Winter Cover Crops Influence Bacterial Community Structure

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INTRODUCTION

Cover crops are grown during the fallow period of the year to reduce soil erosion, prevent nutrient loss, improve soil structure, and increase overall soil health (Roberson et al., 1991; Reeves, 1994). Nitrogen (N) fertilizers are widely used and consequent nitrate loss can be economically and environmentally detrimental. Research has demonstrated that winter cover crops temporarily conserved inorganic N pools including residual N fertilizer applied to corn (Shipley et al., 1992). Microbial processes play important roles in many cover-crop-induced changes, especially in nutrient cycling. Cereal and legume cover crops increased soil mineralizable C and N after sweet corn harvest (Mendes et al., 1999). Mixed winter cover crops enhanced soil microbial biomass and N mineralization, as well as caused shifts in the microbial community structure in soils under a green bean field (Schutter and Dick, 2002).

Genomic analysis using Next-generation sequencing techniques is now commonly used to study microbial communities with more detailed information. 16S rRNA is the target gene used to analyze bacterial community structure. Different cover crop species cause changes in microbial community structure. In this study we investigated the soil bacterial community composition under different N fertilizer and cover crop managements. Our objective was to determine if N fertilization and cover crops influenced changes in bacterial community structure detected in a continuous corn soil. We hypothesized that (1) N fertilization and cover crop applications would stimulate bacterial richness and diversity; and (2) soil bacterial community structure would shift under different winter cover crop treatments.

SITE & METHODS

The study site was located on the Illinois State University Agriculture Research Farm in Lexington, Illinois. The predominant soil was a poorly drained Drummer El Paso silty clay loam with 0-2% slope. There were 3 block replicates within this continuous corn (*Zea mays L.*) field. Cover crop managements were performed in this site for 5 years, between 2011 to 2016. Each block had a complete randomized design with 5 treatments: ZC (zero control: no N and no cover crop); C (control: N applied and no cover crop), CR [N applied and cereal rye (*Secale cereal L.*)], RAD [N applied; radish (*Raphanus sativus L.*)], and CR/RAD (N applied and mixed of CR and RAD). All cover crop treatments received an identical rate of inorganic fertilizer (anhydrous ammonia 200 kg N ha^{-1}) as the control treatment (Fig. 1).

During the spring of 2016, soil samples were collected from the top 15 cm. Total soil DNA was extracted using FastDNA SPIN Kit for soil (QBIOgene, Carlsbad CA). Bacterial 16S rRNA gene amplicons were PCR amplified using 28F-388R primers and sequenced with Illumina MiSeq platform. The data was analyzed by Mothur and Krona software.

Figure 1. Fall application of anhydrous ammonia into living cereal rye radish stand.

Treatments	Chao1 (richness index)	Invsimpson (diversity index)
Zero control (ZC)	3805	209
Control (C)	4544	226
Cereal rye (CR)	4512	229
Radish (RAD)	3387	199
CR/RAD	4242	200

Table 1. Average Chao 1 and Invsimpson indexes of soil bacterial community for all treatments .

RESULTS

- ✓ No statistically significant differences were detected using Chao 1 ($p > 0.05$) and Invsimpson indexes ($p > 0.05$) among the treatments (Table 1).
- ✓ In all treatments, the 4 predominant bacterial phyla in decreasing abundances were *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Actinobacteria* (Fig. 2).
- ✓ There were more *Proteobacteria* in CR (40%) and CR/RAD (38%) than in ZC (35%), C (35%), and RAD (35%) (Fig. 2). The 8 largest identified genera in *Proteobacteria* are shown in Fig. 3a: RAD and CR/RAD had more *Lysobacter* than other treatments; CR and ZC treatments resulted in higher proportion of *Bradyrhizobium*, *Nitrosospira*, and *Devosia* than others; N application increased the proportion of *Caulobacter*, but cover crop with radish may have an inhibitory effect.
- ✓ The relative abundance of *Bacteroidetes* was greater in fertilizer treatments compared to ZC [i.e. ZC (11%), C (17%), RAD (18%), CR (15%) and CR/RAD (20%)] (Fig. 2). *Flavobacterium* (the largest genus in *Bacteroidetes*) was detected with higher relative abundance in the C (27%) compared to other treatments. *Terrimonas was* relatively more abundant in the ZC samples. *Pedobacter* revealed stimulated growth under RAD. Higher proportion of *Haliscomenobacter* was induced by cover crop treatments. *Segetibacter* growth was promoted by the CR, but *Cytophaga* appeared to decrease under it (Fig. 3b).
- ✓ ZC contained higher relative abundance of *Acidobacteria* than other treatments (Fig. 2). *Acidobacterium* was the largest identified genus, which was relatively greater in fertilized soils than in the ZC. There were no *Holophaga* and *Blastocatella* present in the ZC, contrarily *Terriglobus* only detected in the ZC and CR treatments (Fig. 3c).
- ✓ ZC (4%) had more *Actinobacteria* than C, RAD, CR and CR/RAD (each with 3% relative abundance) (Fig. 2). The two largest identified genera representing this phylum, *Microlunatus* and *Hamadaea*, were relatively more abundant in ZC than other treatments. Cover crop treatments stimulated *Nocardioides* (Fig. 3d).

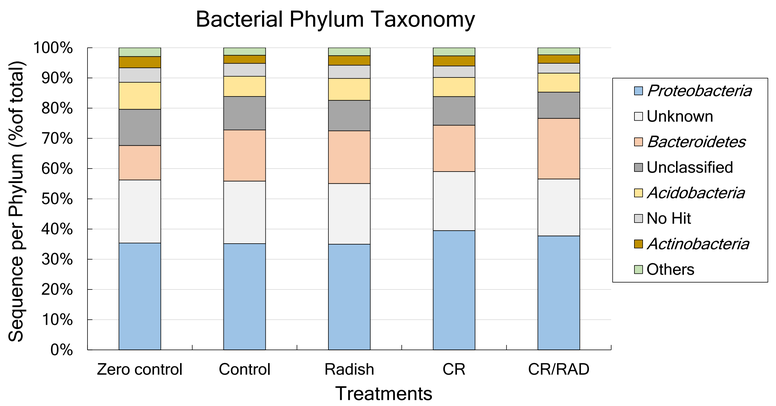


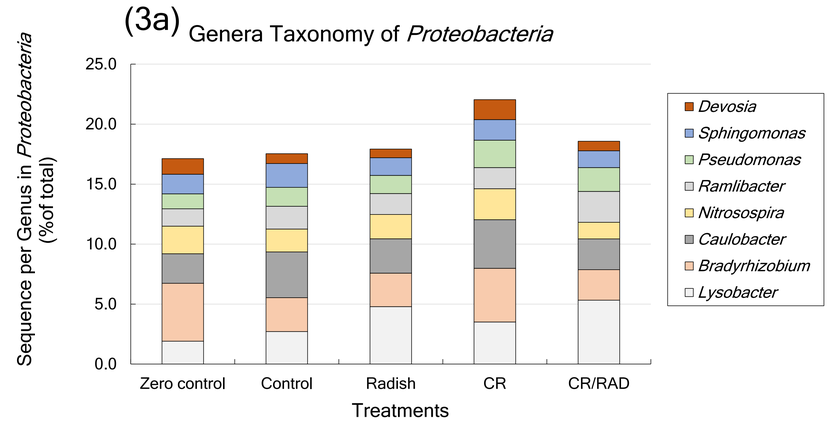
Figure 2. Bacterial phylum-level taxonomic distribution for each treatment: zero control (ZC), control (C), radish (RAD), CR, and CR/RAD.

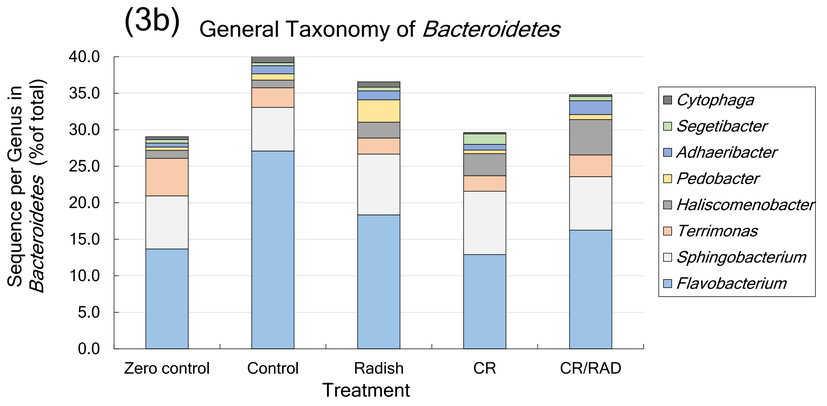
CONCLUSIONS

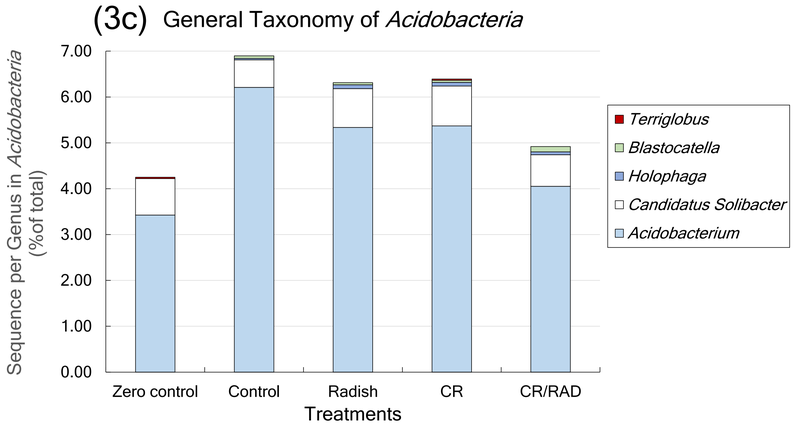
Diversity and richness index measures revealed no statistically significant differences among our treatments. However, over 5 years of consecutive cover crop adoption there were shifts in bacterial community caused by cover crop and N application observed at all taxonomic levels, from phylum to genus. The cereal rye appeared to scavenge residual nitrogen significantly by revealing similar bacterial community structure with the unfertilized ZC treatment.

References

- Mendes I.C. et al. 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops
- Reeves D.W. 1994. Cover crops and rotations.
- Roberson E.B. et al. 1991. Cover crop management of polysaccharide-mediated aggregation in an orchard soil.
- Schutter M.E. and Dick R.P. 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil.
- Shipley P.R. et al. 1992. Conserving residual corn fertilizer nitrogen with winter cover crops.







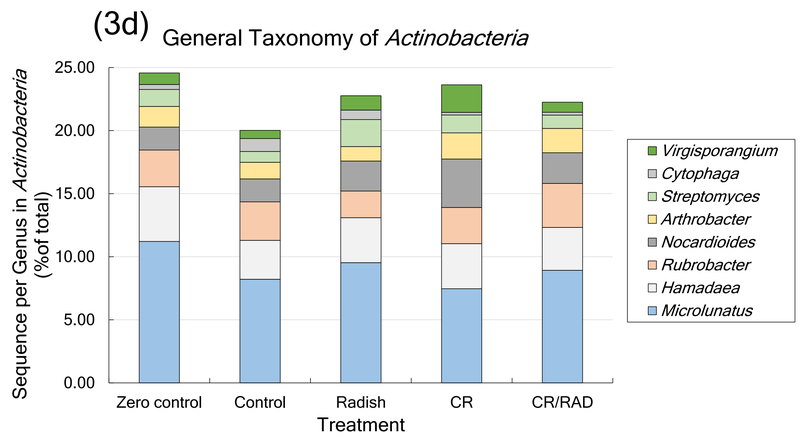


Figure 3. Genus-level taxonomic distribution of *Proteobacteria* (3a), *Bacteroidetes* (3b), *Acidobacteria* (3c) and *Actinobacteria* (3d) in every cover crop treatment: zero control (ZC), control (C), radish (RAD), CR, CR/RAD.

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