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Characterization of microbial community response to cover crop residue decomposition

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ABSTRACT

Cover crop adoption in the U.S. Corn Belt region is a rapidly emerging management practice in corn (*Zea mays*) agroecosystems. However, little is known about the impact of the inclusion of cover crops on the soil microbiome and its relation to the decomposition of the cover crop residue during the cash crop growing season. Therefore, this study sought to determine the impact of cover crop species and residue management practices on soil microbial community composition and structure during winter cover crop decomposition over the corn growing season. Cover crop treatments included hairy vetch (*Vicia villosa* Roth), cereal rye (*Secale cereal*), a hairy vetch/cereal rye mixture, and a no cover crop control. Residue management practices included no-tillage and a 15 cm reduced spring tillage following cover crop termination. Soil samples were collected at five dates during cover crop decomposition that corresponded to an accumulated number of calendar days from cover crop termination, and soil bacterial communities were characterized using the small subunit (16S) rRNA gene sequences. Statistical analyses revealed that sampling date, cover crop treatment, and residue management treatment were significant determinants of soil microbial community composition ($p < 0.05$) and the effect of cover crop treatment increased as the decomposition period progressed. As cereal rye began to decompose and soil β-glucosidase (EC 3.2.1.21) potential activity increased, the relative abundance of bacteria previously identified as cellulolytic, including *Agromyces*, *Agrobacterium*, and *Bacillus*, contributed to the difference among the cover crop treatments (LDA score > 2.0). Data generated from this study leads to a deeper understanding of bacterial responses to cover crop decomposition in corn agroecosystems.

1. Introduction

The incorporation of organic matter amendments and reduced tillage residue management into nutrient management plans of farmers is a growing trend in the United States as the debate surrounding the sustainability of intensive, conventional agriculture practices continues (Cover Crop Survey, 2017). Cover crops, vegetation most commonly grown between cash crop plantings, serve as organic matter amendments to agroecosystems. Likewise, reduced tillage residue management, such as not tilling soils prior to planting cash crops, is used to improve soil health. It is well understood that cover crops increase soil organic carbon (Liu et al., 2005), and studies have demonstrated that organic matter amendments such as cover crops may increase nutrient use efficiency in agroecosystems by mitigating nitrate-nitrogen losses through subsurface drainage (Radicetti et al., 2016; Tonitto et al., 2006; Torstensson et al., 2005). Additionally, benefits such as reduced erosion from wind and water have been documented for this management practice (Alliaume et al., 2014; Panagos et al., 2015).

Understanding soil biological activity dynamics is an important aspect of sustainable agroecosystem management (Cheeke et al., 2012). Cover crops and reduced tillage residue management increase the amount of labile carbon added to the agroecosystem, especially in the spring after cover crop termination (Johnson et al., 2013; Ladoni et al., 2016; Sindelar et al., 2014), and the microbial community is a primary consumer of this labile carbon (Murphy et al., 2007). When labile carbon is added to the soil system, a succession of enzymes, cellulases, act synergistically to depolymerize the labile carbon cellulose. β-glucosidase (EC 3.2.1.21) is the last cellulase enzyme in the cellulase enzyme succession, and this enzyme yields glucose (Hobbie and Hobbie, 2013). This resulting glucose serves as a vital energy source in microbial metabolism (Eivazi and Tabatabai, 1988), and studies have used the potential activity of this enzyme as a measure of decomposition

Abbreviations: Cereal rye, CR; Hairy vetch, HV; Operational taxonomic unit, OTU; Principal coordinate analysis, PCoA; Canonical correspondence analysis, CCA; Variance inflation factor, VIF; PermDISP, Permutational analysis of multivariate dispersions; PERMANOVA, Permutational multivariate analysis of variance

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because soil β-glucosidase activity responds to organic amendment addition. This potential activity has been used to quantify the microbial community response to the influx of labile carbon into soil systems (Blagodatskaya et al., 2016; Knelman et al., 2017). In addition, studies discovered that organic amendments led to increases in total microbial activity (Flieβbach et al., 2007), including increased microbial biomass (Cong et al., 2006) and potential enzyme activity (Stark and Condron, 2008). Another soil analysis used to examine how cover crops and reduced tillage impact the soil microbial community is phospholipid fatty acid analysis. Finney et al. (2017) found that cover crops increased total phospholipid fatty acid concentrations compared to the control plots.

Following cover crop termination, influxes of labile carbon from cover crop residue has resulted in less productive agriculture systems, as measured by cash crop yield, especially for corn (Crandall et al., 2005; Kramberger et al., 2009; Kuo and Jellum, 2002; Roth et al., 2018; Tonitto et al., 2006). In fact, when cereal rye (CR) cover crops have been used, corn productivity has been shown to decrease, as potential labile carbon from the CR residue increases. However, diverse responses of corn yield to different winter cover crop species has been shown, such as corn yield increases after legume cover crops (Miguez and Bollero, 2005), and labile soil organic matter indicators have been found to reflect short-term dynamics of corn cropping systems (Culman et al., 2013). This conundrum has led to a debate regarding the tradeoffs between ecosystem services derived from cover crops and the subsequent costs of less cash crop productivity (Crandall et al., 2005; Roth et al., 2018). Reduction in corn yield in the presence of a CR cover crop has been attributed to a reduction in soil inorganic nitrogen availability due to nitrogen immobilization, which has resulted in less corn nitrogen uptake and lower yields relative to the control (Crandall et al., 2005; Kramberger et al., 2009; Ruffo, 2001). These observations demonstrate the potential of the microbial community to become a major sink for inorganic nitrogen, due to the response of the community to the influx of cover crop residue labile carbon. Thus, there is a critical need to better understand the dynamics and extent of the microbial community response to the influx of cover crop residue carbon during corn development. These dynamics include measuring potential enzyme activity, soil microbial community structure, and soil microbial community composition, with microbial community structure referring to the numbers of populations and their proportional representation within a community, being reported by α- and β-diversity measurements. Microbial community composition is characterized by the abundance of individual OTUs (operational taxonomic units). However, studies using high-throughput sequencing of bacterial ribosomal genes have examined the soil microbial community dynamics in response to organic matter amendments by selectively sampling during the year (Fernandez et al., 2016), but have not captured the extent of the soil microbial community dynamics during corn development (the cover crop decomposition period). In addition, no studies have concurrently measured the influx of cover crop residue and the potential enzyme activity representing the functional capacity of the soil microbial communities' ability to decompose the cover crop residue as the corn develops. Lastly, no studies have examined these dynamics in the first year of the transition of the agroecosystem to no-tillage residue management and cover cropping.

Therefore, we have two objectives: (1) to investigate the influence of cover crop species on soil microbial community structure and composition during the decomposition period relative to a no cover crop control, and (2) to determine if residue management impacts the soil microbial community structure and composition during the decomposition period of different cover crop species.

2. Methods

2.1. Site description and experimental design

The study site was located in Tippecanoe County, Indiana at the Purdue University Agronomy Center for Research and Education (40°28′16″N; 86°59′32″W) on a silty clay loam soil. Precipitation and bare soil temperature measurements were collected from the Indiana State Climate Office (https://iclimate.org). The winter of 2015/2016 was the first year the cover crop and residue management systems were implemented. Prior to these cover crop and tillage regimes, the site was managed in a corn-soybean annual rotation with fall and spring tillage prior to corn planting every other growing season. The experiment had a split-plot design with three field replications. Each plot was 12 rows wide and approximately 80 m in length. There were six treatments used: three cover crops, no cover crop controls, and two tillage practices. Cover crop treatments included hairy vetch (HV) (*Vicia villosa*), CR (*Secale cereale*), and a HV/CR mixture. Cover crops were broadcasted on September 28–30, 2015 in the standing corn cash crop. The HV and CR seeding rates were 33 and 56 kg ha^{-1}, respectively. The HV/CR mixture seeding rate was 16/44 kg ha^{-1}. The cover crops grew over the winter and were chemically terminated on April 14, 2016. Prior to cover crop termination, cover crop spring aboveground biomass sampling was completed to determine the amount of cover crop winter growth. This cover crop aboveground biomass was oven dried at 105°C until constant weight was reached. Following termination, anhydrous ammonia was applied at a rate of 78 kg ha^{-1} of nitrogen, which is common among farmers to ensure adequate nitrogen is available for the corn cash crop. On April 25, 2016, the spring tillage plots were disk tilled twice to a depth of approximately 15 cm. On April 26, 2016, the same plots were cultivated to further prepare the seedbed. The no-tillage plots were not disk tilled or cultivated. On April 26, 2016 corn was planted in all plots.

2.2. Litter bags for residue decomposition measurement

Cover crop aboveground biomass that was used in the litter bag decomposition measurement was collected two days after cover crop termination on April 16, 2016. Only aboveground cover crop biomass was used for the litter bag measurements. Aboveground biomass was air-dried prior to being inserted into the litter bags and applied to the field. Litter bags were created similar to other studies (Jahanzad et al., 2016; Melkonian et al., 2017; Organization for Economic Cooperation and Development, 2006; Varela et al., 2017). Briefly, nylon mesh (1 mm) (Industrial Netting Inc., Minneapolis, MN) was used for litter bag construction and the mesh was sealed with silicon glue. The litter bags were 30 × 15 cm and filled with the air-dried aboveground cover crop residue. The mixture, CR, and HV litter bags were filled with 22 g, 20 g, and 10 g of aboveground biomass, respectively. The 22 g of aboveground biomass in the mixture bag contained 20 g of CR and 2 g of HV residue to simulate the amount of HV residue in the mixture treatments in the field. To simulate the tillage treatment, as completed in other studies (Sievers and Cook, 2018), litter bags were buried approximately 7.5 cm. The litter bags applied in no-tillage residue management treatments were attached to the surface between corn rows with lawn staples. The bags were systematically retrieved from the field during the cover crop decomposition period to determine the percent biomass that had been released from the bags. Litter from each collected bag was air dried and analyzed for total mass loss and total carbon concentrations using the FLASH 2000 Elemental Analyzer (dry combustion method), and therefore the amount of cover crop mass released from the litter bags during the decomposition period was determined.

2.3. Soil sampling

Soil samples were collected on May 5, May 23, June 6, June 28, and August 1 during the 2016 cover crop decomposition period and corn growing season. These sampling dates corresponded to 21, 39, 53, 75, and 109 calendar days after cover crop termination. During each soil sampling, 15 randomly distributed soil cores were removed per plot at a