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Soil bacterial community composition following cover crops and corn nitrogen management

Jeff Rutan | **Noah Rosenzweig** | **Kurt Steinke**

Dep. of Plant, Soil, and Microbial Sciences, Michigan State Univ., East Lansing, MI 48824, USA

Correspondence
Kurt Steinke, Dep. of Plant, Soil, and Microbial Sciences, Michigan State Univ., East Lansing, MI 48824, USA.
Email: ksteinke@msu.edu

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Abstract

Utilizing cover crops (CC) prior to corn (*Zea mays* L.) creates opportunities to increase plant diversity and influence soil bacterial communities, but data are scarce when CC are combined with corn N fertilizer management. Field studies were conducted in 2014–2015 and 2015–2016 to evaluate corn N strategies following daikon radish [*Raphanus sativus* (L.)], forage oat [*Avena sativa* (L.)], or no CC on soil bacterial community composition. Nitrogen strategies consisted of 179 kg N ha^{-1} applied pre-plant incorporated as urea, slowly-available poultry litter (61 kg N ha^{-1}) plus sidedress (SID) N at V11, starter N (45 kg N ha^{-1}) applied 5 cm beside and 5 cm below the furrow (5 × 5) plus SID at V4, V11, or 50:50 (split) V4 and V11, and a zero N control. Each year, soil bacterial community composition was variably influenced by CC and N management. With excessive May–June rainfall, radish and oat CC residues shaped community structure and membership differently regardless of soil sampling zone (between-row or in-row), but these residues influenced operational taxonomic units (OTUs) less under deficit May–June rainfall. Soil disturbance associated with coulter-injection of V4 SID placement influenced bulk soil community structure between-rows, whereas in 1 of 2 yr, the 5 × 5 N placement appeared to induce an in-row corn rhizosphere response resulting in OTU membership differences from other N strategies. Including slowly-available organic N as part of a well-balanced fertility program may stabilize the impact of inorganic N fertilizer to soil bacterial communities.

Since 2000, Michigan mean corn (*Zea mays* L.) production varied from 6.6–10.2 Mg ha^{-1} (USDA-NASS, 2018). Meta-analysis of Midwestern U.S. corn and soybean production (1916–2007) correlated yields with drought occurrence and maximum daily temperature during reproduction, which suggests climate variability may be one factor affecting yield consistency (Mishra & Cherkauer, 2010). Factors that enhance cropping system adaption and resilience to climate variability (e.g., moisture or temperature stress) may improve grain yield consistency (Gaudin et al., 2013; Lin, 2011; Song et al., 2015). Manipulating soil microbial communities in agriculture is one factor that may provide opportunities to influence plant health and build greater soil resilience to address climate variability (Hamilton et al., 2016). Plant health and

Abbreviations: AMOVA, analysis of molecular variance; AOB, ammonia oxidizing bacteria; BR, mid-way between two corn rows to simulate bulk soil; CC, cover crop; IR, between two corn plants within a row; OTU, operational taxonomic unit; PL, poultry litter; PPI, pre-plant incorporated; PCoA, principal coordinates analysis; SID, sidedress; UAN, liquid urea ammonium nitrate.

productivity are influenced by the interaction of plants, soils, and soil microbes (Chaparro et al., 2012). Soil bacteria are one component of the microbial biomass that can affect plant fitness directly as pathogens and beneficial mutualists, or indirectly as decomposers and plant pathogen antagonists (Rosenzweig et al., 2013). Agromanagement factors including crop rotation, tillage, residue management, weed control, organic additions (e.g., livestock or green manures), and N fertilization can alter soil bacterial community composition (Aristilde et al., 2017; Ashworth et al., 2017; Fierer et al., 2012; Figuero et al., 2015; Navarro-Noya et al., 2013; Schmidt et al., 2018; Tao et al., 2017). Increased understanding of interactions between agricultural management, soils, and soil bacterial communities may provide opportunities to influence crop production (Rosenzweig et al., 2013; Tautges et al., 2016).

Nitrogen fertilization is a necessary practice for many corn growers that can also influence soil bacterial communities. Growers who apply N must consider rate, placement, timing, and source (i.e., 4R management) for each application, which often includes pre-plant and in-season combinations in the Northern Corn Belt (Rutan & Steinke, 2018b). Research investigating soil bacterial communities as affected by the use of cover crops (Vukicevich et al., 2016), N fertilizer (Geisseler & Scow, 2014), and the long-term use of both (Guo et al., 2019; Schmidt et al., 2018) is well documented. However, aspects of N fertilizer management in microbial studies often focus on N rate (Zhu et al., 2016) and source (e.g., organic and inorganic N, nitrate-N, ammonium-N, or urea-N) (Giagnoni et al., 2016) with little data existing on the impact from N timing and placement strategies when practiced with cover crops (CCs). Nitrogen fertilization may induce changes to soil pH, increase return of organic materials due to aboveground and belowground biomass production, and influence root exudation and C resources (Geisseler & Scow, 2014). Ammonium-based N fertilizers are a source of soil acidity due to the release of H^+ during NH_4^+ nitrification with acidification varying by N source (e.g., anhydrous ammonia, urea, ammonium sulfate) (Havlin et al., 2014). Zhu et al. (2016) observed increased N rates affected composition of corn root exudates (i.e., increased sugars and phenolics), which altered soil microbial community structure and abundance. Ammonia-oxidizing bacteria and archaea require reduced forms of N as electron donors (Prosser & Nicol, 2012). However, addition of N fertilizer may directly inhibit soil microbial communities soon after application through ammonia toxicity, increased pH, and increased osmotic potential in the vicinity of fertilizer prills or bands (Geisseler & Scow, 2014). In contrast, urea applied up to 60 kg ha^{-1} and banded 7.5 cm to the side and 7.5 cm below the seed furrow at planting had little effect on soil microorganisms (Lupwayi et al., 2011). Analysis of long-term (i.e., 55 yr) inorganic fertilization indicated fertilized soils maintained soil organic C and total N stocks resulting in little impact to bacterial communities, and composition was more influenced by soil organic matter inputs and soil properties (Williams et al., 2013). Recent data suggest soil bacterial operational taxonomic unit (OTU) alpha diversity (i.e., richness) is mediated by soil NH_4–N availability whereas beta diversity (i.e., community composition) is the indirect result of N fertilizer on soil pH (Zeng et al., 2016). The multitude of corn N strategies utilized to address N availability may also impact soil bacterial communities. Studies regarding the impact of N timing and placement strategies when practiced with CCs are few and require further investigation.

Core Ideas

- Radish and oat influence to bacterial communities are seasonally variable.
- Disturbance associated with coulter-injected sidedress affects communities in between-row soil differently than in-row soils.
- Using 5 × 5 starter N induced a corn rhizosphere response affecting bacterial membership.
- Balancing a fertility program with a slowly available N source reduced impact of N management to bacterial communities.

Increasing cropping system diversity through CCs is one factor that increased organic C supply and influenced soil bacterial communities (Schmidt et al., 2018). Cover crops have been reported to increase soil microbial diversity, minimize soil-borne pathogen proliferation, and promote disease suppressive bacteria (Vukicevich et al., 2016). Following winter wheat (*Triticum aestivum* L.) harvest in Michigan, radish (*Raphanus sativus* L.) and oat (*Avena sativa* L.) CCs have been used to sequester residual autumn soil NO_3–N prior to a corn cash crop (Rutan & Steinke, 2018b). Radish and oat represent different CC types (i.e., brassica vs. C3 grass, respectively) and different root systems (i.e., taproot vs. fibrous, respectively), which may have differential impacts on soil microbial communities (Maul & Drinkwater, 2010; Vukicevich et al., 2016). Plants can modulate microbial community composition through litter quality, root morphology, and rhizodeposition, and may depend on the growth stage (Berg & Smalla, 2009; Cavaglieri et al., 2009; Chaparro et al., 2012; Cleveland et al., 2014). However, radish and oat CCs have reduced N availability to the ensuing corn crop and require CC-specific N strategies (Rutan & Steinke, 2018a).

In Michigan, growers have utilized different strategies when applying corn N to synchronize N availability with uptake. On medium-textured soils of the Northern Corn Belt, fertilizer strategies often include a single pre-plant incorporated (PPI) N application or split applications where starter fertilizer at planting is followed by in-season sidedress (SID)

(Rutan & Steinke, 2018a, 2018b). Surface broadcast or subsurface banded are two N placements that are widely used but that may impact soil bacteria differently. For instance, coulter-injection of SID N introduces an additional element of soil disturbance relative to PPI N when conducted simultaneously with conventional tillage. However, liquid urea ammonium nitrate (UAN), often utilized for subsurface band placement, increases fertilizer concentration unlike broadcast urea or poultry litter (PL). High clearance injection equipment has increased interest in delayed N applications up to corn V11 relative to the standard V4 timing (Rutan & Steinke, 2018b). Corn N demand increases during corn vegetative growth and adjusting N placement and timing to supply N may differentially impact soil communities due to mechanical soil disturbance, the ability to influence corn growth, or direct influence to communities. Organic N sources and CC residue supply a source of slowly available N and C creating opportunities to influence soil bacterial communities with different substrate requirements. Increased knowledge may provide additional N application guidance for improved crop health and soil resilience. The objective of this study was to utilize 16S rRNA amplicon sequencing to compare the effects of a radish, oat, and no CC when practiced with corn N timing and placement strategies on soil bacterial community composition.

1 | MATERIALS AND METHODS

1.1 | Experimental sites

Two field studies were conducted in Lansing, MI, in 2014–2015 (Site Year 1 [SY1]) and 2015–2016 (Site Year 2 [SY2]) on a Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalf). At August CC establishment each year, soils were sampled (20-cm depth) for initial analysis of chemical properties, air-dried, and ground (2-mm sieve size). Soil characteristics were 24–34 g kg^{-1} soil organic matter (loss-on-ignition; Combs and Nathan, 2015), 5.8–6.0 pH (1:1 soil/water; Peters et al., 2015), 24–55 mg kg^{-1} P (Bray-P1; Frank et al., 2015), and 122–136 mg kg^{-1} K (ammonium acetate method) (Warncke & Brown, 2015). The study was a split-plot randomized complete block design with four replications. Main plots were CC treatment, and subplots were N strategy. Whole plots measured 27.4 m × 12.2 m in length, and subplots measured 4.6 m × 12.2 m in length. Fields were previously cropped to winter wheat (*Triticum aestivum* L.), chisel plowed after wheat harvest, and disk harrowed (10-cm) prior to CC establishment. Broadcast P and K fertilizer were applied PPI 1 d prior to corn seeding (10-cm depth) as triple super phosphate (0-45-0 N-P-K) and muriate of potash (0-0-62) based on soil test. Corn weed control consisted of acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl) acetamide] and glyphosate [N-(phosphonomethyl) glycine] followed by a second application of glyphosate 17–24 d later. During the growing season, precipitation, soil moisture (0–30 cm), and air temperature were collected using the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI; verified 24 July 2020) from an onsite weather station.

There were eighteen experimental treatments. Cover crop treatments consisted of 'The Buster' daikon radish, 'Magnum' forage oat (Weaver Seed of Oregon), and no CC. Radish and forage oat CCs were drill-planted (14 Aug. 2014, 17 Aug. 2015) at 11.2 and 28.0 kg ha^{-1}, respectively, using a Gandy Orbit-Air Seeder coupled with John Deere double disk openers in 19-cm rows. A single glyphosate application was applied to the no CC treatment in autumn to eliminate vegetative ground cover. To ensure winterkill, CCs were terminated with glyphosate in the autumn after 79–83 d of growth (3 Nov. 2014, 4 Nov. 2015). Nitrogen management strategies were initiated at corn planting and consisted of five strategies previously investigated for the Northern Corn Belt and a zero-N control (Rutan & Steinke, 2018a, 2018b). Nitrogen strategies were balanced to the Michigan 2015 recommended maximum return to N rate of 179 kg N ha^{-1} (https://www.canr.msu.edu/soilfertility/Files/Articles/General-Soil-Fertility/2015%20MRTN%20N%20Rates%20for%20Corn.pdf, Michigan State University, East Lansing, MI; verified 26 June 2021) and consisted of urea-N PPI (46-0-0), dried poultry litter (4-3-2) PPI at 2.2 Mg ha^{-1} (61 kg N ha^{-1} first year available N) plus V11 SID N, and 5 × 5 starter N (45 kg N ha^{-1} urea ammonium nitrate [UAN; 28-0-0], hereafter referred to as 5 × 5) followed by SID N at V4, V11, or split (50:50) V4 and V11. Sidedress N at V4 corn was coulter-injected UAN 5 cm deep and 38 cm to the side of each row. At V11, SID N was UAN mixed with a urease inhibitor [CO(NH2)2 + n-(n-butyl) thiophosphoric triamide] (Koch Agronomic Services) to prevent N volatilization and banded 10–15 cm to the side of each row. Cover crops did not affect soil NO_3^- relative to no CC at subplot initiation, as reported in a subsequent study (Rutan & Steinke, 2018a). Nitrogen sources were plant available during corn growth with the exception of PL, for which treatment total N accounted for first year N availability (61 kg N ha^{-1}). Corn was seeded in 0.76 m rows at 84,510 seeds ha^{-1} with Dekalb DKC48-12 refuge-in-bag (98-d relative maturity; Monsanto) on 1 May 2015 and 17 May 2016, respectively.

1.2 | Data collection

1.2.1 | Soil sampling

In each study, soils were sampled at five time points. At CC establishment, soils were sampled per replication as a baseline analysis. Whole plots were sampled from each replication at

CC termination (autumn) and corn planting (spring). Following corn planting, subplot soils were sampled at growth stages R1 (13 July 2015, 21 July 2016) and R6 (9 Oct. 2015, 12 Oct. 2016) from two sampling zones: 38 cm between two corn rows to simulate bulk soil (BR), and between two corn plants within a row (IR) to simulate soil of the corn rhizosphere. Six soil cores (2.2-cm × 10-cm depth) were collected and homogenized per sample, air-dried, and ground to pass through a 2-mm sieve. Soils were stored at ambient temperature (20°C) in ziptop bags for DNA extraction and 16S sequencing.

1.2.2 | 16S rRNA genomic sequencing

MO-BIO Power Soil kits (MO BIO Laboratories) were used to extract soil genomic DNA from 0.25 g of soil according to manufacturer protocol and stored at −20 °C. Although not required, dried soils were utilized when weighing in order to achieve consistent sample size. Dried soils have also been used previously in the microbial analysis of archived soils (Dolfing et al., 2004). Polymerase chain reaction was used to amplify the V4 hypervariable region (length 250 bp) of the 16S gene using high fidelity polymerase (Accuprime Pfx Supermix; ThermoFisher Scientific) and genomic DNA as a template. Amplicons and libraries were constructed per previously described methods and supplementary protocol (Kozich et al., 2013). The MiSeq Illumina platform was used to sequence amplicon libraries submitted to the Michigan State University Research and Technology Support Facility in East Lansing, MI, for next-generation sequencing using the multiplex coded-primer (tag) approach. Sequence data were processed through the Michigan State University High Performance Computing Center in East Lansing, MI, and analyzed using a previously described analysis pipeline (Kozich et al., 2013) and protocol (https://mothur.org/wiki/miseq_sop/; verified 27 June 2021) with the Mothur software package (version 1.33.2b; Schloss et al., 2009). Additional polymerase chain reaction and library preparation methods can be found in Supplemental File S1. Soil bacterial OTUs were based on a 97% sequence identity and classified to the SILVA database (Quast et al., 2013).

1.3 | Data analysis

Due to computational load (each year contained 316 soil samples), years were analyzed separately. Additionally, a "mock" community was not used in the study and precluded error rate estimation in the analysis (Smith et al., 2016). Replicates were analyzed as individual samples and grouped using design file subroutines in MOTHUR. Sequences were subsampled (i.e., 9,500, Year 1; 6,000, Year 2) to standardize data. The 'make.biom' command in MOTHUR was used to generate BIOM files for visualization in MicrobiomeAnalyst (Dhariwal et al., 2017; https://www.microbiomeanalyst.ca/; verified 29 Jan. 2019). Univariate analyses using Kruskal–Wallis and Mann–Whitney tests of significance were conducted online within MicrobiomeAnalyst and used to indicate treatment-affected taxa with a false discovery rate cutoff of $P < .05$. Beta diversity (i.e., taxa relative abundance between samples) was visualized with unconstrained principal coordinates analysis (PCoA) based on Bray–Curtis similarity coefficients. Bacterial relative abundance for PCoA visualization were low-count filtered to a minimum count of four sequences in 20% of samples, low-variance filtered for variance (10%) based on interquantile range, total sum scaled, and figures generated with MicrobiomeAnalyst online using permutational ANOVA to validate clustering significance (Dhariwal et al., 2017). Centroids representing the interaction of cover crop, N strategy, soil sample zone (i.e., bulk soil whole plots, IR, and BR soils), and soil sample time (i.e., autumn, spring, corn R1, and R6) were pooled across four replications. Ordinations served as a proxy for parceling datasets by soil sampling time by zone for treatment separation. Changes in community structure (i.e., combination of membership and OTU abundance) and membership (i.e., list of OTUs in a community, or presence vs. absence) were analyzed between treatments (Schloss & Handelsman, 2006a). Distance matrices were generated using the Yue and Clayton measure of dissimilarity to compare community structure, and the Jaccard index of dissimilarity to compare community membership for cover crop, N strategy, and their interaction at each sampling time x zone. Hypothesis testing within each sampling zone by time observation was conducted on distance matrices using analysis of molecular variance (AMOVA) to assess the spatial separation of clustering centroids. Analysis of molecular variance tested whether genetic diversity between two populations was significantly different from the variation that would result from pooling the two populations. The metastats command, a non-parametric T-test, was used to investigate CC impact to individual OTUs.

2 | RESULTS AND DISCUSSION

2.1 | Environmental conditions

At May corn seeding in SY1, air temperatures were 10.9% above normal followed by 5.4% below normal from June to August (Figure 1a). Cumulative precipitation in May, June, and August was 28 to 116% above normal resulting in periods of wet soils (Figure 1b). In contrast to SY1, air temperatures following corn planting in SY2 were up to 7.5% above normal from May to August. However, cumulative May–June precipitation was 39 and 80% below normal, respectively, resulting in dry soils that reduced corn N uptake (Rutan &

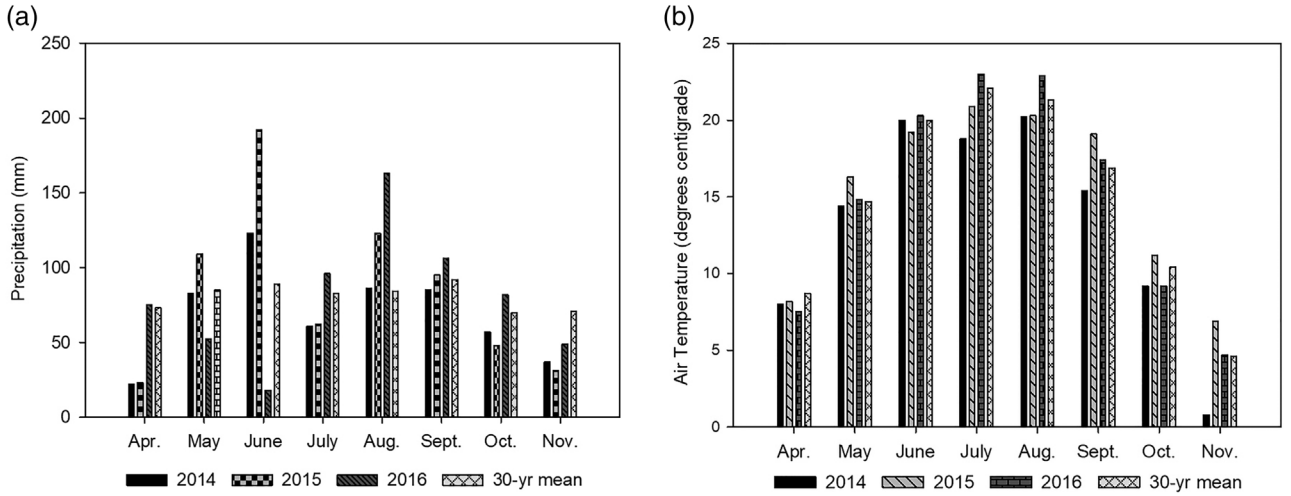

FIGURE 1 Cover crop and corn growing season (a) monthly mean air temperature and (b) monthly cumulative precipitation (mm), including 30-yr mean data, for Lansing, MI, 2014–2016. Air temperature data were collected from the Michigan Automated Weather Network (https://mawn.geo.msu.edu/). Precipitation data were collected from the Michigan Automated Weather Network (https://mawn.geo.msu.edu/). Source for 30-yr mean, NOAA (https://www.ncdc.noaa.gov/cdo-web/datatools/normals)

Steinke, 2018b). Reduced corn N uptake can have implications for the ability of a growing corn crop to influence rhizosphere bacterial communities (Bell et al., 2015; Zhu et al., 2016).

At R1 and R6 soil sampling times, soil moisture varied between years. Despite below normal rainfall in July SY1, soil moisture at R1 was 0.27 cm^3 cm^{-3} as compared with 0.09 cm^3 cm^{-3} (mean of 14 d leading up to soil sample time) in SY2. At R6, soil moisture was 0.17 and 0.24 cm^3 cm^{-3} in SY1 and SY2, respectively. Differences in soil moisture and temperature may result in distinct microbial communities and explain temporal variability of community composition (i.e., at multiple sampling times) (Bainard et al., 2016). Although soil moisture was not collected on each experimental unit in the current study, differences at R1 and R6 each year may help explain community variability at each sampling time. Future studies may be enhanced using redundancy analysis to identify edaphic properties such as soil moisture and temperature predictive of spatial structure and temporal community variation.

2.2 | Soil bacterial community composition

Using a 97% identity cutoff and quality filtering, the mean number of OTUs identified per sample was 3,391 ± 846 in SY1 and 2,810 ± 800 in SY2. Results suggested that previous estimates of 2,000–5,000 OTUs may be conservative (Schloss & Handelsman, 2006b). In SY1 and SY2, a total of 91,376 and 49,203 OTUs, respectively, were identified, but only 11–18% were represented by more than 20 reads, which has been considered a cutoff for rare OTUs (Table 1; Zhao et al., 2014). Sequencing identified 24 and 26 bacterial phyla in SY1 and SY2 (24 phyla appearing across both years), and 531 and 500 genera, respectively.

Sequence reads indicate an abundance of members, whereas OTU numbers represent the distinguishable taxa (Fernandez et al., 2016). Of the phyla detected, 10 accounted for more than 75 and 50% of all sequence reads and OTUs, respectively (Table 2).

The top four phyla shared in each site year (*Acidobacteria*, *Proteobacteria*, *Actinobacteria*, and *Planctomycetes*) represented 40–43% of the total OTUs identified, whereas up to 26% of reads were unclassified at the phylum level. In both site years, the same four phyla dominated the composition of soil bacterial communities collected before and after corn planting regardless of soil sampling zone (except *Planctomycetes* in Year 2 IR samples at R1; Supplemental Table S1). The top three most abundant phyla (*Acidobacteria*, *Proteobacteria*, and *Actinobacteria*) have also dominated agricultural soils in another study (Orr et al., 2012). Similar phyla in BR and IR soils indicate the corn crop did not introduce new phyla but instead modified native communities. The interaction of soil sampling zone by soil sampling time affected the relative abundance of most phyla in both site years ($P < .05$; Supplemental Table S1). After corn planting, relative abundance differences between BR and IR soil bacterial phyla were most apparent at R1. Additionally, variation between R1 BR and IR phyla were greater in SY2 than SY1. Greater variation indicated a stronger influence of the corn rhizosphere in SY2 and was likely influenced by differences in soil moisture (previously discussed). In both site years, similar trends observed at R6 were less apparent than R1 and suggested that the influence of the corn rhizosphere is temporally variable. In SY1

TABLE 1 Summary of 16S sequencing analysis[a] and operational taxonomic units (OTUs) classification for soil samples collected in Site Year 1 and Site Year 2

Year	No. of samples	Total no. of OTUs[b]	Avg. no. OTUs per sample	Classification[c]				
				Phyla	Classes	Orders	Families	Genera
1	316	91,376	3,391	24	68	110	226	531
2	316	49,203	2,810	26	72	112	224	500

[a]Taxonomic sequences were aligned to the Silva-based bacterial database.
[b]Total number of sequences in Year 1, 9,236,936; total number of sequences in Year 2, 7,104,617.
[c]Classifications exclude unclassified samples.

TABLE 2 Relative abundance of phylum composition representing ≥0.8% of total bacterial sequence reads and number of operational taxonomic units (OTUs) within each rank observed in Site Year 1[a] and Site Year 2[b]

Phylum	Year 1		Year 2	
	Reads	OTUs	Reads	OTUs
	%			
Acidobacteria	18.3	8.1	15.6	6.0
Actinobacteria	11.8	7.6	14.2	8.0
Armatimonadetes	1.5	1.0	1.3	1.1
Bacteroidetes	4.3	2.8	3.7	3.8
Chloroflexi	1.0	1.0	1.3	1.4
Firmicutes	3.8	3.5	5.6	5.4
Gemmatimonadetes	0.9	1.4	0.9	1.0
Planctomycetes	8.7	10.7	8.7	6.7
Proteobacteria	17.3	16.8	18.8	19.5
Unclassified	26.3	43.9	24.8	42.0
Verrucomicrobia	5.7	1.9	4.8	2.6
Other[c]	0.4	1.5	0.3	2.5

Note. Bacterial taxa were assigned at a 97% sequence similarity cut-off level.
[a]In Year 1, 9,236,936 sequences, 91,376 OTUs, and 24 bacterial phyla were identified.
[b]In Year 2, 7,104,617 sequences, 49,203 OTUs, and 26 bacterial phyla were identifiedx.
[c]Others represent bacterial phyla <0.8%.

IR soils, differences in phyla relative abundance between R1 and R6 indicated more variation than BR soils. Similar trends were observed in Year 2 but indicate that temporal variation was an important factor that influenced bacterial community composition, and that corn ontogeny (i.e., R1 and R6) affects the influence of the corn rhizosphere (Yang et al., 2017).

Ammonia-oxidizing bacteria (AOB) are a functionally important group regarding biological oxidation of NH_3 to NO_2–N and NO_3–N, and consist of two major genera including *Nitrosomonas* and *Nitrosospira* (Liu et al., 2018). Other autotrophic bacteria, *Nitrosolobus*, *Nitrospira*, and *Nitrosovibrio*, can also oxidize NH_4–N (Havlin et al., 2014). Although plant available N was contained in the urea ammonium nitrate source utilized in 5 × 5 starter and SID N, urea and NH_4–N still require oxidation. Nitrogen fertilizer application can affect AOB structure and impact potential nitrification rates (Ai et al., 2013). Sequencing indicated the presence of *Nitrobacter* (0.007 and 0.012% relative abundance), *Nitrosomonas* (0.002 and 0.001% relative abundance), *Nitrosospira* (0.261 and 0.237% relative abundance), and *Nitrospira* (0.234 and 0.196% relative abundance) in SY1 and SY2, respectively. When genera were analyzed across CCs in SY1, univariate analyses of R1 soils indicated that CCs influenced the relative abundance of *Nitrospira* (radish, 0.26%; oat, 0.20%; no CC, 0.23%; $P \leq .05$). In a similar manner, analyses indicated relative abundance of *Nitrosospira* was influenced by N management where the zero-N control, PL, and starter + V4, V11, or split SID resulted in 0.19, 0.31, 0.36, 0.30, 0.41, and 0.43%, respectively. In SY2, CC did not impact AOB, whereas similar results were observed with N management and was consistent with other research indicating *Nitrosospira*-like AOB prefer an enriched N environment (Liu et al., 2018). However, relative abundance of *Nitrosospira* (0.23%) in PL was less than other N strategies where it is likely dry soils reduced mineralization of organic C sources (i.e., CC and PL). Although influence of N management was more consistent between years on AOB, magnitude of management impacts may be weather dependent.

2.3 | Factors affecting soil bacterial community beta-diversity

Data observed at R1 and R6 were explored with PCoA ordination at the OTU level. Plots of centroids representing each combination of CC, N management, soil sampling zone, and sample timing indicated that cluster separation improved when grouped using sample zone by timing factors (Figures 2a,b). Centroids of autumn and spring soil samples were included and clustered separate from other sampling times at the class level (data not shown).

Across both years, centroid clusters at the OTU level were most apparent according to soil sampling time followed by sampling zone. The separation suggests that variation in bacterial community structure was most associated with

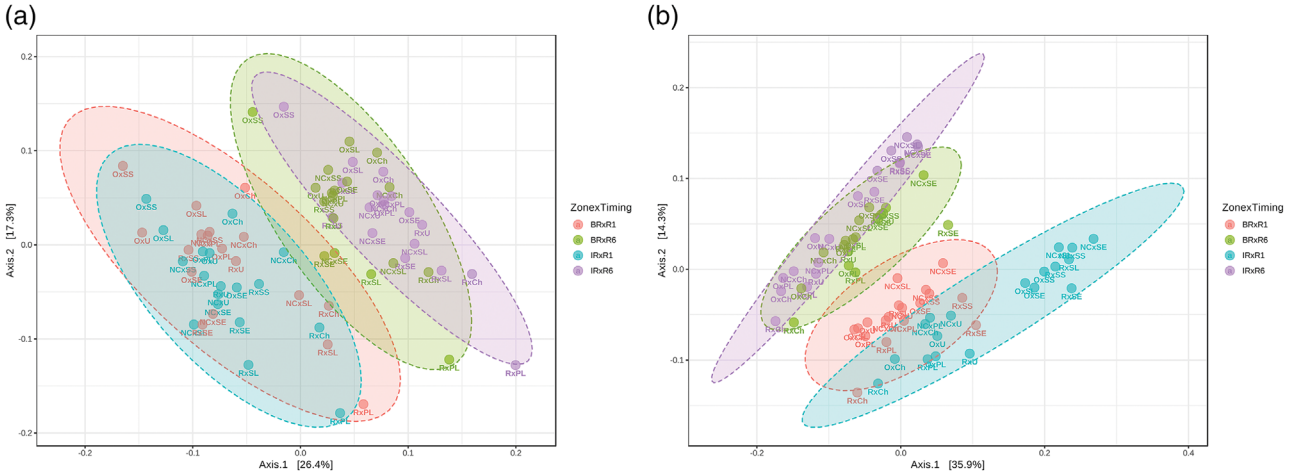

FIGURE 2 (a) Year 1 and (b) Year 2 ordination by principal coordinates analysis plots of operational taxonomic unit-level soil bacteria illustrating community clustering by soil sample zone and timing combinations (PERMANOVA $P \leq .001$). BR, between-row; IR, in-row; R1 and R6 designate corn growth stages. The principal coordinates analysis plot was built based on Bray–Curtis distances for each four-factor combination of cover crop, N management, zone, and timing combined across four replications. The percentage of total variance explained by each axis is shown. Centroids are labeled by cover crop (R, radish; O, oat; NC, no cover crop) followed by N management (Ch, zero-N control; U, urea; PL, poultry litter+V11 sidedress; SE, 5 × 5+V4 sidedress; SL, 5 × 5+V11 sidedress; and SS, 5 × 5+split sidedress) separated by "x"

temporal followed by spatial differences. Maul et al. (2014) noted that season of the year had the strongest influence on soil microbial community structure. Greatest overall separation between centroid clusters occurred between IR soils sampled at corn growth stage R1 and R6 (i.e., purple relative vs. blue on plots). Clusters representing BR soil samples collected at either R1 or R6 were separated but less apparent than IR soils (i.e., red vs. green). Separation of BR samples reflected the effect of season on structuring soil bacterial community structure. The interaction between soil sample timing and zone suggests that season has variable influence on BR as compared with IR soil bacterial community structure. Season appeared to interact with corn ontogeny and influenced rhizosphere community structure to a greater degree than bulk soil. Greater cluster separation between R1 and R6 IR samples suggests communities are dynamic and the influence of the corn rhizosphere on community structure varies temporally between R1 to R6. Cluster separation indicated the influence of CC and N strategies were less than the influence of soil sampling location and time. Data are consistent with previous studies indicating plant development influences rhizosphere community structure (Cavaglieri et al., 2009). Distinct root exudate phytochemicals excreted at specific growth stages structure the rhizosphere microbiome (Jacqueline et al., 2014). However, net corn rhizodeposited C is dependent upon photosynthesis rate and suggests seasonal variation of corn growth and development may likewise result in a seasonably variable rhizosphere influence (Amos & Walters, 2006).

In SY2, separation of OTU centroids clustered by soil sampling zone × time (i.e., purple vs. green; red vs. blue) were more apparent than in SY1 (Figure 2a). Greater separation in SY2 was evident between BR and IR samples collected at either R1 or R6 and suggests a greater rhizosphere influence. Inconsistent separation between site years indicated rhizosphere influence from corn may not be similar each year.

Nitrogen management practices that influence corn growth may indirectly impact the ability of the corn crop to influence the rhizosphere community (Zhu et al., 2016). Plot centroids were labeled according to CC by N management to investigate the impact of applied treatments to -diversity. Centroid groups according to N management were more apparent than CC. Within each sampling zone by timing cluster, centroids representing N management strategies that included 5 × 5 starter N were distinct from those consisting of PL, urea, and zero-N, which tended to group in close proximity. Grouping indicated that N management in addition to soil sample zone by timing was causing dispersion of centroids and influenced soil bacterial community structure. Grouping was more apparent in SY2 relative to SY1, further suggesting that management factors may not have similar impacts on soil communities each year. Additional ordinations averaged across soil sample zone and timing and grouped by CC further emphasized the separation of N management centroids in SY2 relative to SY1 (Supplemental Figures S1 and S2). Although ordinations are a visual observation tool and do not provide statistical evidence of differences in community structure, differences in distance between centroids in Supplemental Figure S2 provides further evidence suggesting that N management strategies, which include 5 × 5 starter N, are distinct from strategies which did not.

TABLE 3 Analysis of molecular variance (AMOVA) on soil bacterial community structure and membership as affected by cover crop (CC) (no CC, radish, or oat) in whole plots (WP) prior to corn crop establishment (Pre), at corn silking (R1) and at physiological maturity (R6) sampled from between-row (BR) and in-row (IR) soils in Site Year 1[a]

Treatment comparisons	Structure[b]					Membership[c]				
	WP[d]	BR		IR		WP	BR		IR	
	Pre	R1	R6	R1	R6	Pre	R1	R6	R1	R6
	AMOVA *p* value[e]									
Global[f]	.01*	.02*	.01*	<.01*	<.01*	.02*	<.01*	<.01*	<.01*	<.01*
No CC, radish	.19	.13	.07	.04*	.05*	.19	.06	.06	.03*	.04*
No CC, oat	.25	.12	.09	.20	.29	.33	.04*	.02*	.02*	.06
Radish and oat	<.01*	<.01*	<.01*	<.01*	<.01*	.01*	<.01*	<.01*	<.01*	<.01*

[a]No differences between cover crops were observed in Year 2.
[b]Analysis of molecular variance based on Yue and Clayton measure of dissimilarity between community structures.
[c]Analysis of molecular variance based on the Jaccard similarity coefficient based on observed richness.
[d]Compared with soils sampled prior to CC establishment, WP community structure combined across autumn and spring observations was affected by oat ($P < .01$), whereas membership was affected by radish ($P = .05$) and oat ($P = .02$).
[e]Probability that bacterial communities were similar between pair-wise comparisons.
[f]Global test represents significant effect of treatments at $\alpha = .05$.
*Significant at the .05 probability level.

2.4 | Analysis of community structure and membership

2.4.1 | Cover crops

Analysis of molecular variance on OTU structure and membership confirmed differences due to CCs only in Year 1 (10 of 10 observations). Global tests of significance in Year 1 indicated community composition (i.e., structure) and membership were affected by CCs at each soil sampling time by sampling zone (Table 3).

Results suggest community structure and membership were more sensitive to CCs in a wet year (i.e., SY1) rather than N management. Despite a lack of differences relative to no CC, differences in structure and membership occurred between radish and oat CCs. After corn establishment, oat only affected community membership whereas radish also affected community structure, suggesting the selection of CC species can have differential impacts to biological communities. Radish affected community structure and membership only within IR soils regardless of sampling time. In March, radish CC residues were decomposed unlike oat residues likely due to the reduced C/N ratio as a result of less lignin content contained in radish residue (Jahanzad et al., 2016; Rutan & Steinke, 2018a). Despite reduced March biomass, radish CCs were still able to impact communities through corn maturity. Recent evidence suggests that CC litter quality affects corn mediation of CC litter decomposition and may stimulate corn rhizosphere C allocation utilized by bacteria (Rosenzweig et al., 2017; Schipanksi et al., 2017). Data suggest changes to community structure and membership of IR soils due to radish CC may be an indication of corn rhizosphere priming. However, no community changes due to CCs were observed in SY2, emphasizing the interannual variability growers may encounter when using CCs to influence biological communities. In the current study, CCs were terminated in the autumn and incorporated the following spring. Cover crop residue management methods including incorporation, shredding, or termination date may affect the influence and consistency CCs have on community structure (Buyer et al., 2010). Further research is needed to generalize trends across years.

2.4.2 | Nitrogen management

In contrast to CCs, differences due to N management on OTU structure and membership were more prevalent in a dry year (i.e., SY2; seven of eight observations) relative to a wet year (i.e., SYI; one of eight observations), illustrating the impact of weather variability on community response to N (Table 4). Global tests of significance indicated that N management affected community structure 2 of 2 yr and membership in 1 of 2 yr.

In contrast to IR soils, BR soil analysis in SY1 indicated community structure differences where treatments included V4 SID relative to the zero N control. No differences were observed between treatments receiving full SID at V4 or split N applications. Similar results were observed in SY2, suggesting weather did not impact the consistency of results; our results agree with other studies that observed consistent impact of N inputs to community diversity over multiple years (Ramirez et al., 2010; J. Zhou et al., 2017). When compared with PL, treatments receiving V4 SID affected R1

TABLE 4 Analysis of molecular variance (AMOVA) on soil bacterial community structure and membership as affected by N management (zero-N, pre-plant incorporated N [urea], poultry litter + V11 sidedress [PL+V11], and subsurface banded starter N followed by sidedress at V4 [5 × 5+V4], V11 [5 × 5+V11], or 50:50 split [5 × 5+split]) at corn silking (R1) and at physiological maturity (R6) sampled from between-row (BR) and in-row (IR) soils in Site Year 1[a] (SY1) and Site Year 2 (SY2)

	Structure[b]			Membership[c]		
Treatment comparisons	SY1, BR, R1	SY2, BR, R1	SY2, BR, R6	SY2, IR, R1	SY2, IR, R6	SY2, BR, R6
	AMOVA p value[d]					
Global[e]	.01	.02	.03	<.01	<.01	.04
Zero-N vs. urea	.09	.42	.10	.23	.11	.08
Zero-N vs. PL+V11	.24	.16	.23	.53	.37	.14
Zero-N vs. 5 × 5+V4	.02*	.02*	<.01*	<.01*	<.01*	<.01*
Zero-N vs. 5 × 5+V11	.69	.21	.13	<.01*	<.01*	.10
Zero-N vs. 5 × 5+split	.01*	.03*	.02*	<.01*	<.01*	.02*
Urea vs. PL+V11	.16	.37	.64	.64	.57	.56
Urea vs. 5 × 5+V4	.35	.07	.07	.02*	.03*	.18
Urea vs. 5 × 5+V11	.21	.70	.73	.03*	.05*	.77
Urea vs. 5 × 5+split	.37	.17	.34	.02*	<.01*	.59
PL+V11 vs. 5 × 5+V4	.07	.04*	.08	.02*	<.01*	.12
PL+V11 vs. 5 × 5+V11	.42	.23	.47	.04*	.02*	.51
PL+V11 vs. 5 × 5+split	.03*	.04*	.20	.02*	<.01*	.21
5 × 5+V4 vs. 5 × 5+V11	.08	.07	.04*	.89	1.00	.11
5 × 5+V4 vs. 5 × 5+split	.16	.82	.28	.86	.90	.31
5 × 5+V11 vs. 5 × 5+split	.03*	.16	.45	.97	1.00	.74

[a]Community structure was not affected by N management in IR soil samples at R1 ($p = .37$) and R6 ($p = .22$) or BR soils at R6 ($p = .08$) in Year 1. Membership was not affected in Year 1 ($p \geq .10$) in addition to IR soils at R1 in Year 2 ($p = .28$).

[b]Analysis of molecular variance based on Yue and Clayton measure of dissimilarity between community structures.

[c]Analysis of molecular variance based on the Jaccard similarity coefficient based on observed richness.

[d]Probability that bacterial communities were similar between pair-wise comparisons.

[e]Global test represents significant effect of treatments at $\alpha = .05$.

*Significant at the .05 probability level.

BR soil community structure in three out of four observations across years, but no differences occurred between PL and starter plus full V11 SID. Results suggest BR soil community structure differences due to treatments containing V4 SID were due to soil disturbance associated with coulter-injection at SID V4 (Smith et al., 2016). Soil disturbance can affect microbial communities through habitat modifications including pore space, oxygen movement, and physical disruption between organisms and the soil pore network (Young & Ritz, 2000). Coulter-injection creates a semi-opened furrow in bulk soil between corn rows. Similar trends associated with coulter-injected SID in both years indicate soil disturbance as a likely mechanism that influenced community structure response. In SY2, global tests for R6 BR soil data were significant, and indicated community structure was still different between treatments receiving V4 SID and the untreated control. Due to deficit May–June rainfall in SY2, injection furrows remained visible through corn reproductive stages. Differences in BR soils at R6 illustrate the longevity soil disturbance can have on community structure, which may be prolonged in a dry year.

Global tests of significance indicated N management affected soil bacterial membership only during SY2 (Table 4). At R1, differences in the IR and not BR soil, as indicated by AMOVA, suggest an actively growing corn crop influenced community membership. In IR soils at R1 and R6, treatments receiving 5 × 5 starter N resulted in different community membership relative to other treatments, whereas no differences were observed between urea PPI, PL, and the zero-N control. In contrast to BR soils, IR soils were not disturbed by coulter-injection. Instead, data indicate 5 × 5 starter N may have influenced the capacity of the corn to influence the rhizosphere community. No differences observed between 5 × 5 starter treatments provide further support. A lack of influence on IR soils in SY1 illustrates the interannual variability N management may have on soil biological health. Whereas the 5 × 5 placement is a recommended practice to supply sufficient N for early corn growth and provide yield

TABLE 5 Analysis of molecular variance (AMOVA) on soil bacterial community structure[a] as affected by cover crop N management (no N fertilizer [zero-N], pre-plant incorporated N [urea], poultry litter + V11 sidedress [PL+V11], and subsurface banded starter N followed by sidedress at V4 [5 × 5+V4], V11 [5 × 5+V11], or 50:50 split [5 × 5+split]) at corn silking (R1) and at physiological maturity (R6) sampled from in-row soils in Site Year 2[a]

	R1			R6		
Treatment comparisons	**No cover**	**Radish**	**Oat**	**No cover**	**Radish**	**Oat**
	AMOVA p value[b]					
Global[c]	.014	.014	.014	.004	.004	.004
Zero-N vs. Urea	.98	.16	.44	.42	.32	.32
Zero-N vs. PL+V11	.88	.34	.38	.72	.15	.69
Zero-N vs. 5 × 5+V4	.08	.03*	.09	.05*	.03*	.13
Zero-N vs. 5 × 5+V11	.14	.01*	.08	.05*	.06	.13
Zero-N vs. 5 × 5+split	.11	.03*	.05*	.03*	.07	.04*
Urea vs. PL+V11	.88	.76	.83	.70	.98	.70
Urea vs. 5 × 5+V4	.11	.24	.16	.12	.26	.29
Urea vs. 5 × 5+V11	.20	.13	.17	.16	.12	.66
Urea vs. 5 × 5+split	.14	.28	.19	.05*	.10	.18
PL+V11 vs. 5 × 5+V4	.12	.13	.13	.06	.14	.22
PL+V11vs. 5 × 5+V11	.20	.09	.17	.11	.07	.26
PL+V11vs. 5 × 5+split	.10	.12	.12	.03	.06	.09
5 × 5+V4 vs. 5 × 5+V11	.81	.97	.87	.83	.84	.86
5 × 5+V4 vs. 5 × 5+split	.81	.98	.91	1.00	.92	.85
5 × 5+V11 vs. 5 × 5+split	.94	.98	.85	.92	.99	.71

[a]Analysis of molecular variance based on Yue and Clayton measure of dissimilarity between community structures.
[b]Probability that bacterial communities were similar between pair-wise comparisons.
[c]Global test represents significant cover crop × N management interaction ($p \leq .01$). Global tests did not indicate a significant interaction of cover crop and N management in Year 1.
*Significant at the .05 probability level.

consistency (Rutan & Steinke, 2018b), N management that includes PL may likewise provide community stability during corn growth.

Analysis of IR soils in SY2 indicated community structure was significantly affected by a CC and N management interaction at R1 and R6 sampling times (Table 5).

The interaction indicated a previous CC influenced corn rhizosphere response to N management. Cover crop residues can interact with an annual crop to alter rhizosphere microbial community structure due to a combination of stimulated root exudation and nutrient release during decomposition (Buyer et al., 2010). Bell et al. (2015) observed correlations between soil N availability and tissue N levels with rhizosphere bacterial community structure and concluded that reduced soil N availability due to plant N uptake selected for soil bacteria able to survive decreased N conditions. In a similar manner, differences in soil N availability due to N strategies may likewise influence the capacity of the corn to structure its rhizosphere (i.e., IR samples) as compared with bulk soils (i.e., BR samples). Most prevalent were CC × N management contrasts within the zero-N control. Relative to the zero-N control at R1, community structure following a radish CC was significant when treatments received 5 × 5 placed starter N, as well as split-applied SID following oat. Unlike BR soils, V11 SID following 5 × 5 starter influenced IR soil community structure when preceded by a radish CC, suggesting different mechanisms due to CC × N strategy structure communities. However, structure was unaffected with strategies containing slowly available N as PL or with PPI (100% urea). Rutan and Steinke (2018a) collected and reported agronomic corn yields from the current study. In that publication, PPI N applied as 100% urea to medium-textured soils reduced corn yields unlike with PL, which suggested growers incorporating a slowly available N source may stabilize impact to soil bacterial communities and maintain corn yields.

A post-hoc Mann–Whitney/Kruskal–Wallis analysis indicated the relative abundance of seven phyla were significantly affected by the interaction of CC and N management ($P \leq .05$), which explains influence to community structure. Relative to the zero-N control, the relative abundance of *Acidobacteria*, *Armatimondetes*, *Verrucomicrobia*, and WS3 declined where 5 × 5 starter followed CCs whereas the relative abundance of *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* increased (data not shown). The addition of N fertilizer may

TABLE 6 Number of significantly different operational taxonomic units (OTUs) from top 1,000 abundance OTUs due to cover crops during corn growth[a] in Site Year 1 and Site Year 2

Cover crop[b] comparison	Top 1,000 OTUs	
	no.	
No cover vs. radish	322	271
No cover vs. oat	293	272
Radish vs. oat	535	200

[a]Data are relative to observations collected at R1 and R6 corn.
[b]Operational taxonomic units were averaged across N strategy, soil sampling time, and soil sampling zone.

have implications regarding soil disease suppression. In general, members of the genus *Streptomyces* (of Actinobacteria) promote recalcitrant organic matter decomposition and produce secondary metabolites, which act as antibiotics (Rosenzweig, 2014). Results at R6 contrasted with R1. Within each CC at R6, strategies that included 5 × 5 starter reduced *Acidobacteria* relative to the zero-N control, whereas the magnitude of reduction was less following a CC and was consistent with other studies (Y. Zhou et al., 2017). Despite CC termination the previous November (approximately 11 mo earlier), data suggest CCs and N management interact to influence corn rhizosphere community structure. Future studies should include soil sampling of the following rotational crop to determine the longevity of community response to management.

2.5 | Differentially abundant features

Metastats analyses compared differences in OTU relative abundance due to CC, thus providing an indication of cropping system diversity (Table 6).

In SY1, metastats analyses of the top 1,000 OTUs indicated more OTUs were significantly different from the no CC when corn was followed by oats than radish. Data suggest the oat CC produced fewer shifts in OTU abundance relative to no CC (i.e., the most similar communities). There was an increase in the number of dissimilar communities between radish and oat CCs indicating these two species were influencing different taxa. Differences between radish and oat CCs in SY1 suggest that CC species selection may be an important tool growers may use to manipulate the soil microbiome to increase plant health (Chaparro et al., 2012). Results in SY2 contrasted with SY1 where radish and oat CC resulted in fewer dissimilar communities between each other relative to no CC. In fact, the number of dissimilar communities were nearly the same when radish or oat was compared with no CC, and corresponds to AMOVA results indicating no impact to community structure and membership in SY2. Differences in soil moisture each year may have affected community composition more than crops (Bainard et al., 2016) and likely affected CC mineralization and substrate availability. Cover crop litter quality has also influenced community composition (Vukicevich et al., 2016). Although radish resulted in the most biomass production (Rutan & Steinke, 2018a), CC chemical composition varied each year. The C/N ratio of radish was 18:1 and 23:1 in SY1 and SY2, respectively, whereas oat was 23:1 and 17:1 in SY1 and SY2, respectively. Differing C/N ratios each autumn may impact mineralization rates and variably influence succession of copiotrophs and oligotrophs during corn growth the following year (Bastian et al., 2009). Whereas weather can be difficult to predict, differences due to soil moisture may have implications for growers of irrigated crops (e.g., potato [*Solanum tuberosum* L.]), where CC species selection may have different results.

3 | CONCLUSIONS

Application of N fertilizer requires growers to consider the 4Rs of corn management, which include rate, placement, timing, and N source. Unlike many studies that have investigated N fertilizer impacts to the soil bacterial communities, the current study utilized high throughput sequencing technology to highlight the impact of N timing and placement strategies in conjunction with CCs. The current analysis highlights the interannual variability affecting soil bacterial community response to N inputs, and growers using similar treatments may not expect the same results each year. Soil moisture was presumably a large factor regulating community composition. Under deficit rainfall conditions, CC species selection affected soil bacterial community structure response to N management. Community composition to CCs and N management varied by sample time and sample zone each year but demonstrated that community structure accounting for relative abundance was consistently sensitive to N timing and placement combinations. Different mechanisms may govern community structure of bulk and rhizosphere soils, which have N management implications. Soil disturbance associated with coulter-injection appeared to be a strong factor structuring bulk soil communities, whereas 5 × 5 starter placement influenced a corn-mediated response affecting OTU membership. However, including an organic N source such as PL may reduce impacts to soil community composition and provide stability regardless of time or location. Growers are likely to encounter more seasonal variability to soil biological communities when planting CCs as opposed to N management.

Additional studies that investigate associations between changes in soil bacterial community composition and ecosystem functioning and stability over time are warranted. Redundancy analysis relating soil chemical properties and soil bacteria may provide further insight on mechanisms regulating community composition and choice of N management strategy.

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AUTHOR CONTRIBUTIONS

Jeff Rutan: Data curation; Formal analysis; Investigation; Writing-original draft. Noah Rosenzweig: Methodology; Resources. Kurt Steinke: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Kurt Steinke https://orcid.org/0000-0003-3150-1660

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