treatments with two controls. For subsequent analyses, the 14 cover crops will be referred to as "cover crop treatments." Cover crop diversity refers to whether the treatment was a mixture, monoculture, or control. Seeding application rates were as described by Holmes et al. (55). Cover crops were planted in early (PrariErth) or late (Kinnikinnick) April by hand-broadcasting, and seeds were lightly incorporated using gravel rakes and drag harrows. Cover crops grew for ∼2 months before termination by mowing and rotavation to a depth of 15 cm.

Aboveground cover crop biomass was measured from two randomly-tossed quadrats (45.7 cm by 61 cm) immediately before termination, as previously described (55). Weeds, which were treated as a single "species," were separated from cover crops and weighed separately. Dry weights were calculated for each cover crop species and used for subsequent analyses.

We sought to investigate the short-term impacts of cover crops in the period between termination and when typical cash crops would emerge. We collected soil samples from plots for three time points after cover crop termination: within 1 week (immediate effects), after 1 or 2 weeks (medium-term effects), and after 4 weeks (at typical crop emergence). Precise sampling dates varied for each site-year, depending on weather and soil conditions. In 2015, soils from each plot were collected at 3, 7, and 34 days post-termination at PrariErth and 6, 18, and 32 days post-termination at Kinnikinnick. In 2016, samples were collected 3, 17, and 33 days post-termination at PrairiErth and 5, 14, and 34 days post-termination at Kinnikinnick. From each plot, we collected 16 randomly-spaced soil cores down to depth of 10 cm, and we combined these cores to obtain a single composite sample for each plot. For microbial community composition (see below) a subsample of ∼20 g was collected immediately from each composite sample, and then frozen at −20°C and freeze-dried for DNA extraction. Approximately 50 g of the remaining soil was air-dried for subsequent nitrogen content analysis.

Soil Inorganic Nitrogen Analyses

Soil inorganic nitrogen content was assessed using standard methods for plant-available, exchangeable ammonium and nitrate through KCl-extraction (56) followed by colorimetric quantification of nitrate and ammonium. For each sample, two subsamples of 10 ± 0.05 g were weighed into 50 mL centrifuge tubes. One subsample was incubated anaerobically to quantify potentially mineralizable organic nitrogen (see below), while the other was processed immediately for inorganic nitrogen content.

For inorganic nitrogen extraction, 40 mL 1 M KCl was added and samples were shaken at approximately 240 rotations per minute at room temperature for 50 min. Nitrate and ammonium contents were quantified by colorimetric reactions. Nitrate analysis followed Doane and Horwath (57): a solution of sulfanilamide and N-(1-napththyl)-ethylenedaimine dihydrochloride in saturated vanadium (III) chloride was combined with each KCl extract and incubated in the dark for 4 h. Ammonium analysis followed Weatherburn (58): a solution of sodium salicylate, sodium citrate, sodium tartrate and sodium nitroprusside was combined with each KCl extract and with a 2% bleach:1.5 M sodium hydroxide solution and incubated for 50 min at room temperature. Absorbance values were measured at 540 nm for nitrate and 650 nm for ammonium to colorimetrically quantify nitrogen concentration using Epoch Biotek plate reader spectrophotometer and Gen5 software. Standard curves of known concentrations of KNO_3 and $(NH_4)_2SO_4$ were used to measure nitrate and ammonium concentrations, respectively. For all nitrogen assay results, concentrations were converted to mg/kg soil.

Potentially mineralizable nitrogen (PMN) was measured following protocols adapted from Drinkwater et al. (59) and Moebius-Clune et al. (60). The anaerobic incubation subsamples were combined with 10 mL ddH_2O and the headspace was cleared of O_2 with the addition of He gas to create a waterlogged, anaerobic environment in order to inhibit the oxidation of ammonium. These subsamples were incubate anaerobically at 37°C for 7 days in order to accumulate mineralized ammonium. Total PMN was determined by measuring the ammonium concentration following the protocol described above. PMN was calculated as the difference in ammonium concentration after and before the 7-day incubation.

DNA Extraction, Sequencing, and Analysis

Whole-community microbial DNA was extracted from freeze-dried soil samples using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH) following the manufacturer's protocol. Extracted DNA was purified at 65°C for 15 min with 1% cetyl-trimethylammonium bromide (CTAB) to remove humic acids. Samples were further extracted with 24:1 chloroform: alcohol to remove residual impurities. DNA was precipitated and washed three times with ethanol, then dried in a vacuum concentrator and dissolved in 1 x Tris-EDTA buffer. The purified DNA was adjusted to ∼20 ng/μL and stored at −80°C until further analysis.

To prepare samples for sequencing, 10 μL of each sample was added to a 96-well PCR plate and sequenced on a single flow cell using Illumina MiSeq V3 platform at W. M. Keck Center for Comparative and Functional Genomics at

the University of Illinois at Urbana-Champaign. For bacteria and archaea, the V4-V5 region of 16S rRNA was sequenced using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3') (61). For fungi, the internal transcribed spacer (ITS) region between the 18S and large subunit rRNA genes was sequenced using primers ITS3-F (5'-GCATCGATGAAGAACGCAGC-3') and ITS4-R (5'-TCCTCCGCTTATTGATATGC-3') (62). Samples for 2015 and 2016 were sequenced separately and combined for downstream analyses. A total of 22,722,058 raw reads were obtained from samples in 2015 and 21,685,014 in 2016 from both bacterial and fungal sequences. Library size ranged from 3,979 to 112,830 sequences per sample for the fungal ITS region with a mean of 16,997 sequences per sample, and 3,551–102,839 sequences per sample from the bacterial V4 region with a mean of 12,280 sequences per sample.

Sequence files were obtained as fastq files. Paired-end 16S sequences were merged using Fast Length Adjustment of Short reads (FLASH) software (63). Quality filtering of fastq files was performed using the FASTX-Toolkit software; sequence reads with a quality score of <30 and with fewer than 90% of bases were removed (64). Sequences were binned into discrete operational taxonomic units (OTUs) based on 97% similarity using usearch (65). Quantitative Insight into Microbial Ecology (MacQIIME version 1.9.2) was used for aligning and assigning of sequences (66). Sequences were aligned using the basic local alignment search tool (BLAST), and taxonomy was assigned based on the Greengenes reference database for bacteria and archaea and the UNITE database for fungi (67–69). Sequences identified as plants, protists, chloroplasts, and mitochondria were removed. Read counts were rarefied to 5,100 for bacterial sequences and 2,900 for fungal sequences. After rarefying, there were 527 samples from which 16,069 unique bacterial and 112 unique archaeal OTUs and were detected from the 16S rRNA gene. For the fungal sequences, there were 560 samples from which 4,932 fungal OTUs were identified from the ITS region after rarifying. Sequences have been uploaded to GenBank, BioProject # PRJNA503856.

Data Analysis: Soil Nitrogen

Data sets and R code to perform the following analyses are available online at https://github.com/acyann/post-termination-cover-crops.

We examined whether soil nitrogen levels differed between mixtures, monocultures and controls. We combined cover crop treatments by functional group and/or mixture, and therefore these analyses used the following treatment groups: brassica monocultures (mustard and turnip), grass monocultures (wheat and oat), legume monocultures (pea and bean), mixtures, and controls (plant-free or weedy). We also examined whether these patterns changed over time after termination. For these analyses, we used three complete site-years and one partial site-year due to experimental problems at PrairiErth in 2016 (55). Each complete site-year included 56 plots (12 treatments x 4 blocks), sampled at three time points. For PrairiErth 2016, we only included soil data for brassica monocultures (mustard and turnip) and the two controls (4 plots x 4 blocks), sampled at three time points. All data were analyzed using R software version 4.1.1 (70). Linear mixed effects models were used to determine how cover crop type influenced measures of soil nitrogen and total soil phenolic content using the packaged *nlme* version 3.1-153 (71). We fit separate models for each of the three time points (1, 2, and 4 weeks following termination), and we evaluated each of the three nitrogen species (nitrate, ammonium, and PMN) separately. Cover crop type was treated as the fixed effect and year, site, and replicate as nested random effects. Models were fit using the maximum likelihood approach. To test for mean differences between treatment groups (i.e., cover crop type), Tukey's Honestly Significant Difference (HSD) *post-hoc* tests were run using the package *multcomp* version 1.4-18 (72). Results of the linear mixed models and Tukey HSD tests were considered significant at the level of alpha < 0.05.

Data Analysis: Microbiome

Data sets and R code to perform the following analyses are available online at https://github.com/acyann/post-termination-cover-crops.

To parallel the soil nitrogen analyses described above, we examined whether soil nitrogen levels differed between mixtures, monocultures and controls, and also over time following termination. We used the same samples as described above (three complete site-years, plus a limited set of samples from PrairiErth 2016), and we used the same treatment groups defined by plant functional group. We used permutational multivariate analysis of variance (PERMANOVA) with the function "adonis" from the R package *vegan* version 2.5-7 (73). All analyses used the Bray-Curtis distance matrix with 999 permuations to construct the null distribution. We first ran a PERMANOVA model including site, year, and their interaction in order to determine if these random effects influenced microbial communities. We then tested for the effect of time and cover crop treatment using a restricted permutation scheme by stratifying on the random effects site and year (for the test of time since termination) or site, year, and time (for the test of cover crop treatment). We visualized patterns in microbial community composition through two-dimensional non-metric multidimensional scaling of the Bray-Curtis matrix.

To provide a more in-depth analysis of microbial responses, we also examined correlations between microbial taxa and various environmental drivers in our data set, including soil nitrogen, site/year/time, and cover crop biomass. For this analysis, we conducted a weighted gene co-expression network analysis (WGCNA), which has previously been used for soil microbiome analysis in a variety of soil environments (74–77). We only used samples from the three complete site-years, because we did not have biomass data from PrairiErth in 2016 (55). We further restricted this analysis to the most abundant microbial taxa in our sample set. For both 16S and ITS, OTU tables were either filtered to exclude OTUs with a relative abundance of <0.01% or to include only the top 1000 OTUs, whichever method was more restrictive. The abundances of these top OTUs were normalized using the total sum scaling (TSS) method and then log2 transformed.

We then conducted a weighted gene co-expression network analysis (WGCNA) to determine patterns of co-occurrence among OTUS and between these OTU groups and our environmental data. WGCNA first uses network analysis to identify highly correlated "modules" of OTUs that respond in

concert, and then it seeks to identify environmental correlates for each of these modules. For this analysis, we used the package *WGCNA* version 1.70-3 (78, 79). The network was constructed based on the patterns of interactions across OTUs. A dendrogram was constructed, which creates a hierarchical topology for the network. From this point, a soft threshold was applied, which guides where the dendrogram is cut, and that cut separates the network into modules that display co-abundance. The location where the dendrogram is cut is determined by the topological features, expressed as the variable β, which is selected based on where graphs describing the scale independence and mean connectivity level off. This value sets the power for blockwise module construction (80). For the 16S network, $\beta = 5$ was used; for the ITS network, $\beta = 6$ was used. The minimum module size was set to 20 OTUs. This generated four modules for the 16S network and three modules for the ITS network. Correlation values between modules and environmental data (cover crop type, soil nitrogen, etc.) were generated on a heat map. Hub taxa were identified as the taxa in each module with a module correlation of $R > 0.70$ or $R < -0.70$. Taxonomy was visualized using *ggplot2* (81). Heatmaps were visualized using *WGCNA*.

RESULTS

Soil Nitrogen Dynamics

Across all site years, soil nitrate concentrations were greatest in the plant-free control plots within the 1st week after cover crop termination (**Figure 1A**). Soil nitrate in brassica plots was significantly lower than in the plant-free control plots during the 1st week, but there was no significant difference in soil nitrate across any of the cover crop monocultures or mixtures. Soil nitrate levels decreased over the 4-week post-termination period, particularly for the plant-free control plots, which were indistinguishable from cover crop plots by the 2nd week after termination (**Figure 1A**). By the 4th week, all soil nitrate levels were statistically indistinguishable across plots.

Soil ammonium levels were not statistically different across any cover crop treatment or controls at any time point after termination (**Figure 1B**). Soil ammonium levels tended to be highest during the 2nd week after termination, although the levels in plant-free controls were also high within the 1st week (**Figure 1B**).

Levels of potentially mineralizable organic nitrogen (PMN) were elevated in all cover crop plots relative plant-free controls, although this elevation was only statistically significant for cover crop mixtures (**Figure 1C**). All cover crop monocultures and mixtures had significantly higher PMN than plant-free controls by the 2nd week (**Figure 1C**), and PMN levels were highest overall at this time. By week four, only the brassica and weedy-control plots had significantly higher PMN than plant-free controls, with all other cover crop treatments having intermediate PMN values.

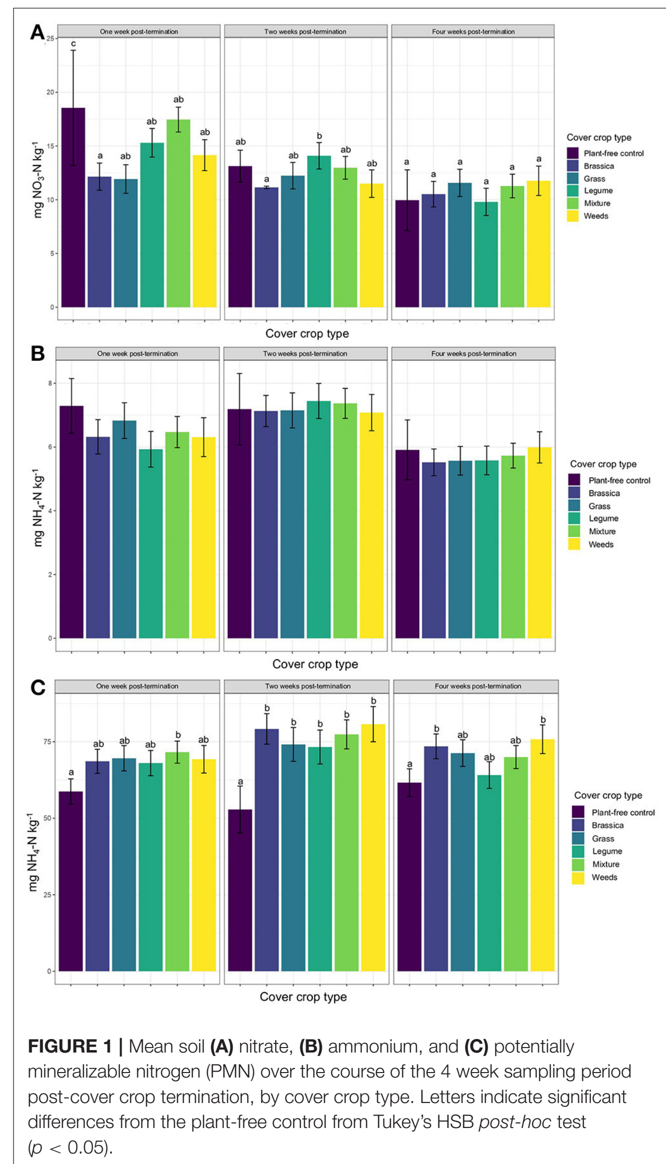

FIGURE 1 | Mean soil **(A)** nitrate, **(B)** ammonium, and **(C)** potentially mineralizable nitrogen (PMN) over the course of the 4 week sampling period post-cover crop termination, by cover crop type. Letters indicate significant differences from the plant-free control from Tukey's HSB *post-hoc* test ($p < 0.05$).

Overall Patterns in Soil Microbiome Composition

Site, year, and their interaction were significant predictors of soil bacterial and fungal community composition (**Table 1**), so we used stratification in subsequent models to test for effects of time and cover crop treatment. When stratified within site and year, time since termination was significant for both bacterial and fungal community composition (**Table 1**). When stratified within site, year, and time, cover crop type was a significant predictor of fungal community composition, but not of bacterial community composition (**Table 1**). Non-metric multidimensional scaling of microbial communities primarily reflected the overwhelming influence of site-year differences (**Supplementary Figures 1, 2**).

Coordinated Bacterial Responses: 16S WGCNA

A total of four modules were identified by the WGCNA analysis for 16S data (**Figure 2** and **Table 2**). The composition of the four modules was taxonomically distinct, and the relative proportions of phylum-level representation of the modules differed greatly

TABLE 1 | PERMANOVA tests were carried out on the entire dataset to evaluate the influences of site, year, cover crop type, cover crop diversity or sample date influenced bacterial and fungal community composition.

	Bacterial community				Fungal community			
	df	*F*	R^2	*p*	*df*	*F*	R^2	*p*
Site	1,526	31.67	0.051	0.001*	1,559	96.11	0.131	0.001*
Year	1,526	32.02	0.052	0.001*	1,559	50.50	0.069	0.001*
Site x year	1,526	29.03	0.047	0.001*	1,559	31.29	0.043	0.001*
Sample date *stratified by site and year	2,526	4.13	0.016	0.001*	2,559	13.37	0.046	0.001*
Cover crop type *stratified by site, year and sample date	5,526	1.46	0.014	0.087	5,559	2.11	0.019	0.001*

The Bray-Curtis distance method was applied to community data. df, degrees of freedom: numerator, total; F, F statistic; R^2, R^2-value; p, p-value. Results were considered significant at the $p < 0.05$ level and are indicated with an asterisk.

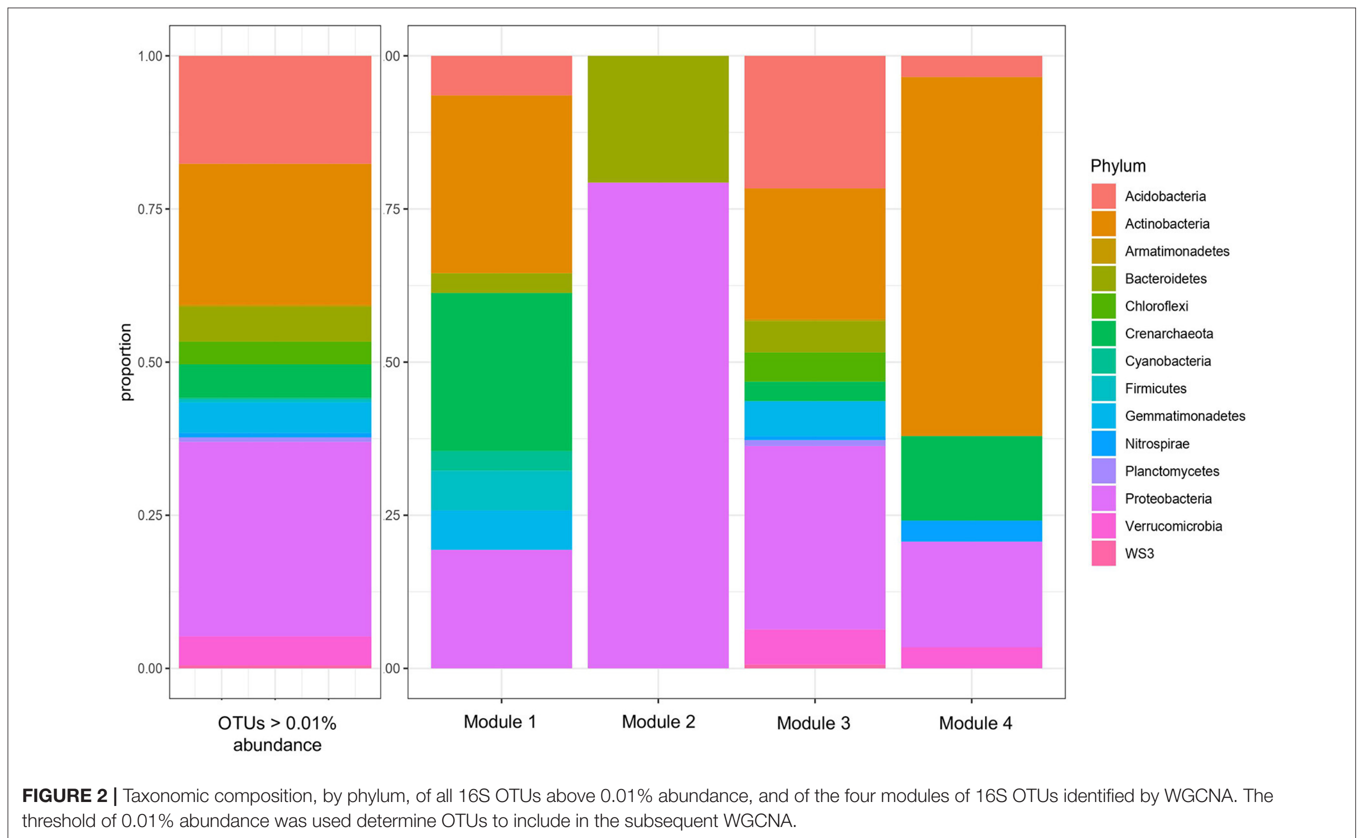

FIGURE 2 | Taxonomic composition, by phylum, of all 16S OTUs above 0.01% abundance, and of the four modules of 16S OTUs identified by WGCNA. The threshold of 0.01% abundance was used determine OTUs to include in the subsequent WGCNA.

with that of the overall soil microbiome (**Figure 2**). Module 1 was largely composed of Actinobacteria, Crenarcheaota, and Proteobacteria; Module 2 Proteobacteria and Bacteroidetes; Module 3 Acidobacteria, Actinobacteria, and Proteobacteria; and Module 4 Actinobacteria, Crenarchaeota, and Proteobacteria. Module 1 was most positively correlated with 2015 sampling year ($R = 0.19$, $p < 0.05$), Kinnikinnick farm ($R = 0.50$, $p < 0.05$), and weed biomass ($R = 0.31$, $p < 0.05$) (**Figure 3**). The hub taxa in Module 1 included mostly unclassified *Candidatus Nitrososphaera*, a type of ammonia-oxidizing Archaea (**Table 2**). Module 2 was most positively correlated with 2016 sampling year ($R = 0.64$, $p < 0.05$) and most negatively correlated with soil nitrate ($R = -0.36$, $p < 0.05$), soil ammonium ($R = -0.36$, $p < 0.05$), soil PMN ($R = -0.23$, $p < 0.05$), weed biomass ($R = -0.41$, $p < 0.05$), and total biomass ($R = -0.26$, $p < 0.05$). The hub taxa in Module 2 included both *Flavobacterium* spp. (Bacteroidetes) and Beta- and Gamma-proteobacteria. Module 3 was most positively correlated with PrariErth farm ($R = 0.86$, $p < 0.05$), 2015 sampling year ($R = 0.18$, $p < 0.05$), soil nitrate ($R = 0.38$, $p < 0.05$), and total cover crop biomass ($R = 0.36$, $p < 0.05$). This was the largest module and hub taxa were diverse: negative hub taxa included *Candidatus Nitrososphaera* spp. while positive hub taxa included mostly Proteobacteria, Actinobacteria, and Acidobacteria. Module 4 was most positively correlated with 2015 sampling year ($R = 0.29$, $p < 0.05$), PrariErth farm ($R = 0.58$, $p < 0.05$), soil nitrate ($R = 0.51$, $p < 0.05$), total cover crop

TABLE 2 | 16S hub taxa by module.

Module	16S OTU #	Taxonomy	Module correlation
Module 1	116	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, unclassified *Candidatus Nitrososphaera*	0.815
Module 1	1677	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, unclassified *Candidatus Nitrososphaera*	0.757
Module 1	407	Bacteria, Actinobacteria, Rubrobacteria, Rubrobacterales, Rubrobacteraceae, unclassified Rubrobacter	0.747
Module 1	134	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, unclassified *Candidatus Nitrososphaera*	0.743
Module 1	389	Bacteria, unclassified Gemmatimonadetes	0.717
Module 2	112	Bacteria, Bacteroidetes, Flavobacteriia, Flavobacteriales, Flavobacteriaceae, *Flavobacterium succinicans*	0.865
Module 2	2332	Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, unclassified Xanthomonadaceae	0.854
Module 2	7145	Bacteria, Bacteroidetes, Flavobacteriia, Flavobacteriales, Flavobacteriaceae, unclassified *Flavobacterium*	0.786
Module 2	12739	Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, unclassified *Pseudomonas*	0.783
Module 2	451	Bacteria, Bacteroidetes, Flavobacteriia, Flavobacteriales, Flavobacteriaceae, unclassified *Flavobacterium*	0.777
Module 2	91	Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, unclassified Xanthomonadaceae	0.770
Module 2	20	Bacteria, Proteobacteria, Betaproteobacteria, Burkholderiales, unclassified Oxalobacteraceae	0.753
Module 3	432	Bacteria, Chloroflexi, unclassified Gitt-GS-136	0.820
Module 3	49	Bacteria, Proteobacteria, Betaproteobacteria, unclassified MND1	0.819
Module 3	333	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.792
Module 3	14539	Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, unclassified *Kaistobacter*	0.792
Module 3	78	Bacteria, Acidobacteria, [Chloracidobacteria], unclassified RB41	0.789
Module 3	67	Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, unclassified Sinobacteraceae	0.785
Module 3	108	Bacteria, Acidobacteria, Acidobacteria-6, unclassified iii1-15	0.781
Module 3	6933	Bacteria, Actinobacteria, MB-A2-108, unclassified 0319-7L14	0.778

(Continued)

TABLE 2 | Continued

Module	16S OTU #	Taxonomy	Module correlation
Module 3	110	Bacteria, Actinobacteria, Thermoleophilia, unclassified Solirubrobacterales	0.772
Module 3	151	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, unclassified *Candidatus Nitrososphaera*	0.759
Module 3	94	Bacteria, Acidobacteria, iii1-8, unclassified DS-18	0.747
Module 3	47	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.747
Module 3	120	Bacteria, Actinobacteria, MB-A2-108, unclassified 0319-7L14	0.741
Module 3	103	Bacteria, Acidobacteria, Acidobacteria-6, unclassified iii1-15	0.724
Module 3	90	Bacteria, Proteobacteria, Alphaproteobacteria, unclassified Rhizobiales	0.724
Module 3	27	Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae, unclassified *Balneimonas*	0.724
Module 3	14	Bacteria, Bacteroidetes, [Saprospirae], [Saprospirales], unclassified Chitinophagaceae	0.721
Module 3	2600	Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae, unclassified *Balneimonas*	0.717
Module 3	207	Bacteria, Gemmatimonadetes, unclassified Gemm-1	0.711
Module 3	8571	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.703
Module 3	155	Bacteria, Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae, unclassified *Agromyces*	0.702
Module 3	822	Bacteria, Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae], unclassified *DA101*	−0.701
Module 3	1882	Bacteria, Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae], unclassified *DA101*	−0.703
Module 3	2583	Bacteria, Acidobacteria, [Chloracidobacteria], unclassified RB41	−0.705
Module 3	683	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	−0.711
Module 3	1255	Bacteria, Actinobacteria, Thermoleophilia, unclassified Gaiellales	−0.713
Module 3	148	Bacteria, Acidobacteria, Solibacteres, unclassified Solibacterales	−0.713
Module 3	17862	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, *Candidatus Nitrososphaera SCA1170*	−0.716
Module 3	458	Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales, Koribacteraceae, unclassified *Candidatus Koribacter*	−0.731

(Continued)

TABLE 2 | Continued

Module	16S OTU #	Taxonomy	Module correlation
Module 3	3221	Bacteria, Gemmatimonadetes, Gemmatimonadetes, unclassified Ellin5290	−0.733
Module 3	243	Bacteria, Gemmatimonadetes, Gemmatimonadetes, unclassified Ellin5290	−0.734
Module 3	1145	Bacteria, Proteobacteria, Alphaproteobacteria, unclassified Ellin329	−0.734
Module 3	122	Bacteria, Bacteroidetes, [Saprospirae], [Saprospirales], unclassified Chitinophagaceae	−0.734
Module 3	255	Bacteria, Acidobacteria, Solibacteres, Solibacterales, Solibacteraceae, unclassified *Candidatus Solibacter*	−0.735
Module 3	880	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	−0.750
Module 3	5425	Bacteria, Gemmatimonadetes, Gemmatimonadetes, unclassified N1423WL	−0.760
Module 3	3517	Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, unclassified *Kaistobacter*	−0.762
Module 3	10	Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, unclassified *Kaistobacter*	−0.779
Module 3	1805	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	−0.784
Module 3	118	Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales, unclassified Koribacteraceae	−0.787
Module 3	17124	Bacteria, Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae], unclassified *DA101*	−0.789
Module 3	1302	Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales, unclassified Koribacteraceae	−0.792
Module 3	552	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	−0.795
Module 3	384	Bacteria, Proteobacteria, Alphaproteobacteria, unclassified Ellin329	−0.796
Module 3	3	Bacteria, Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae], unclassified *DA101*	−0.812
Module 3	11121	Bacteria, Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae], unclassified *DA101*	−0.814
Module 3	421	Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales, unclassified Koribacteraceae	−0.814
Module 3	96	Bacteria, Proteobacteria, Betaproteobacteria, A21b, unclassified EB1003	−0.822

(Continued)

TABLE 2 | Continued

Module	16S OTU #	Taxonomy	Module correlation
Module 3	289	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	−0.826
Module 3	66	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, *Candidatus Nitrososphaera*	−0.836
Module 3	77	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, *Candidatus Nitrososphaera*	−0.859
Module 3	168	Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales, unclassified Koribacteraceae	−0.869
Module 4	374	Bacteria, Actinobacteria, Actinobacteria, unclassified Actinomycetales	0.842
Module 4	1704	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.829
Module 4	738	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.782
Module 4	128	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.777
Module 4	338	Bacteria, Actinobacteria, MB-A2-108, unclassified 0319-7L14	0.776
Module 4	8090	Archaea, Crenarchaeota, Thaumarchaeota, Nitrososphaerales, Nitrososphaeraceae, unclassified *Candidatus Nitrososphaera*	0.755
Module 4	366	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.748
Module 4	199	Bacteria, Actinobacteria, Thermoleophilia, unclassified Solirubrobacterales	0.747
Module 4	13312	Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, unclassified *Rhodoplanes*	0.715
Module 4	46	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.711
Module 4	102	Bacteria, Actinobacteria, Thermoleophilia, Gaiellales, unclassified Gaiellaceae	0.706

Correlation > 0.70 or < -0.70.

biomass ($R = 0.32$, $p < 0.05$), and most negatively correlated with sampling days post-cover crop termination ($R = -0.27$, $p < 0.05$). Hub taxa for Module 4 were comprised mostly of unclassified Gaiellaceae.

Coordinated Fungal Responses: ITS WGCNA

Three modules were identified among ITS OTUs in the WGCNA analysis (**Figure 4**). At the class level, the three modules were broadly similar in their relative taxonomic composition. They were also compositionally similar to the overall fungal community, although Sordariomycetes and Dothidiomycetes tended to be over-represented in modules relative to the overall community, and Agaricomycetes tended to be under-represented

in modules. Module 1 was most positively correlated with 2015 ($R = 0.42$, $p < 0.05$), PrariErth farm ($R = 0.97$, $p < 0.05$), soil nitrate ($R = 0.52$, $p < 0.05$), and total cover crop biomass ($R = 0.43$, $p < 0.05$) (**Figure 5**). Hub taxa in this module included Sordariomyctes and Leotiomycetes (**Table 3**). Module 2 was most positively correlated with 2016 ($R = 0.96$, $p < 0.05$) and Kinnikinnick farm ($R = 0.46$, $p < 0.05$) and most negatively correlated with soil nitrate ($R = -0.61$, $p < 0.05$), soil ammonium ($R = -0.33$, $p < 0.05$), weed biomass ($R = -0.46$, $p < 0.05$), and total cover crop biomass ($R = -0.48$, $p < 0.05$). The top hub taxa in Module 2 included *Cystolepiota adulterine* and Dothidiomycetes. Module 3 generally had much weaker correlations to environmental variables, and it was positively correlated with 2016 ($R = 0.36$, $p < 0.05$) and Kinnikinnick farm ($R = 0.21$, $p < 0.05$) and negative correlated with soil nitrate ($R = -0.20$, $p < 0.05$) and total cover crop biomass ($R = -0.20$, $p < 0.05$). The hub taxa in Module 3 included *Mortierella capitate*, *Trichocladium asperum*, and other Sordariomyctes.

DISCUSSION

Compared to plant-free controls, cover cropping had a significant impact on soil nitrogen levels in these short-term, springtime trials, but we found very few overall differences between plots that used different functional groups of cover crops (brassicas vs. grasses vs. legumes). By far, the most important differences appeared to be between plots that contained some kind of plant cover (including weeds) and the plant-free control plots. Plots with plant cover had lower soil nitrate and higher potentially mineralizable nitrogen than plant-free controls in the first few weeks following termination, although these differences largely disappeared by the 4th week. Microbial community composition in our study was largely driven by site and year, suggesting that large scale spatial and temporal effects are the primary determinants of soil microbial species pools. Nevertheless, our network analysis revealed coordinated responses in highly-correlated modules of soil bacteria and fungi in cover cropped systems, and we discuss these in more detail below.

Soil Nitrogen by Cover Crop Type

Soil nitrate concentrations were initially greater under the plant-free control plots than any plots with cover crops. The inclusion of cover crops in this system should therefore minimize risk of nitrate leaching in the weeks following termination, as has been previously observed (20, 38, 40). Significant mineral nitrogen uptake by brassicas and weeds during the growing season may have further supported greater PMN concentrations during the fallow period prior to subsequent crop establishment (20). From an ecological and environmental sustainability perspective, lower concentrations of nitrate in the spring, during times of heavy rainfall and increased risk for leaching, may be advantageous. The five-species mixtures all contained at least one brassica, one legume, and one grass species, in addition to volunteer weed growth. It was, therefore, not surprising that the post-termination effects of mixtures on soil mineral nitrogen were consistently moderate. Holmes et al. (55) found that mixtures were consistently productive throughout the study, generating neither the most nor least biomass. Like the other more productive cover crops (brassicas, weeds, and grasses), nitrate losses were low under mixtures following termination. Similar to grasses, ammonium concentrations following mixtures declined steadily, though less dramatically.

Under mixtures, PMN concentrations were also moderate, and the relative dominance of brassicas in some of the mixtures (55) could have influenced the post-termination effects of those mixtures. PMN concentrations have been reported to decrease in mixtures with increasing proportions of grasses like rye or rye grass (82), and the mixtures in this study were heavily influenced by high biomass producers such as brassicas and weeds instead of grasses (55). This difference in PMN may also be reflected by the lower C:N ratios of legumes than grasses, and contribute to higher PMN due to more easily mineralizable content form tissues (20). Since mixtures contained tissues with variable C:N ratios, decomposition was occurring at different rates during the four-week sampling period. Organic nitrogen mineralization from legumes was likely more rapid due to low C:N ratios (83), lowering the overall PMN content when averaged across the 4-week sampling period. The quick release of nitrogen from legumes likely contributed to increased soil nitrate and slightly decreased soil PMN under mixtures as compared to monocultures like brassicas.

Organic farmers must prioritize their goals for planting spring-sewn cover crops. If the objectives are to reduce potential nitrate leaching and increase the potential for nitrogen mineralization throughout the upcoming growing season, then our study shows that a brassica monoculture such as Idagold mustard would accomplish this goal. However, while low soil nitrate concentrations post-cover crop incorporation may be beneficial for suppressing weed establishment, low mineral nitrogen could potentially hinder future crop growth if nitrogen demands are not met. If the goal of cover cropping is to increase nitrogen fixation, and subsequently inorganic nitrogen supply for crops, then a legume monoculture or mixture would allow for increased inorganic nitrogen available to subsequent crops while reducing the growth and establishment of weeds in legume monocultures. Determining the correct legume to use and ensuring that it will establish effectively when planted in a mixture will also be necessary to ensure successful establishment of all species.

Soil Microbial Community Response

Year and site were the strongest drivers of microbial community composition, which was illustrated in the PERMANOVA analysis. Therefore, it wasn't surprising to find that year and farm were often the strongest drivers in the WGCNA analysis. For the bacterial and archaeal OTUs, module 2 was strongly associated with the year 2016, and subsequently Kinnikinnick since biomass data that year was only available from that farm. Likewise, module 3 was strongly influenced by PrariErth farm. Castle et al. (84) also found that site-specific controls were most influential on short-term responses of soil bacterial communities under different cover crop treatments. The other correlations (nitrate, PMN, cover crop biomass) were considerably weaker, and thus challenging to disentangle the overarching strong effects

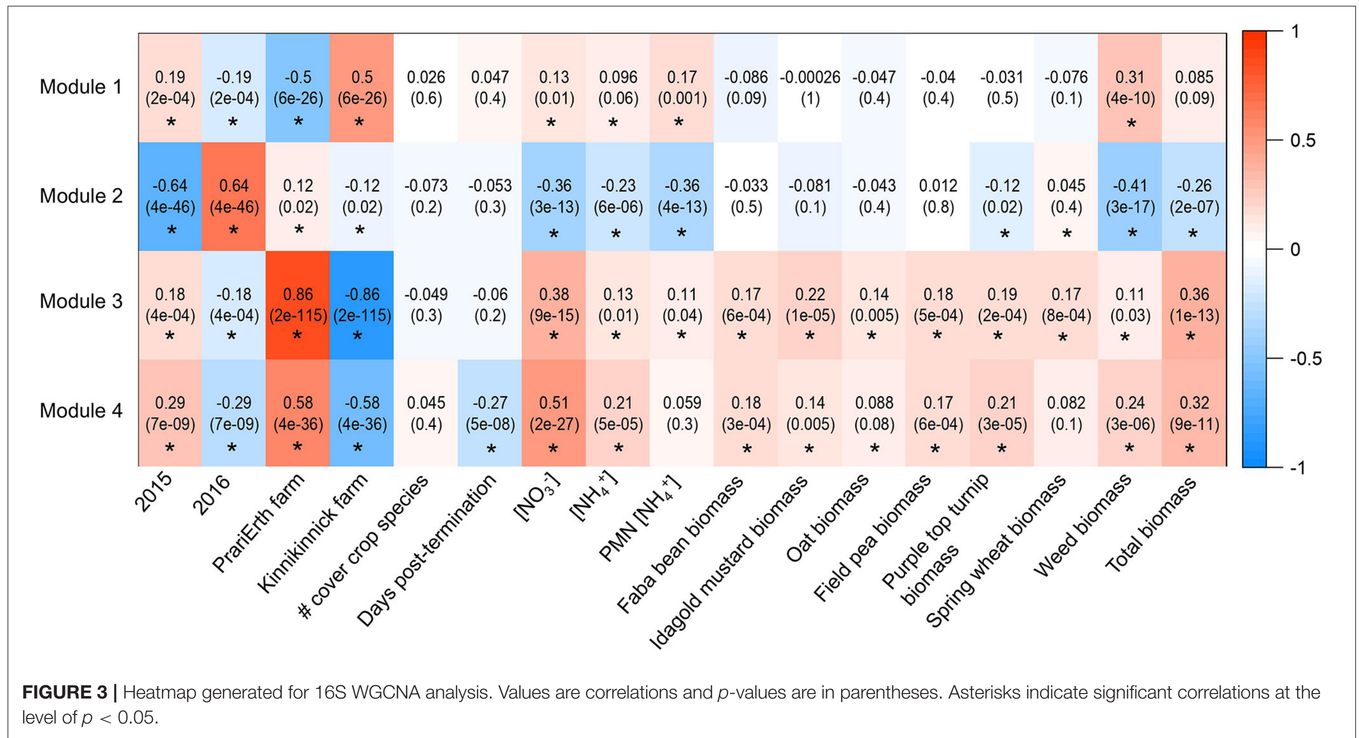

FIGURE 3 | Heatmap generated for 16S WGCNA analysis. Values are correlations and *p*-values are in parentheses. Asterisks indicate significant correlations at the level of $p < 0.05$.

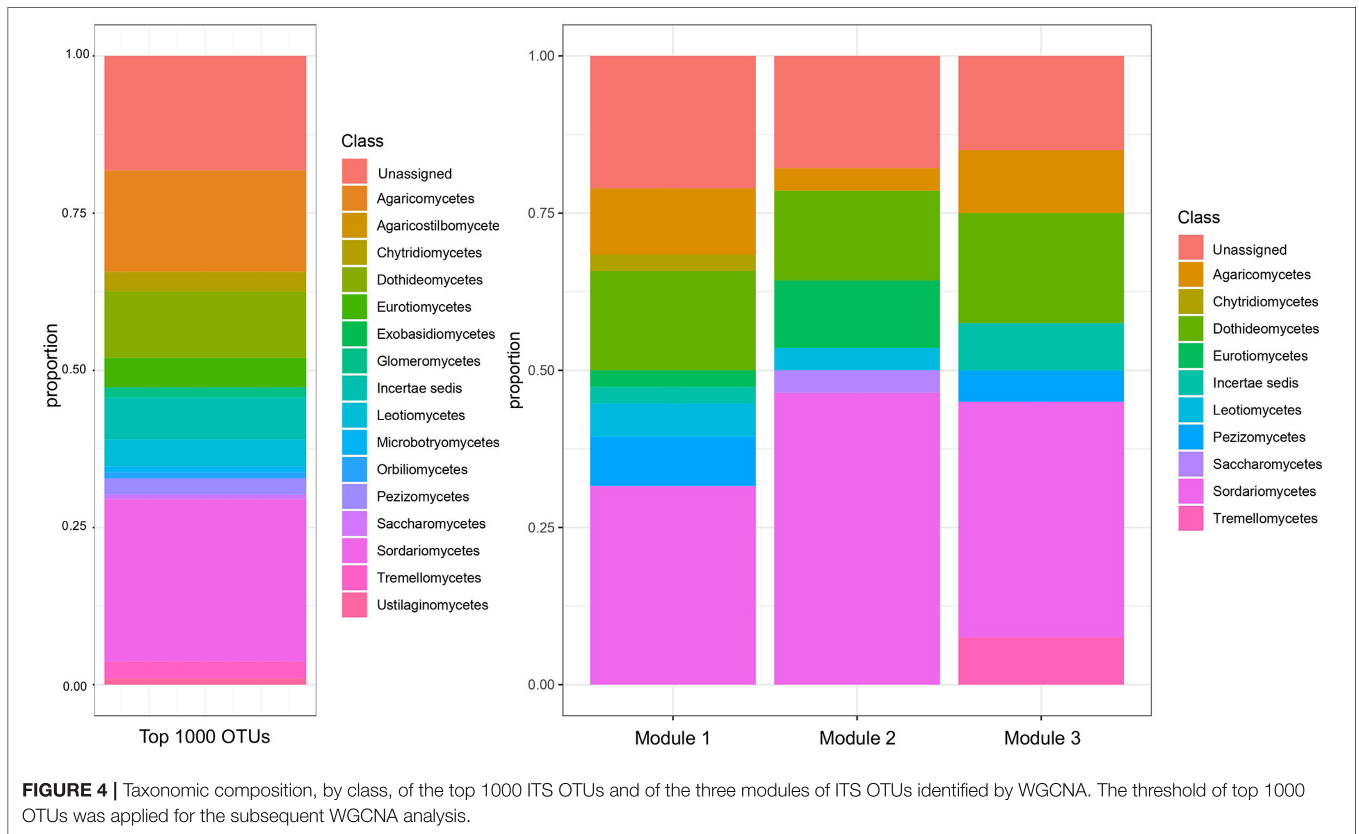

FIGURE 4 | Taxonomic composition, by class, of the top 1000 ITS OTUs and of the three modules of ITS OTUs identified by WGCNA. The threshold of top 1000 OTUs was applied for the subsequent WGCNA analysis.

of site and year. For the fungal OTUs, module 1 was very strongly influenced by PrariErth farm and module two by the year 2016. It is important to recognize these strong site and year effects, which reflect large scale temporal and spatial drivers of soil microbial communities. However, because these effects are idiosyncratic to our study, they hinder our ability to speculate about how cover

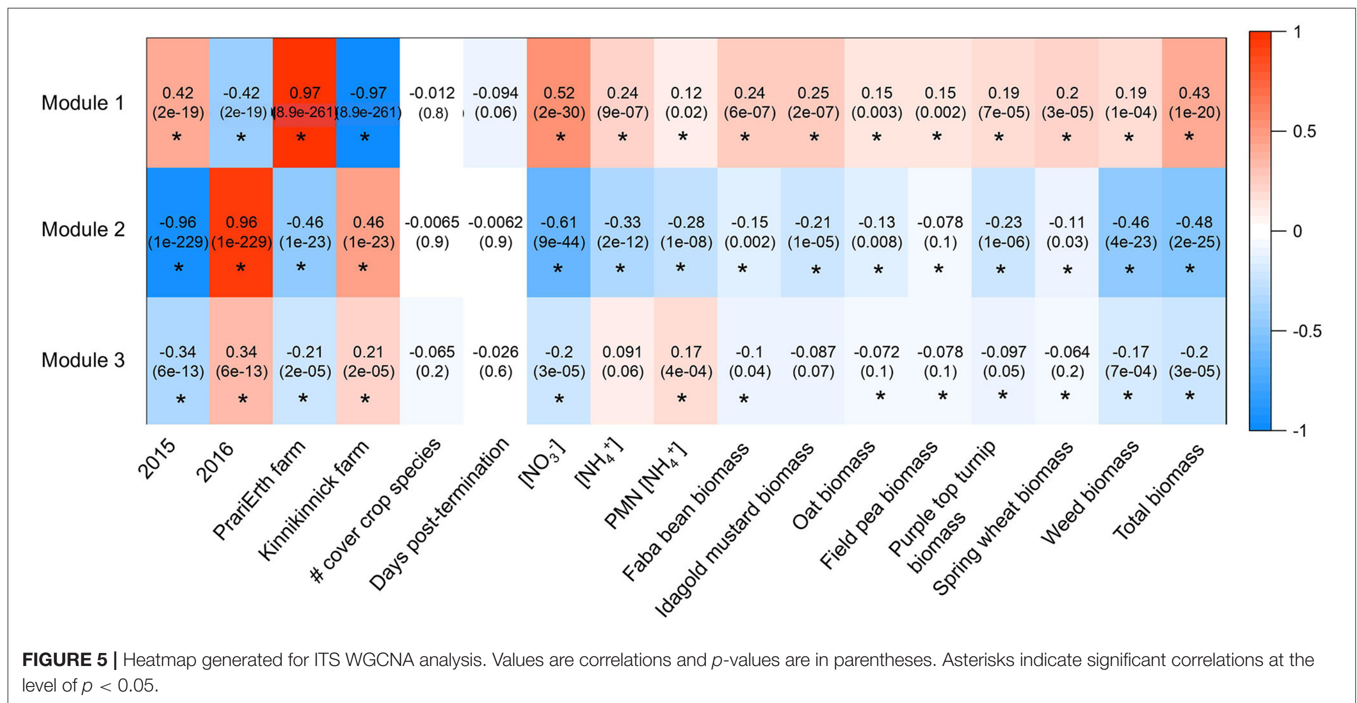

FIGURE 5 | Heatmap generated for ITS WGCNA analysis. Values are correlations and *p*-values are in parentheses. Asterisks indicate significant correlations at the level of $p < 0.05$.

cropping may affect microbial communities more generally. Therefore, we focus the remainder of our discussion on the remaining modules that showed much weaker correlations with site and year, and may therefore better reflect more general features of microbial response to cover cropping.

In the analysis of bacterial and archaeal OTUs, module 1 had a relatively strong positive correlation with weed biomass. There is a prevalence of ammonia-oxidizing organisms within the hub taxa from this module. The most abundant taxa from 16S module 1 were ammonia-oxidizing archaea of the genus *Candidus Nitrosphaera*. Individual ammonia-oxidizing bacterial and archaeal OTUs displayed individualistic responses to cover crop biomass, for example they were found in other modules of the analysis (module 3, module 4). In a concurrent study at this site, similar concentrations of soil ammonium across all cover crop types were reported, so detection of ammonia-oxidizing microorganisms across various cover crop biomasses was not entirely surprising. Though archaea made up <1% of the total "bacterial" 16S sequences that were analyzed in this study, they are ubiquitous in soils and are generally resistant to changing environmental conditions (85–87).

Ammonia-oxidizing bacteria and archaea are responsible for the first step of nitrification, conversion of ammonium to nitrite. This pathway is particularly important in agricultural systems, where nitrogen loss via nitrification decreases the pool of available inorganic nitrogen for subsequent crop uptake (12). The positive correlation between these ammonia-oxidizing OTUs and weed biomass may suggest that weeds, when at high biomass, can further support nitrification in soils. This may be an important discovery in the effort to reduce inorganic nitrogen losses in agriculture, which is a major concern in the Midwest.

Other hub taxa of note in 16S module included a single Rubrobacter, which are widely distributed in soils, such as grasslands, prairies, and pastures (88). Hub taxa for this module also included a Gammatimonadetes OTU, a taxon that has been found may be adapted to low soil moisture (89).

16S module 4 had negative correlation with sampling date (positive week 1), positive correlation with soil nitrate (nitrate levels also higher in week 1, across all cover crop types) and positive correlation with total cover crop biomass. The hub taxa in this module were overwhelmingly unclassified OTUs of the family Gaiellaceae (phylum: Actinobacteria). OTUs of the order Gaiellales have been shown to predominate in extreme environment, including saline-alkaline soils (90), wastewater treatments plants (91), and marine ecosystems (92). There was a single Actinomycetales (phylum: Actinobateria). Members of this order are often found in soil habitats and can support plant growth via biological nitrogen fixation (93, 94). There was one Rhizobiales (phylum: Proteobacteria), which also includes nitrogen-fixing associative taxa (95–98). There was also an ammonia-oxiziding archaea in this module, a potential producer of increased soil nitrate levels, which module 4 was also positively with. Rhodoplanes was also identified as a hub taxa in this module, and taxa of this family are photosynthetic with denitrification properties (99).

Fungal module 3 was negatively correlated with soil nitrate and total cover crop biomass, which likely links it to the plant-free control plots or the cover crop treatments with lower successful establishment. One of the hub taxa was *Mortierella capitate* (phylum: Zygomycota), which has been found to promote crop growth (100). There were two taxa identified as *Trichocladium asperum*, a polyphyletic genus of the family Chaetomiaceae

TABLE 3 | ITS hub taxa by module.

Module	ITS OTU #	Classification	Module correlation
Module 1	98	Fungi, Ascomycota, Leotiomycetes, unclassified Helotiales	0.852
Module 1	72	Fungi, Ascomycota, Sordariomycetes, Sordariales, unclassified Lasiosphaeriaceae	0.805
Module 1	257	Fungi, Basidiomycota, Agaricomycetes, Sebacinales, Sebacinales Group B, unclassified *Serendipita*	0.800
Module 1	2669	Fungi, Ascomycota, Sordariomycetes, Hypocreales, unclassified Nectriaceae	0.761
Module 1	65	Fungi, unclassified Ascomycota	0.760
Module 1	181	unclassified Fungi	0.752
Module 1	153	Fungi, Ascomycota, Leotiomycetes, Helotiales, Incertae sedis, *Pyrenopeziza revincta*	0.719
Module 1	46	Fungi, Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae, *Aspergillus fischeri*	0.711
Module 1	2872	Fungi, Ascomycota, Sordariomycetes, Hypocreales, unclassified Nectriaceae	0.702
Module 2	526	Fungi, Basidiomycota, Agaricomycetes, Agaricales, Agaricaceae, *Cystolepiota adulterina*	0.829
Module 2	316	Fungi, Ascomycota, Dothideomycetes, unclassified Pleosporales	0.798
Module 2	398	Fungi, unclassified Rozellomycota	0.741
Module 2	1216	Fungi, Ascomycota, Eurotiomycetes, Onygenales, Incertae sedis, unclassified *Myceliophthora*	0.727
Module 2	444	Fungi, Ascomycota, unclassified Leotiomycetes	0.721
Module 2	542	Fungi, Ascomycota, Sordariomycetes, Xylariales, Xylariaceae, unclassified *Xylaria*	0.721
Module 2	634	Fungi, Ascomycota, Dothideomycetes, unclassified Pleosporales	0.721
Module 2	853	Fungi, unclassified Ascomycota	0.721
Module 2	1017	Fungi, Ascomycota, Saccharomycetes, Saccharomycetales, Trichomonascaceae, unclassified *Blastobotrys*	0.721
Module 2	1616	Fungi, Ascomycota, Dothideomycetes, Tubeufiales, Tubeufiaceae, unclassified *Helicoma*	0.721
Module 3	100	Fungi, Zygomycota, Incertae sedis, Mortierellales, Mortierellaceae, *Mortierella capitata*	0.927
Module 3	5870	Fungi, Ascomycota, Sordariomycetes, Sordariales, Chaetomiaceae, *Trichocladium asperum*	0.828
Module 3	3	Fungi, Ascomycota, Sordariomycetes, Sordariales, Chaetomiaceae, *Trichocladium asperum*	0.808
Module 3	86	Fungi, unclassified Ascomycota	0.795
Module 3	11	Fungi, Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae, unclassified *Fusarium*	0.782
Module 3	5999	Fungi, Ascomycota, Sordariomycetes, Hypocreales, Clavicipitaceae, *Metarhizium marquandii*	0.740
Module 3	4674	Fungi, unclassified Ascomycota	0.732
Module 3	131	Fungi, Basidiomycota, Tremellomycetes, Cystofilobasidiales, Cystofilobasidiaceae, *Mrakia frigida*	0.721
Module 3	53	Fungi, Ascomycota, Sordariomycetes, unclassified Sordariales	−0.711
Module 3	39	Fungi, Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae, unclassified *Fusarium*	−0.750

Correlation > 0.70 or < -0.70.

that has been found a number of habitats, including soils and decomposing plant material (101). Other members of the family are commonly found in decomposing plant material and play a role in plant degradation (102). Some other species in the family have causes neurological disease in humans (103). Another hub taxa belongs to the family Nectriaceae, which also includes important human and plant pathogens (104). Hub taxa *Metarhizium marquandii* includes plant-growth promoting fungi (105).

Taken together, changes in these 16S and ITS modules suggest that the more general coordinated responses of soil microbial communities in our study were primarily associated with changes in soil nitrate concentrations and overall plant biomass. Given that plant cover was also a key driver of soil nitrate in our study, we conclude that a major impact of springtime cover cropping is to drive changes in soil nitrate levels, and that soil nitrate, in turn, is a key driver of microbial community composition, particularly for bacteria. Cover cropping also promoted an increase in potentially mineralizable nitrogen pools in soils, and the presence of saprotrophic fungi in the hub taxa of module 3 suggests that the decomposition of cover crop residues may drive subsequent soil microbial changes over time.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, BioProject # PRJNA503856.

AUTHOR CONTRIBUTIONS

AH, SW, and AY designed the research. AH maintained the experimental research plots. AH and AY collected the samples. EL produced the data. EL and AY conducted the data analysis and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsoil.2022.824087/full#supplementary-material

Supplementary Figure 1 | NMDS plot of bacterial communities representing all cover crop treatments. Each point represents a single sample and the bacterial community from that sample. Due to the significant effects of site and year, points are labeled by their site-year interactions. The Bray-Curtis distance method was used to perform the NMDS, with a stress level of 0.164. Ellipses represent the 95% confidence interval around the centroid for the given site-year.

Supplementary Figure 2 | NMDS plot of fungal communities representing all cover crop treatments. Each point represents a single sample and the fungal community from that sample. Due to the significant effects of site and year, points are labeled by their site-year interactions. Bray-Curtis distances were used to perform the NMDS, with a stress level of 0.214. Ellipses represent the 95% confidence interval around the centroid for the given site-year.

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