Research Note

SOIL MICROBIAL COMMUNITIES AS AFFECTED BY A COMMERCIAL ORGANIC FERTILIZER AND SUNN HEMP AS A COVER CROP IN ORGANIC SWEET PEPPER PRODUCTION IN PUERTO RICO[1, 2]

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Organic farming relies on practices that sustain and improve soil productive capacities, with soil microbial communities playing an important role. Nutrient cycling, residue decomposition, nitrogen fixation, and carbon sequestration are primarily controlled by microbes, which are the main source of enzymes capable of carrying out these activities (Gardner et al., 2011; Moeskops et al., 2010). Thus determining the status of soil microbial communities is essential in order to evaluate soil's health as affected by organic farming management. This study examines the effects of a commercial fertilizer and a cover crop on soil microbial communities after a cycle of organic sweet pepper production. The fertilizer used was Bioflora Dry Crumbles 6-6-5[6], which is made from poultry manure and is approved for use in organic farms; the cover crop used was Sunn hemp (*Crotalaria juncea*). In this study, two methods were used in the characterization of the soil microbial community structure in order to detect early changes resulting from management: the phospholipid fatty acid (PLFA), and the ester-linked fatty acid methyl ester (EL-FAME) analyses.

Field studies were established at the University of Puerto Rico, Agricultural Experiment Station in Lajas (latitude: 18° 01' 55" N, longitude: 67° 04' 18" W, elevation: 26 meters above sea level) in April 2012. The experimental site is certified organic and has a Vertisol clay soil type of the Fraternidad series classified as a fine, smectitic, isohyperthermic Typic Haplusterts (NRCS, 2010). Treatments were arranged in a 2 x 4 factorial with a randomized complete block design and four replicas. No cover crop (NCC) and cover crop (CC) represent the two levels of one factor, and the nitrogen fertilization rates (0, 56, 112 and 168 kg N/ha) supplied by the commercial fertilizer (BioFlora Dry Crumbles™

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[6]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

6-6-5) represent the four levels of the second factor. Sunn hemp was incorporated 56 days after sowing (DAS) in the assigned sub-plots (3 m x 4 m), whereas half of the sub-plots remained fallow. After incorporation, fertilizer was applied to every sub-plot according to the treatment. Nine days later sweet pepper seedlings were transplanted and managed equally until harvest.

After harvesting sweet pepper, one composite soil sample was taken (0- to 10-cm depth) from every experimental unit (32 soil samples total). Fatty acids were extracted from soil microbial communities using PLFA and EL-FAME analyses. Specific types of fatty acids are associated with distinct microbial groups such as fungi, gram positive (G+) and gram negative (G-) bacteria, as well as actinomycetes; all of which can provide useful information on microbial community composition (Zelles, 1999). The PLFA method followed procedures described by Buyer and Sasser (2012), and only examines the phospholipid content that belongs to living microbiota. For the EL-FAME method, the extraction procedure described by Schutter and Dick (2000) was used. This method extracts fatty acids originating not only from phospholipids, but also from glycolipids and neutral lipids including those from dead organic matter. Fatty acids obtained from the two extraction methods are converted into fatty acid methyl esters (FAME). The FAMEs are more volatile than the original fatty acids and therefore were analyzed using gas chromatography (Perkins and Colwell, 2008).

In this Vertisol soil, the sum of total FAMEs ranged from 190.38 nmol/g to 229.47 nmol/g for EL-FAME, which can provide an indication of the soil microbial community size. According to ANOVA, the microbial community size was not affected by cover crop, fertilization rate or their interaction. Principal component analyses (PCA) comparing all FAMEs (a total of 45 extracted from the soil) together, to better represent the microbial community structure, showed no separation due to fertilization rate (Figure 1A). However, a trend toward separation in the soil microbial community structure due to cover crop was evident (Figure 1B).

There was a significant difference between extraction methods, with the average concentration of total FAMEs for the PLFA method (109.65 nmol/g soil) substantially lower than that using the EL-FAME method (209.20 nmol/g soil). According to the PLFA procedure, a total of 34 different FAMEs were identified in this soil, 23 of which were consistently present in the samples and were used for data analysis. On the other hand, the EL-FAME procedure identified 58 FAMEs; 45 were used for data analysis. Twenty-two fatty acids were common to both methods.

Regarding the abundance of each microbial group according to indicator FAMEs, those typical for G+ bacteria were most abundant and corresponded to approximately 28% and 26% of total PLFA and EL-FAME concentrations, respectively. The next abundant microbial groups were G- bacteria and actinomycetes. Total bacterial abundance, obtained from the sum of G- bacteria, G+ bacteria and actinomycetes, corresponded to 65% and 48% of total PLFA and EL-FAME concentrations, respectively. Fungal markers constituted 10% and 13% of the total FAMEs using the PLFA and El-FAME methods, respectively. FAMEs that correspond to saprophytic fungi were most abundant compared to arbuscular mycorrizal fungi (AMF) for this soil. In order to calculate fungal:bacterial (F:B) ratios, a distinction between fungal groups was made and AMF was excluded from the F:B ratio since it does not share the same ecological role as saprophytic fungi. The F:B ratio obtained was ~0.1 for both methods, which indicates bacterial dominance over fungal dominance in this soil.

Long-term studies have provided important information on how different management practices (i.e., cover crops, cropping systems, tillage) can affect soil microbial community size and structure (Bossio et al., 1998; Ndiaye et al., 2000; Schutter et al., 2001; Acosta-Martínez et al., 2010). However, studies determining changes in soil microbial

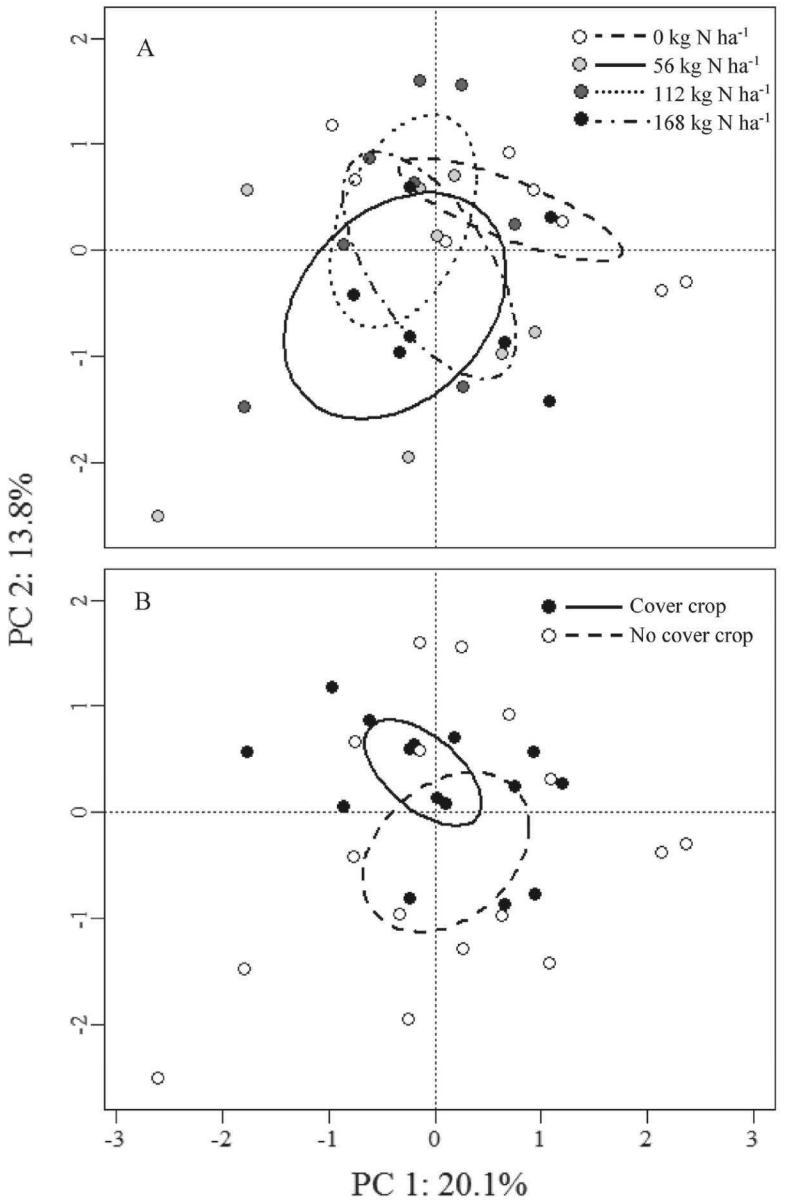

FIGURE 1. Principal component analysis (PCA) of the soil microbial community structure as affected by the use of N fertilization rate (A) and cover crop treatment (B) according to the EL-FAME method using 45 FAMEs. The percent variance explained by each principal component (PC) axis is included.

communities within the first years of implementing the management practices can aid in management redirection and decision-making. This information is especially important since organic farming is a recent management practice in Puerto Rico. Cover crop and fertilizer rates in this experiment were tested within the first year of establishment, and the use of cover crops revealed a trend of microbial community shifts compared to other treatments. No differences were found due to the fertilization levels. These early results for the Vertisol soil evaluated confirm previous reports by Ocio et al. (1991) that the microbial communities will first use available nutrients from plant residues rather than from the soil nutrient pool. Regarding the use of the commercial organic fertilizer (BioFlora 6-6-5), no effects were found in soil microbial community abundance or structure. Although studies generally address the effects of organic amendments after some years of applications, it is possible to observe early shifts in the microbial community structure and activities within the first year of application. Many researchers have reported the high levels of nutrients found in poultry manure and the shifts in microbial community structure toward higher fungal populations within the first three years of application compared to the non-treated soil (Acosta-Martínez and Harmel, 2006). The lack of an early response of the soil microbial communities to the commercial fertilizer used can be due to its manufacturing process, which includes composting and heating to temperatures over 83° C for pelletization (Global Organics Group, 2012). This process may reduce the microbial and enzyme load of the fertilizer. Using both EL-FAME and PLFA analyses, results showed that one cover crop cycle was too early to detect significant changes in microbial community composition according to differences in microbial groups. However, changes in microbial community composition could have been detected by methods such as pyrosequencing that characterize microbial diversity at the species level. Overall, compared to other indicators of soil quality such as physical (e.g., texture and soil aggregation) or chemical (e.g., pH, CEC, and organic matter) parameters, biochemical parameters that indicate size, structure and activity of soil microorganisms provide a rapid index of soil quality (Sparling, 1992). This rapid determination is essential in soil management because it allows early detection of soil degradation or improvement and hence is an efficient tool for monitoring soils. The trend of early shifts in microbial community structure due to the presence of cover crops is ecologically significant because of the implications of improved biogeochemical cycling and soil organic matter (SOM) dynamics in this Vertisol soil under organic farming.

Although a comparison of the trends in shifts in microbial community structure using both methods (PFLA and EL-FAME) was planned, this was not possible due to the lack of significant effects of cover crop and fertilization rates on the FAMEs evaluated. The quantitative differences in FAME indicators of abundance (concentration) between the methods were expected, since PLFA only extracts the fatty acids found in phospholipids and, therefore, the concentration of FAMEs will be smaller. Compared to the original PLFA extraction method, this high throughput PLFA procedure released in 2012 by Buyer and Sasser provides an advantage in optimizing time and costs. However, more research will help validate the method for different soils. When it comes to the EL-FAME method, the fact that fatty acids are extracted from all lipids and not only from phospholipids is an issue (Zelles, 1999; Schutter and Dick, 2000), and as seen in this experiment, EL-FAME extracted 45 different fatty acids whereas PLFA only extracted 25. However, many studies have successfully used this method to evaluate shifts in microbial community structure and when it is used to determine changes in abundance due to treatments, attention should be paid to the interference caused by fatty acids that are not part of the microbial community. The fact that the F:B ratios were the same for both methods suggest that the extraction patterns are similar and they are suitable for microbial community structure characterization, especially for tropical soils. According

to the comparison established here for PLFA and EL-FAME methods, both are suitable for evaluating the microbial community in soils from Puerto Rico under organic farming practices.

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