QUANTIFYING THE EFFECTS OF NUTRIENT MANAGEMENT AND COVER CROPS ON SOIL MICROBIAL COMMUNITIES AND SOYBEAN PRODUCTION

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Abstract

Agricultural productivity relies upon microbial communities to cycle nutrients from soil to plant yet little information is available concerning how nutrient management and cover cropping practices influence microbial activity and crop production. Lack of consistent soybean (*Glycine max* L.) yield response to applied fertilizer has resulted in a need to further investigate management practices focused upon enhancing soil biological activity which in turn may feed the crop. A field study was initiated to determine the effects of cover crops and soil fertility on soybean soil biological activity, microbial composition, and plant production. Three cover crop treatments and six fertilizer programs were evaluated prior to soybean. Cover crop treatments included oilseed radish (*Raphanus sativus* L.), hairy vetch (*Vicia villosa* Roth), and no cover crop. Six soil amendment treatments included a non-fertilized check, a biotic fertilizer containing mycorrhizal inoculum, chicken manure, a biological soil inoculant applied to seed, a biological soil inoculant applied in-furrow, and inorganic N-P-K fertilizer application based on first-year nutrient mineralization of biotic and manure treatments. Soil DNA extraction occurred pre-plant, R1, and pre-harvest from within and between soybean rows and will be used to taxonomically classify soil bacteria. Data collection included soil respiration to assess temporal changes in microbial activity, R1 tissue sampling and nutrient analysis, R3 nodulation evaluation, grain measurements at physiological maturity (i.e., pods per node), and grain yield. Preliminary results will be summarized from cover crop and fertilizer treatment effects on soybean production in addition to preliminary information on soil bacterial classification.

Introduction

Mean Michigan soybean yields have increased at a rate of 1% per year from 1980 to 2013 while mean Michigan corn yields have increased nearly twice as rapidly over the same time period (Fig. 1). When at or above critical nutrient levels, soybean yield response to fertility programs has been inconsistent with fields in close proximity under similar management practices and similar soil *physical* properties often exhibiting contradictory yield results. These phenomena beckon the need to increase the breadth of soil fertility research. The key to more sustained and predictable soybean yield gains may be found in the living aspect of the soil -- a critical yet often ignored portion of soil health that is still far from understood.

Soil microbial life plays an imperative role in production agriculture. Microbes are able to break down nutrients into plant available forms and increase crop resiliency to physical and biological stresses (Podile and Kishore, 2006; Miransari, 2011). Microbial community composition may be of specific importance to soybean production due to the symbiotic relationships with rhizobia (*Bradyrhizobium japonicum*) that soybean plants form which can result in most of the nitrogen (N) needs of the soybean crop to be fixed from N_2 gas in the atmosphere (Duong et al., 1984).

Little research has been conducted to determine what effects varying categories of cover crops, soil amendments, or their interaction have on soil bacterial communities and soybean yield. Illumina Sequencing technology is now advanced and affordable enough to perform DNA extractions from soil samples and monitor changes between treatments throughout the growing season. The results of the DNA extraction procedure will report bacterial diversity to the specificity of genus level for each sample analyzed.

The objectives of this study were to 1) measure and characterize temporal changes in soil and rhizosphere microbial community composition as affected by cover crop, 2) determine what effects soil amendment substrates have on soil microbial community composition and biomass, and 3) determine whether incremental changes in soil biological activity may impact overall plant productivity.

Methods and Materials

The study was initiated at the MSU agronomy farm in East Lansing, MI on a Capac Loam (45% sand; 41% silt; 14% clay) following wheat to allow sufficient time for cover crop planting and establishment. Pre-plant soil properties included 3.0% OM, 41 ppm P, 118 ppm K, and 6.5 pH. Soybeans were planted in 30" rows at 154,500 seeds per acre. The trial was arranged as a split-plot randomized complete block with four replications. Three cover crop treatments including oilseed radish (N scavenger), hairy vetch (N producer), and no cover treatment were arranged vertically with 6 soil amendment sub-plot treatments consisting of a biotic fertilizer at 1 T/A, organic chicken manure at 2 T/A, a biological soybean inoculant applied as a seed treatment, a biological soybean inoculant applied in-furrow, inorganic NPK fertilizer based on nutrient levels of the biotic and manure treatments, and a non-fertilized check. Soil amendment treatments were not equalized according to nutrient additions as this was not the focus of this investigation but rather effects on soil biological activity as stated previously.

Cover crops were planted 9 Aug 2012 using a grain drill at 12 and 20 lb/A for oilseed radish and hairy vetch, respectively. Remaining cover crop residues and any weeds were sprayed with glyphosate approximately 1 week prior to soybean planting, which occurred on 14 May 2013. Six and eight inch soil samples were taken in each cover crop strip prior to winterkill or dormancy, and a 12" field composite sample was taken prior to winter for base total N analysis. Six-inch soil samples were analyzed for microbial respiration using Solvita® CO_2 test kits. Twelve-inch soil samples were analyzed for nitrate content and were taken monthly throughout the growing season in each non-fertilized check plot.

Four-inch soil samples were taken in each main plot prior to soybean planting, as well as in every sub-plot at R1 and soybean harvest. For the latter two sampling dates, samples were obtained from the rhizosphere (within the row, between two soybean plants) and from soil between soybean rows. DNA was extracted from each sample using a Mo Bio 101 DNA extraction kit (Mo Bio Laboratories Inc., Carlsbad, CA). Soil genomic DNA was used as a template for PCR amplification of the 16S rRNA variable regions using a previously described primer pair (Rosenzweig et al., 2012) for total bacterial community analysis. Generated sequence data will be analyzed with an Illumina MiSeq™ Personal Sequencing System at the MSU

Genomics Core. Additional community analyses will be performed using the MOTHUR software package.

Stand counts were taken eight days after planting from one meter of row to quantify differences in emergence. Anion strips were installed in each check plot approximately 2 weeks after soybean planting to monitor changes in available N. Anion strips were removed and replaced every two to three weeks until harvest. Chlorophyll meter readings were collected at V4 using a Minolta SPAD-502 chlorophyll meter (Spectrum Technologies Inc., Plainfield, IL). Twenty-five plants were sampled per plot and the average recorded. A relative SPAD output was calculated for each plot by dividing the plot SPAD reading by the highest SPAD reading from each replication. Plant height was collected at V4 from 20 plants per plot and mean height recorded. Plant height and SPAD measurements were repeated at R5.

Twenty trifoliate tissue samples were collected from each plot at R1 and analyzed for N, P, K, S, Ca, Mg, B, Cu, Fe, Mn, and Zn. Soybean root nodules were counted at R3. At physiological maturity, plant measurements included grain yield, test weight, and moisture. Ten plants were collected from each plot to determine mean total nodes, pod producing nodes, pods per node, beans per pod, and weight of 100 beans. Grain was also sampled to measure percent oil and protein.

Statistical analysis for all data aside from the DNA results was performed using PROC MIXED in SAS. All measurements were analyzed assuming fixed main effects of cover crop and sub-plot fertilization and random replication effects. When significant ($P < 0.10$) main effects were indicated, treatment means were separated using LSMEANS and compared using the PDIFF command.

Preliminary Results and Discussion

A significant yield increase was observed with oilseed radish preceding soybean, but a significant yield decrease was observed following hairy vetch (Fig. 2). The number of soybean nodules per plant correlated with yield data when separated by cover crop treatments (Fig. 3). Nodule counts can be an indirect method of measuring available soil N, as soybean nodulation can be strongly restricted if a large amount of inorganic N is present in the soil profile (Streeter, 1998). Hairy vetch has been shown to provide N credits of 89 lb/A (Ebelhar et al., 1984) or higher to a subsequent crop. Data from the present study reflect an N credit from hairy vetch through decreased nodule numbers and suggest that oilseed radish may *not* be providing large quantities of N to subsequent crops as nodulation numbers were similar between radish and no cover crop treatments.

Solvita® respiration kits were used to determine the amount of CO_2 released from 40g of oven dried soil in 24 hours after 20ml of deionized water was added. Elevated levels of CO_2 released from the soil results from higher microbial respiration activity. Plots with hairy vetch cover crop treatments showed higher average respiration values than oilseed radish and no cover in December 2012, and May through July 2013 (Fig. 4). Hairy vetch green manure that was worked into the soil in the spring may have provided a food source for microbiota that persisted throughout the first half of the growing season.

Soil amendment treatments also had an effect on early-season respiration data (Fig. 5). Chicken manure had the greatest soil respiration test values in mid-June (approximately one month after planting). The two organic-based amendments (e.g., biotic fertilizer and chicken manure) exhibited similar patterns of reduced soil respiration in August followed by increased soil respiration values in Sept. (Fig. 6). Late season microbial activity could play a role in crop production when considered in the context of sustainable agriculture and crop yields throughout an entire crop rotation. Greater rates of microbial respiration or cycling during the winter months may lead to increased nutrient availability the following season and reduced inputs.

Project Continuation

A second year of research is currently underway for this project and will continue to investigate management impacts on soil biology and whether plant production may be influenced using this approach. Soil DNA extraction methodology has taken some time to decipher and comprehend results but data from both study years should be available in early 2015. It is important to reiterate that the primary goal of this project is to gain a better understanding of the microbial response to cover crop and soil amendment practices in lieu of balancing NPK nutrition across soil amendment treatments.

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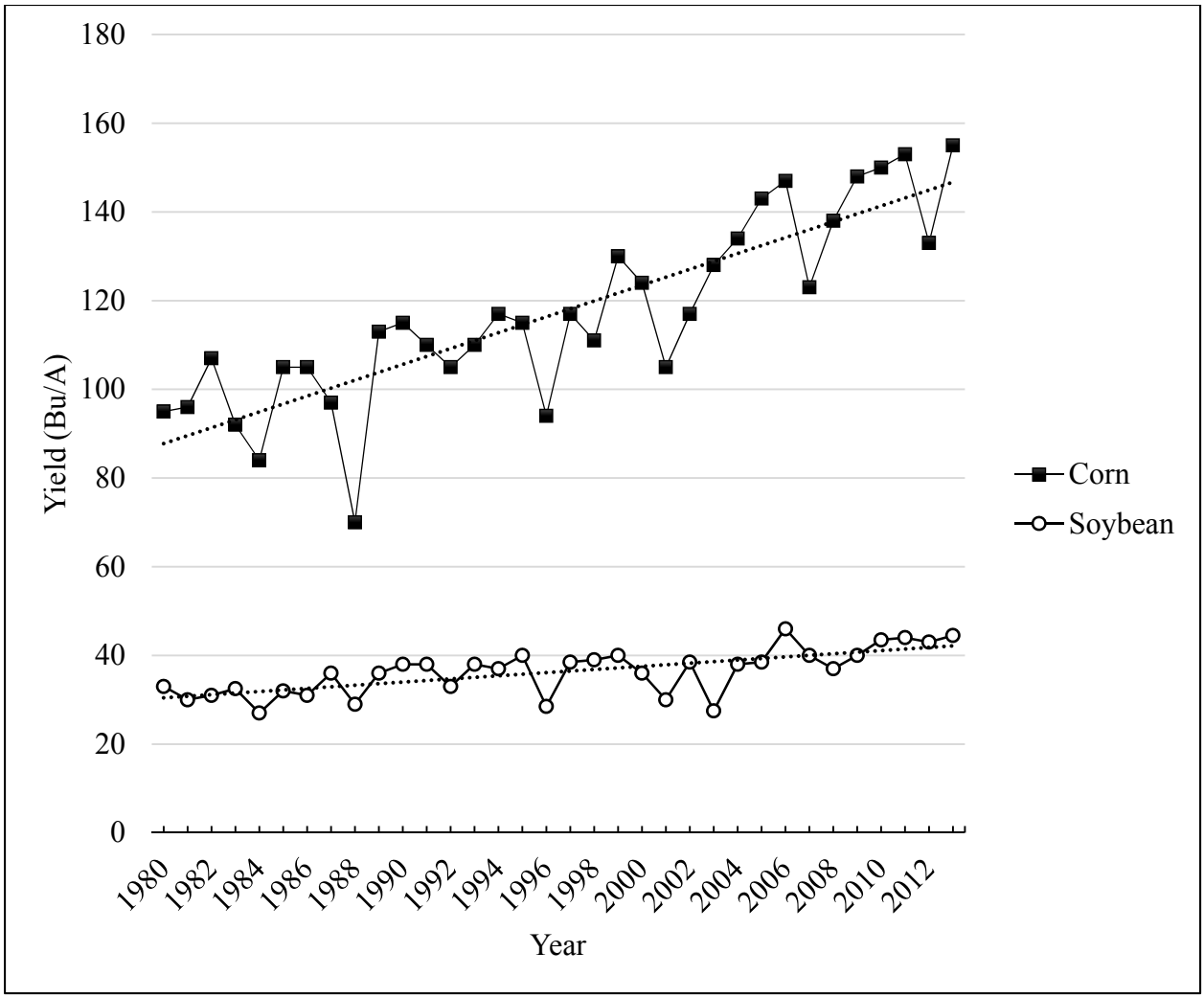

Figure 1. Michigan soybean and corn mean grain yield (bu/A) from 1980 to 2013 with individual trend lines. (Source: USDA National Agricultural Statistics Service).

Table 1. Cover crop and soil amendment effects on soybean yield, moisture, and test weight, East Lansing, MI, 2013.

Treatment	Yield (bu/A)	Moisture (%)	Test Weight (lbs/bu)
Cover Crop			
Oilseed Radish	69.8 a*	13.9 a	53.1 b
Hairy Vetch	64.1 c	13.7 a	54.5 a
No Cover	68.7 b	13.9 a	53.5 b
Amendment			
Biotic CNEF	65.0 a	13.7 a	53.6 a
Chicken Manure	69.2 a	13.9 a	53.9 a
Inoculant Seed Treatment	67.2 a	13.7 a	54.2 a
Inoculant In-Furrow	68.3 a	13.8 a	53.9 a
Inorganic N, P, K	68.2 a	13.8 a	53.3 a
Untreated Check	67.4 a	13.8 a	53.4 a
Treatment Effects (*P>F*)			
Cover Crop	.000	.463	.055
Amendment	.285	.560	.928
Cover Crop x Amendment	.234	.043	.069

*Values in the same column followed by the same letter are not significantly different (α=0.1).

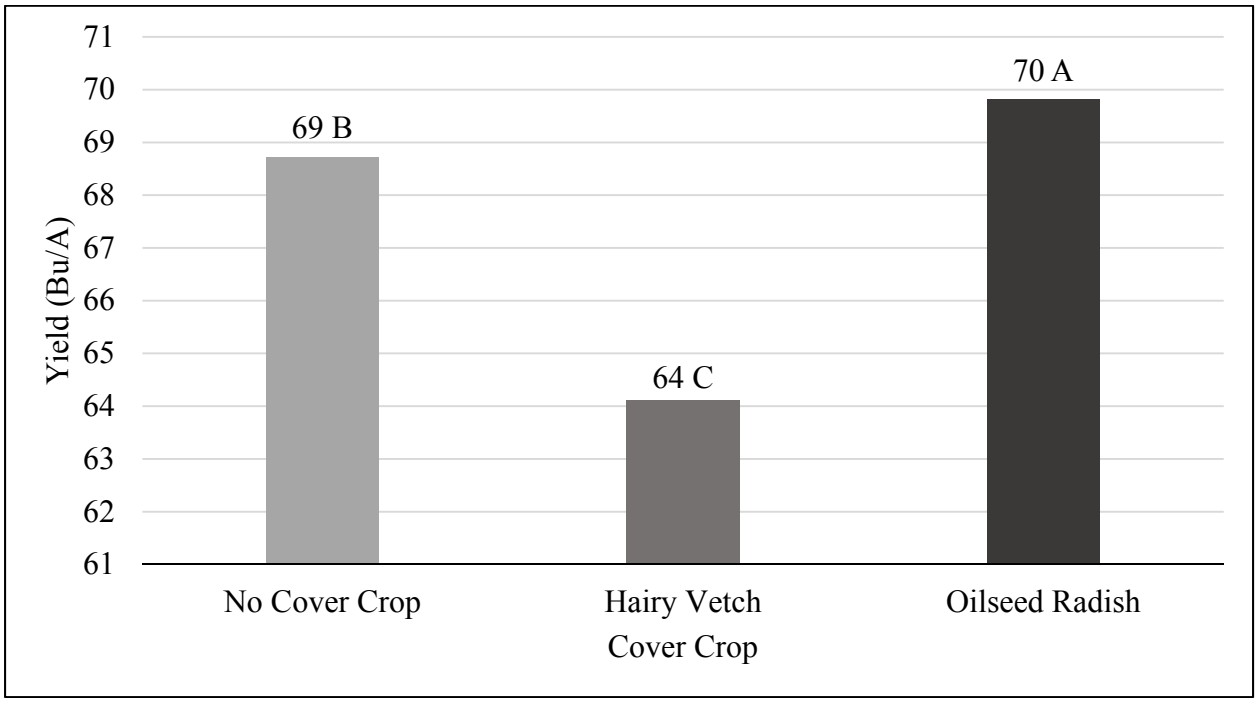

Figure 2. Cover crop effects on soybean yield, East Lansing, MI, 2013. Values followed by the same letter are not significantly different (α=0.1).

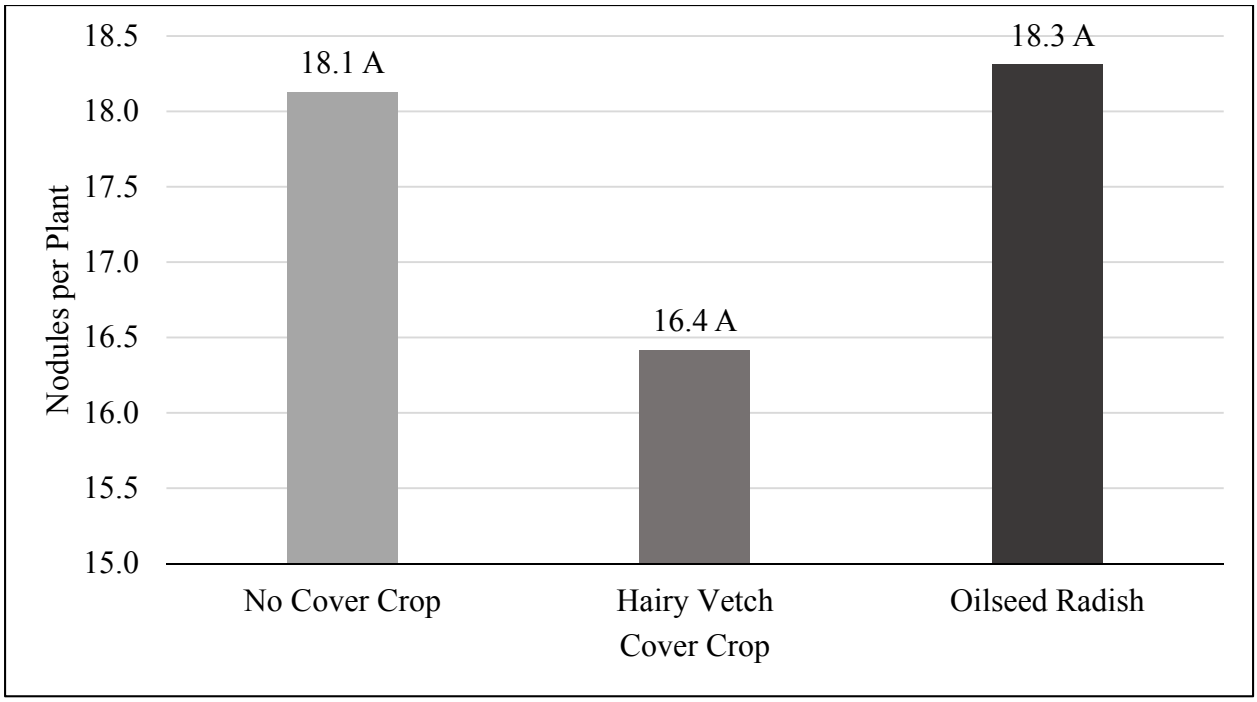

Figure 3. Cover crop effects on the number of root nodules per plant, East Lansing, MI, 2013. Values followed by the same letter are not significantly different (α=0.1).

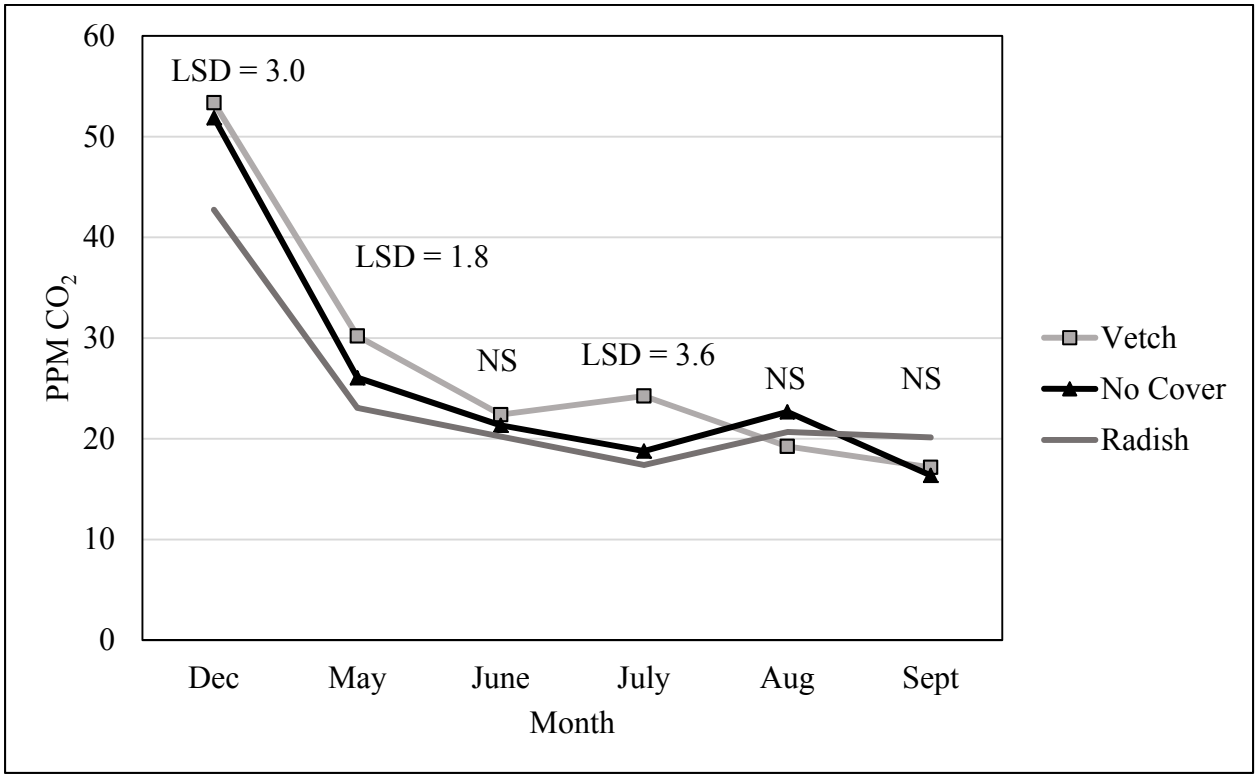

Figure 4. Impact of cover crop on monthly mean rate of soil respiration, East Lansing, MI, 2012-2013. LSD values listed are significant at α=0.10.

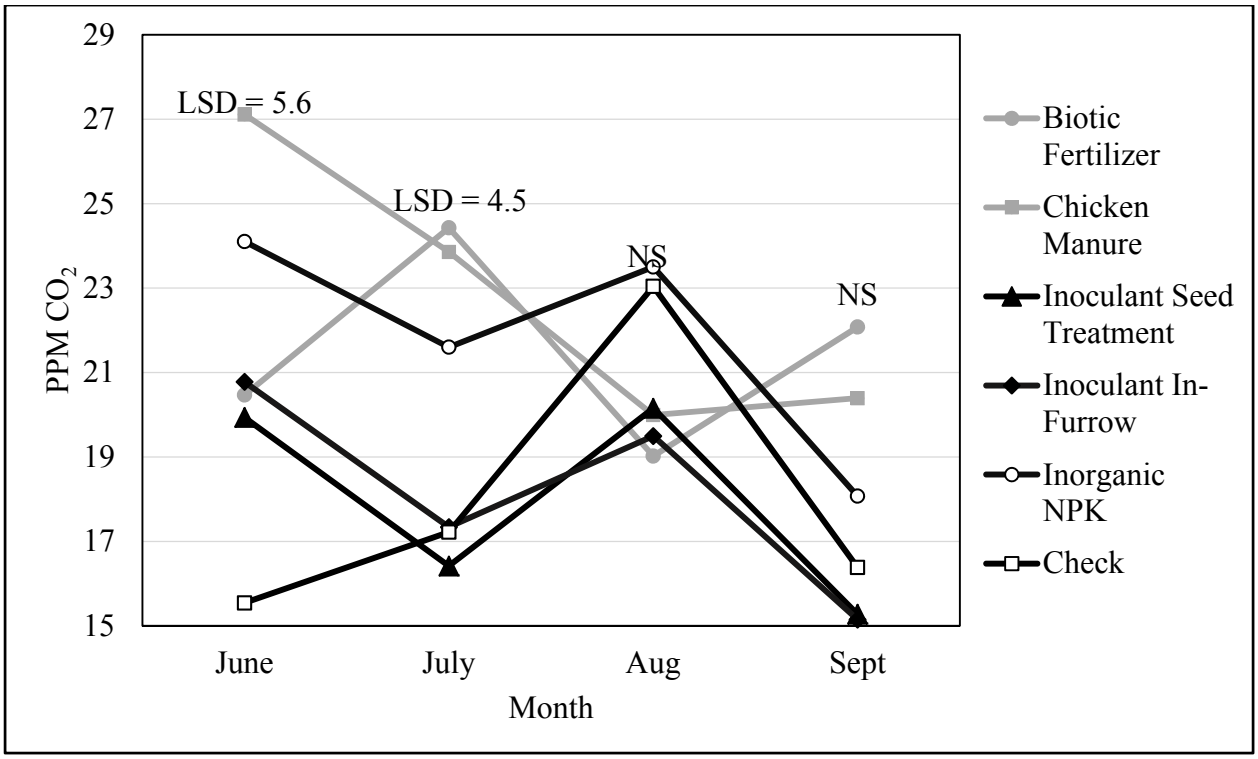

Figure 5. Impact of soil amendment on monthly mean rate of soil respiration, East Lansing, MI, 2013. LSD values listed are significant at α=0.10.

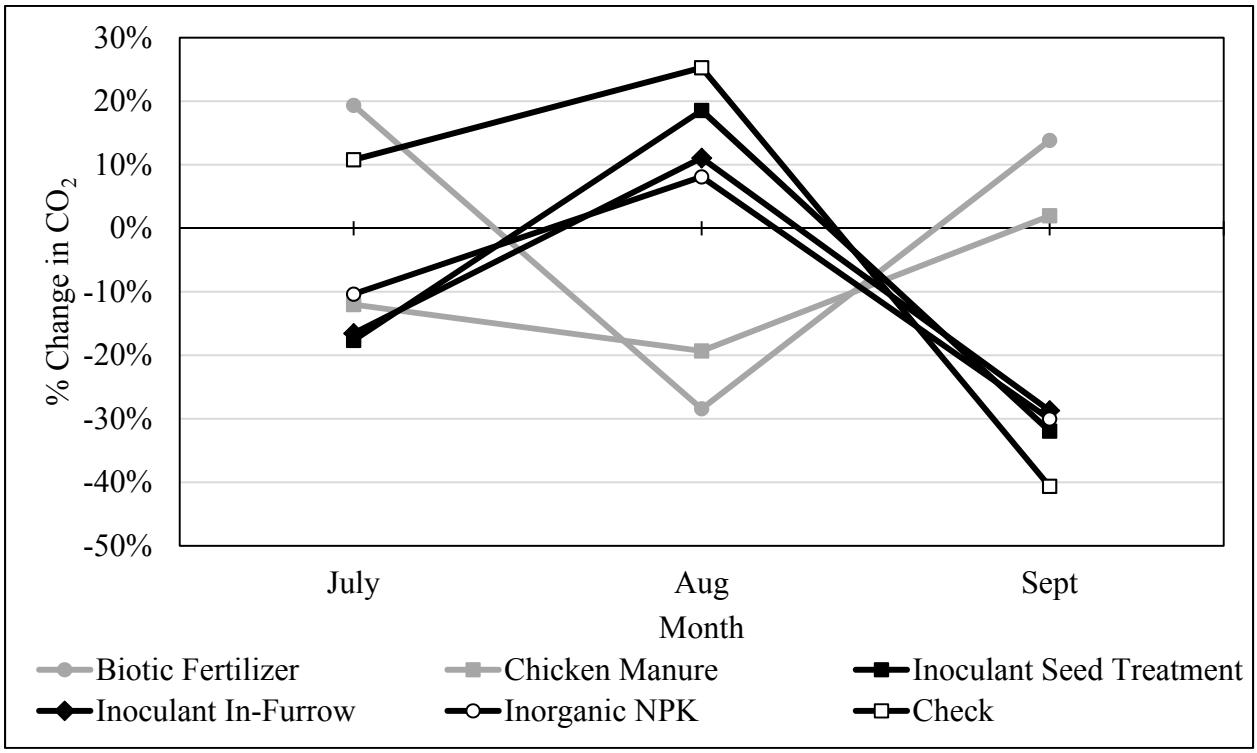

Figure 6. Percent change in CO_2 respired from previous month by soil amendment, East Lansing, MI, 2013.

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