ORGANIC SWEET PEPPER YIELD AND SOIL MICROBIAL COMMUNITIES AS AFFECTED BY A COMMERCIAL ORGANIC FERTILIZER AND SUNN HEMP AS A COVER CROP IN PUERTO RICO

by

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ABSTRACT

Sunn hemp (*Crotalaria juncea*) is a legume cover crop that produces large amounts of biomass which, combined with its relatively high nitrogen (N) content and N-fixing capacity, can potentially offset N fertilizer needs by providing nitrogen to the subsequent crops in organic production. This can be economically beneficial if fertilizer inputs are reduced without a yield reduction. This is especially true when organic growers have the need to buy commercial organic fertilizers, and it is more critical in an island like Puerto Rico, where all imports must be shipped in, adding to the cost. Furthermore, the numerous benefits to soil health and the environment contribute to the system's sustainability.

This study was conducted at the Agricultural Experimental Station of Lajas, University of Puerto Rico for two growing seasons, the first one starting in December 2011 (2011 Experiment) and the second one in April 2012 (2012 Experiment). The specific objectives of this experiment were to: 1) determine fruit yield of sweet pepper (*Capsicum chinense*) as affected by a commercial organic fertilizer (BioFlora Dry Crumbles™ 6-6-5) and the use of sunn hemp as a cover crop; 2) determine the status of the soil microbial community size and structure under cover crop and N fertilization treatments; and 3) assess other soil parameters such as pH, organic matter, and enzyme activity.

To evaluate cover crop and N interactions a 2x4 factorial arrangement of treatments was used. No cover crop and cover crop were the two levels of one factor, and 0, 56, 112, and 168 were the four levels of the second factor, which indicated the kilograms of N per hectare (ha) supplied by the commercial fertilizer BioFlora (6-6-5). There was no significant effect of cover crop for both experiments, however, the 2011 Experiment indicated a trend

toward reduced yields when the cover crop was used, which could be attributed to sunn hemp's allelopathic properties. N fertilization did have a significant effect on sweet pepper yields, with 112 and 168 kg N ha^{-1} the fertilization rates that resulted in the highest yields for the 2011 Experiment with no significant difference between them. In the 2012 Experiment, the 168 kg N ha^{-1} resulted in highest yield. Differences in N fertilizer needs between experiments could be attributed to different factors such as soil properties, weather conditions (temperature and precipitation) and pest incidence.

Regarding soil microbial community size and structure, there were no significant differences found among treatments according to individual evaluation of total FAMEs. However, the principal component analysis (PCA) indicated an early shift in the microbial community structure due to cover crop use. Other studies have demonstrated that soil microbial composition is affected by different management practices which indicate that replicating these experiments could confirm the early differences seen in this study over time. The rest of the soil parameters assessed indicate favorable conditions, even though organic matter content was lower than expected.

RESUMEN

Sunn hemp (*Crotalaria juncea*) es una leguminosa comúnmente usada como cultivo de cobertura que produce grandes cantidades de biomasa. Combinado con su alto contenido de nitrógeno (N) y su capacidad de fijar N, esta leguminosa podría compensar las necesidades de fertilización al proveer N a cultivos en producción orgánica. Esto puede resultar en beneficios económicos si se logra reducir la cantidad de fertilizante utilizado sin afectar los rendimientos. Esto es de especial importancia para productores orgánicos que compran fertilizantes y la situación es más crítica en una isla como Puerto Rico, donde estos productos deben ser importados, aumentando su costo. Adicionalmente, los diversos beneficios para la salud del suelo y el ambiente contribuyen a la sustentabilidad del sistema.

Este estudio se realizó en la Estación Experimental Agrícola de Lajas de la Universidad de Puerto Rico durante dos épocas diferentes; la primera inició en diciembre del 2011 (Experimento 2011) y la segunda en abril del 2012 (Experimento 2012). Los objetivos específicos de este estudio fueron: 1) determinar los rendimientos de ají dulce (*Capsicum chinense*) cultivado bajo diferentes tasas de aplicación de un fertilizante orgánico comercial (BioFlora Dry Crumbles™ 6-6-5) y el uso de sunn hemp como cultivo de cobertura; 2) determinar el tamaño y estructura de las comunidades microbiológicas del suelo en los diferentes tratamientos de fertilización e incorporación de sunn hemp; y 3) evaluar otros parámetros del suelo como pH, contenido de materia orgánica y actividad enzimática.

Para evaluar los efectos del cultivo de cobertura y fertilización se usó un arreglo de tratamientos factorial de 2x4. El uso y no uso de sunn hemp representaron los dos niveles del

primer factor y las cuatro tasas de fertilización (0, 56, 112, y 168 kg N ha^{-1}) suplidas por el fertilizante comercial BioFlora (6-6-5) representaron los cuatro niveles del segundo factor. No hubo diferencias significativas en relación al uso de sunn hemp para ambos experimentos, sin embargo, en el Experimento 2011 se identificó una tendencia hacia menores rendimientos con el uso de sunn hemp lo cual podría ser ocasionado por las propiedades alelopáticas de esta especie. Por otro lado, sí hubo diferencias significativas entre las tasas de fertilización. En el Experimento 2011 los rendimientos más altos se obtuvieron con 112 y 168 kg N ha^{-1} y no hubo diferencias significativas entre estos dos niveles. En el Experimento 2012 los rendimientos más altos se obtuvieron con 168 kg N ha^{-1}. Las diferencias entre las tasas de fertilización requeridas por ambos experimentos pueden atribuirse a varios factores, incluyendo propiedades del suelo, condiciones climáticas (temperatura y precipitación) e incidencia de plagas.

En relación al tamaño y estructura de la comunidad microbiológica del suelo tampoco se encontraron diferencias significativas entre tratamientos, sin embargo, el análisis de componentes principales mostró leves cambios en la estructura microbiana como resultado del uso de sunn hemp. Otros estudios han demostrado que la composición microbiana del suelo es afectada por diferentes prácticas de manejo lo cual induce a pensar que réplicas de estos experimentos podrían confirmar los leves cambios en este estudio. Los demás parámetros mostraron condiciones favorables, a pesar de que el contenido de materia orgánico fue menor a lo esperado.

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1 INTRODUCTION

` Applications of chemical fertilizers produced with fossil energy have contributed to an increase in crop yields for the past 40 years (Pimentel, 1996). There is no doubt that the green revolution made impressive contributions to food production but its apparent gains are now opaqued by the serious environmental problems it has caused. Additionally, not only does it rely on finite fossil fuel reserves, but also the costs of agricultural chemicals have increased considerably during the past years, making this not only an ecological but also a social concern (Sherwood and Uphoff, 2000). This situation becomes more critical on an island like Puerto Rico, where all imports must be shipped in, adding to the cost.

Nitrogen (N) is an essential nutrient for crop growth, and also the most difficult to manage because of its mobility in soils and the large amounts needed by plants (Campiglia et al., 2010). Approximately 80 million tons of commercial N is used in world agriculture every year, and of the total N applied to crops only 25-50% is harvested in the crop. Of the remainder, 20-50% is lost by erosion, 10-50% by leaching and 10-50% by volatilization, making N a major environmental pollutant from the agricultural industry (Pimentel, 1996). As a general rule, agriculture in the tropics is expected to be more dependent on N fertilizers because of heavy rains and rapid decomposition of organic matter (Döbereiner, 1997). Keeping in mind these environmental concerns, tropical countries have the added pressure to meet their food demand while their population is in constant increase.

During the 1940s and 50s, Puerto Rico began its industrialization process, resulting in a gradual devaluation and abandonment of agriculture as a viable economic activity. The past 30 years have represented a loss of approximately 30% of agricultural lands to other uses and

currently about 85 to 90% of all the food consumed in Puerto Rico is imported (Padín et al., 2009). In addition, Puerto Rico has a population density of almost 449 inhabitants per square kilometer which is one of the highest population densities in the world and the fourth highest in America (United Nations Statistics Division, 2012). With the challenge to produce a greater percentage of the food the island's residents consume and the concerns about the long-term sustainability of conventional production methods, the potential for organic farming has received increasing attention.

Organic farming is just emerging in Puerto Rico and thus, there are many factors regarding this production method that need to be explored. Organic farming relies on practices that will sustain and/or improve the soil's productive capacities and the soil microbial communities play an important role in doing so. Nutrient cycling, residue decomposition, N fixation, and carbon sequestration are primarily controlled by microbes because they are the main source of necessary enzymes capable of carrying out such activities (Gardner et al., 2011; Moeskopsa et al., 2010). Therefore, determining the status of soil microbial communities is essential in order to evaluate the soil's health as affected by organic farming management.

Another ongoing concern in Puerto Rico is the constant need to import materials. Sustainable agricultural practices limit off-farm inputs while maintaining and enhancing soil health. Practices can no longer be based on adding prescribed nutrients to the soil in order to obtain desired yields (Pimentel, 1996). Organic production systems use different sources of N, including manure, compost, legumes, and animal by-products. All of these sources differ in cost, nutrient content, mineralization rate, and environmental impact. The advantage of using legumes as cover crops is that they can reduce N losses and increase N supply for succeeding crops,

reducing or eliminating fertilizer requirements. Furthermore, cover crops are a cost efficient source of N (Campiglia et al., 2010; Campiglia et al., 2011).

Soil fertility improvement capacity of different legumes has been researched in many parts of the tropics. A wide range of legume species has been researched including sunn hemp (*Crotalaria juncea*), which is the most widely used cover crop species in this genus. In North America, this species is grown as a summer crop in Hawaii, Texas, Florida, and Arkansas. The use of this cover crop is highly recommended not only due to its nitrogen contributions to the soil but because it suppresses weeds and nematodes, improves soil tilth and water holding capacity, and reduces erosion (Giller, 2001). Studies in southeastern United States have suggested that sunn hemp is well suited for use as a cover crop and green manure and it can contribute to up to half of the N needed by the subsequent crop (Balkcom and Reeves, 2005; Mansoer et al., 1997; Schomberg et al., 2007). Additionally, the use of sunn hemp in the tropical country of Zimbabwe has proven to increase maize grain yields 8 to 27% compared with continuous production, reducing fertilizer needs by 18 to 36 kg N ha^{-1} (Jeranyama, 2000).

When it comes to soil quality indicators several properties can be evaluated in order to determine if different management practices as affecting soil quality. Among these properties is the soil microbial structure and abundance which has proven to be an early indicator of soil quality. Background data for soil microbial community structure and composition is lacking especially in Puerto Rico, which could potentially help explain the effect of cover crops and the overall soil health.

2 OBJECTIVES

The first objective of this study was to determine fruit yield of sweet pepper (*Capsicum chinense*) as affected by a commercial organic fertilizer (BioFlora 6-6-5) applied at four different N rates and the use of a cover crop (*Crotalaria juncea*). Additional objectives were to determine the status of the microbial community size and structure in this soil under the treatments evaluated, and to assess other soil parameters such as pH, organic matter, and enzyme activity.

3 LITERATURE REVIEW

3.1 Environmental impacts of conventional (industrial) and organic agriculture

Humans have practiced agriculture for more than 10,000 years, but only in the past 50 years have agricultural practices changed to what is currently known as conventional agriculture. Consequently the terms “conventional” and “industrial” agriculture are used interchangeably to describe the food production practices that emerged during the green revolution (Beus and Dunlap, 1990; Horrigan et al., 2002). Conventional farming systems share many characteristics: farms are highly capitalized, especially in developed countries; small units are usually discouraged since it is believed that larger units are more efficient; single crops (monoculture) are grown continuously over many seasons; uniform high-yielding hybrid or genetically modified crops are usually planted; and it requires many inputs which are usually applied in increasing amounts and are manufactured from inorganic sources (Altieri, 2011; Horrigan et al., 2002). The consequences of these agricultural developments can be narrowed to two fundamental problems: (1) reduced diversity within production units and (2) the breakdown of self-sustaining systems (Kiley-Worthington, 1980; Altieri, 2011).

Despite its crucial role in providing food, conventional agriculture is a major cause of genetic erosion as its focus is to maximize the output of a limited amount of species. During the last decades, agriculture has become highly mechanized, requiring farms to have uniform crops and management practices leading to a reduction in crop diversity. Farms with low crop diversity, or monocultures, have poor "associated diversity", including natural enemies of

potential pests. This makes a farm dependent on synthetic pesticides which have impacts far beyond their target organisms (Picone and Van Tassel, 2002).

Monocultures, large production units, and lack of rotation, among other practices, have caused conventional agriculture to rely heavily on external inputs making the system unsustainable. Inputs used in conventional agriculture include synthetic pesticides and fertilizers which are major environmental pollutants. Among inputs used in agriculture, N is of great concern as excess nitrate is water soluble and can easily be leached, making nitrate contamination a widespread problem where N fertilizers are frequently used. A clear example of this can be seen in the Northern Gulf of Mexico which is one of the largest hypoxic zones in the western Atlantic Ocean as a consequence of nutrient enrichment. This zone measures on average 17,000 km^2 and agricultural sources contribute to more than half of the inorganic N carried by the Mississippi and Atchafalaya rivers which eventually pollutes this area (Ribaudo et al., 2005). Unfortunately, N applications are one of the most effective tools for increasing yields, and its use worldwide has grown more than five times from 1960 to 1990. Furthermore, fertilizer consumption in developing countries is projected to double by 2020 (World Resources Institute, 2012).

As consumers become increasingly concerned about the negative impacts of conventional farming practices on the environment, attention has shifted towards organic agriculture. In the United States and Puerto Rico, the National Organic Program (a program of the USDA Agricultural Marketing Service) codifies organic production methods that are based on certified practices verified by independent third-party reviewers. These systems give consumers assurance on how their food is produced and enables them to choose foods based on the methods by which they were produced (Pimentel et al., 2005). The National Organic Program prohibits the use of

synthetic chemicals, genetically modified organisms, and sewage sludge in organically certified production (USDA, 2000). Additionally, a well-managed organic farming system provides favorable environmental conditions such as soils with higher organic matter content and biological activity, significantly lower rates of nitrate leaching compared to conventional farming, no risk of ground and surface water pollution through synthetic pesticides, and higher on-farm biodiversity both of plant and animal species (Alfoeldi et al., 2002).

Organic farming is a rapidly growing industry worldwide. From 2001 to 2011, the total number of hectares under organic production in the world has grown by 135%, meaning a compounded annual growth of 8.9% over this decade. Data from 2011 indicates that there are 37.2 million organic agricultural hectares in a total of 160 countries world-wide (Paull, 2011). Organic agriculture in Puerto Rico is at an early stage, with only five certified organic farms, however the number of growers who are moving away from conventional agriculture is increasing. As a result, growers are in need of sound information to guide their management decisions. A survey conducted in 1997 by the Organic Farming Research Foundation (OFRF) identified fertility management, soil health, and crop rotations as the three most important research areas within crop and soil management (Walz, 1999). These topics were later included in their National Organic Research Agenda to inspire research in these areas (Sooby et al., 2007).

3.2 Nitrogen management on organic farms

Fertilization plans on organic farms are limited to organic sources of N or those derived from natural processes. Such sources include: fixed N from cover crops and green manures, compost produced from on-farm or off-farm materials, manures from on-farm or off-farm sources, and purchased organic fertilizers. All these materials differ in cost, nutrient content,

mineralization rate, and environmental impact, so the goal is to define a fertilization method that results in the greatest crop yield with the least amount of fertilizer, lowest cost, and least negative impact on the environment (Campiglia et al., 2011; Hochmuth, 2003). The OFRF survey identified the use of cover crops as the most common fertility management practice on organic farms followed by compost application (Walz, 1999).

Cover crops are traditionally defined as crops grown to cover the ground in order to protect the soil from erosion and loss of nutrients through leaching and runoff. However, cover crops have multiple benefits including weed suppression, carbon sequestration, reduction of ground water contamination and integrated pest management (Giller, 2001; Scholberg et al., 2010). Basically, cover crops are plants growing and covering the soil surface. When these plants are tilled into the soil they are sometimes referred to as green manures. However, for the purpose of this study the term cover crop, which alludes to its multiple uses and benefits will be used.

The effect of cover crops on N dynamics has been of great interest for many years and has aimed mainly at understanding how to prevent N leaching and how to improve N supply to subsequent crops. Nitrate lost from agriculture to the environment causes nitrate concentrations to increase both in aquifers used for drinking water and in surface waters such as streams, lakes and coastal waters. One of the benefits of growing cover crops is that they can remove nitrate from soil water, thereby reducing nitrate content in the water percolating from the soil. However, there is a large variation on the N uptake by cover crops, which is mostly due to: (1) variable cover crop growth and N uptake potential under a given set of climatic conditions, (2) variable root growth and contact with available N and, (3) available nitrogen in the soil (Thorup-Kristensen et al., 2003).

Even though there are factors affecting N uptake, it has been well documented that cover crops can considerably reduce nitrate leaching loss. On the other hand, the effect of cover crops on N supply for subsequent crops is less clear. Legume cover crops normally increase the N supply for a subsequent crop since they get their N through biological N fixation and by taking inorganic N from the soil. However, the amount of N added to the system through N fixation varies significantly depending on the legume species and environmental conditions (Thorup-Kristensen et al., 2003).

Organic farmers rely on cover crops that must be broken down and transformed by soil microorganisms into inorganic nutrient forms before plants can absorb them. Soluble inorganic fertilizers used in conventional agriculture are available immediately to plants, so nutrient application is timed to coincide with crop uptake need. An ongoing challenge for organic farmers regarding N availability for subsequent crops is the lack of synchrony between N release by the cover crop and N demand by the subsequent crop. This can happen because: (1) Residue materials decompose slowly and therefore N supply may come too late for the crop demand or, (2) residue materials will decompose rapidly and N supply comes too early for the crop demand and therefore is lost to the environment (Baijukya et al., 2006). These aspects of N supply have normally been explained by the effect of N mineralization and immobilization during decomposition of the cover crop. However, this approach only considers the effect of cover crops from the time they are incorporated and ignores their effect on the soil before it is incorporated. Therefore, whether a cover crop will increase or decrease N supply for a subsequent crop is a result of complex interactions that must be closely analyzed to avoid making erroneous assumptions (Thorup-Kristensen et al., 2003).

In addition to biological N fixation, nutrients may be brought into the organic system as manures, composts and allowed commercial fertilizers. Composted manure is recommended in order to control weeds, pests, and diseases; however, the composting process results in physical and chemical changes, causing a significant reduction in N availability (Watson et al., 2002). N losses during composting occur through volatilization in the form of ammonia; on the other hand, soluble N components are stabilized and hence less liable to leaching. Composted manure thus has a more long-term role in building soil fertility, and has been shown to be more effective in building soil microbial biomass and increasing activity than un-composted manure (Watson et al., 2002). In addition to composts made from on-farm materials, composts may originate from commercial sources and their value lies in the relatively rapid availability of nutrients contained. When applying compost, the same issues may arise as when using cover crops, which is timing N release to crop demand. On the plus side, the low N content of composts minimizes the risk of leaching (Briggs, 2008).

3.3 Sunn hemp as a cover crop in Puerto Rico

There are many species of legumes that can be used as cover crops and that are adapted to different day length, temperature, radiation, rainfall, soil, and pests. For this reason, a legume's performance and beneficial effects will depend on the selection of the appropriate species and its management. Overall, they can be grouped as being adapted to temperate/cold or tropical/warm regions.

Approximately half of the world area which is planted with grain legumes is located in tropical countries (Baligar and Fageria, 2007). Tropical legumes contribute about 35% to world production of legumes which is higher than for tropical cereals (30%) (Baligar and Fageria,

2007). Although there are many legume cover crops adapted to tropical biotic stresses such as low soil pH, low levels of soil nutrients, high temperatures, and drought, few species have actually been researched (Baligar and Fageria, 2007). The tropical legume species of interest, which have been studied for their rates of N fixation and contribution of N in corn and vegetable systems include: cowpea (*Vigna unguiculata*), canavalia (*Canavalia ensiformis*), soybean (*Glycine max* L.), hairy indigo (*Indigofera hirsutum*), velvetbean (*Mucuna deeringiana*), lablab (*Lablab purpureus*), and sunn hemp (*Crotalaria juncea*) (Baligar and Fageria, 2007; Dabney et al., 2010; Giller, 2001).

There are more than 600 species of the genus *Crotalaria* and the majority of them are found in Africa. There are several species that can be used as cover crops but the most widely used is sunn hemp (*C. juncea*). Sunn hemp is an erect shrubby annual which can grow up to 3 m in height. It is drought resistant, is adapted to different soil types, and can grow on poor soils with either high or low pH. It is photosensitive and flowering occurs in response to short days, while long day lengths favor vegetative growth (Mannetje, 2012). On average, sunn hemp produces 14.8 to 21 tons of dry mass per hectare which means that the amount of nitrogen potentially available to subsequent crops varies between 380 to 490 kilograms per hectare (Li et al., 2006).

In Puerto Rico, there is limited information on the use of sunn hemp in cropping systems. Results of biomass and N accumulation studies are similar to what has been seen in other regions. Therefore, due to sunn hemp's photosensitivity it is suggested that if the intended use is as a cover crop and not for seed production, sunn hemp should be planted during the summer (when the days are longer) since biomass production will be higher (Chan, 2010; Santos, 2010).

Unfortunately, the effect of sun hemp as a cover crop for subsequent cash crop has not been evaluated in Puerto Rico. However, research studies in Florida have indicated that the use of sunn hemp significantly increased tomato fruit yield from the second harvest in comparison to the fallow treatment (Wang et al., 2003). Another study with corn in Alabama also evaluated the benefits of using sunn hemp as a cover crop, indicating that corn following sunn hemp out-yielded corn following fallow. Averaged over three years, maize grain yields were 1.2 Mg ha^{-1} greater when following sunn hemp (Balkcom and Reeves, 2005). Studies with other species of the same genus have shown similar results. In Uganda, *Crotalaria ochroleuca* increased corn yield by 39% (Fischler et al., 1999); while in Kenya, averaged over two years, *C. ochroleuca* and *Mucuna pruriens* improved maize grain yield by 1.5 t/ha compared to no incorporation (Ojiem et al., 2000). These studies indicate the positive outcomes the use of sunn hemp has had in other regions which could potentially be translated to Puerto Rico.

3.4 Commercial fertilizers for organic crop production

Regarding soil fertility and crop nutrient management, the USDA National Organic Program states that if raw animal manure is applied to soil, an organic crop cannot be harvested for 90 days, if the edible portion does not have direct contact with the soil or for 120 days if it does. However, if the manure is composted according to the regulation, there is no harvest restriction (USDA, 2000). The regulation states that animal and plant materials must be composted through a process that:

(i) Establishes an initial C:N ratio of between 25:1 and 40:1; and

(ii) Maintains a temperature of between 131 °F and 170 °F for 3 days using an in-vessel or static aerated pile system; or

(iii) Maintains a temperature of between 131 °F and 170 °F for 15 days using a windrow composting system, during which period, the materials must be turned a minimum of five times (USDA, 2000).

If growers wish to produce their own compost, records must be kept indicating that they are in compliance with these composting requirements. Many growers believe this process to be too complicated so they prefer to buy commercial organic fertilizers that have been verified by a third party to be in compliance with this rule. In the United States, the Organic Materials Review Institute (OMRI) provides an evaluation of products intended for use in certified organic production, handling, or processing and verifies whether they are acceptable under the USDA National Organic Program.

Commercial organic fertilizers are usually byproducts of fish, livestock, food and other processing industries. Commercial organic N fertilizers provide more concentrated sources of N than compost; however, the cost is higher (Gaskell and Smith, 2007). Additionally, there are few research studies that have evaluated these commercial products or their source materials in controlled field trials. Bioflora Dry Crumbles™ (6-6-5) is an OMRI listed, dry granular fertilizer made from feather meal, dry composted poultry litter, sulfate of potash and seaweed (*Ascophyllum nodosum*) (Global Organics Group, 2012). Mineralization rates for feather meal and pelleted poultry manure at different temperatures have shown that mineralization is faster at higher temperatures, which could be an advantage in tropical climates. Furthermore, it was shown that N release rates are similar between green manures and high N organic fertilizers such as feather meal. However, the amount of N release from the fertilizers is greater (Gaskell et al., 2006).

Mineralization is a complicated process in which timing and N rate of release from organic sources is hard to predict. It is even more complicated when it comes to organic fertilizer materials, because these materials vary considerably with respect to particle size, moisture content, and nutrient distribution. This variability is a result of the production process and the fact that these materials continue to change during transport and storage. Despite these challenges organic vegetable growers rely on organic fertilizers to achieve acceptable yields (Gaskell and Smith, 2007; Gaskell et al., 2006).

3.5 Sweet pepper production in Puerto Rico

The genus *Capsicum* is native to the American tropics and is now cultivated worldwide. Some of the members of *Capsicum* are used as spices, vegetables, and medicines. Sweet pepper (*Capsicum chinense*) is a small, light green pepper that turns red when ripe. In the tropics, this plant can grow as a perennial, although most of the commercial production is with annual systems. These flavorful sweet peppers are preferred for Puerto Rican, Dominican Republic and Cuban cuisines which typically do not use hot peppers (Orengo et al., 1999). However, the Agricultural census (2007) in Puerto Rico indicate sweet pepper harvest has decreased from 14 million kg in 2002 to 11 million kg in 2004, which could be a result of overall decline of the agricultural industry.

Sweet pepper production is started with transplants. Growers can produce their own transplants or they can buy them from a supplier, and it takes approximately six weeks for the transplants to be ready. Suggested field fertilization rate is 112 kg ha^{-1} of N, 112 kg ha^{-1} of P_2O_5 and 90 kg ha^{-1} of K_2O (Orengo et al., 1999). Sweet pepper needs somewhere between 1.3 to 5.0 cm of water weekly, and a weed control program should be in place to avoid competition and

eventual decrease in yields. Major concerns in sweet pepper production are defoliating and virus transmitting insects and diseases caused by fungi such as *Phytophtora capsici* and *Colletotrichum capsici*. Flowering starts 90 to 100 days after planting, fructification begins about 7 days later, and the grower can expect about 10 harvests, though these parameters may change depending on the location and season (Orengo et al., 1999).

3.6 Organic farming benefits to soil quality

3.6.1 Soil fertility

Nutrients derived from cover crops are usually released gradually over time, which enhances nutrient efficiency. Legume cover crops provide supplementary N due to biological N fixation and their relatively low C:N ratio also increases mineralization, making N available to subsequent crops. The use of legume cover crops has been a topic of debate for many years with the opposing view that their contribution to subsequent crops is not significant and that utilization of N released by cover crops can be poor if nutrient release is not synchronized with the subsequent crop demand. Regardless, the amount of N that the legume will fix and that will ultimately be available for the subsequent crop is highly variable. This variability is a result of: 1) the N content of the cover crop; 2) plant available N in the soil; 3) the genetic potential of the legume species; and 4) soil factors such as microbial activity, pH, moisture content and temperature (Fageria et al., 2005).

Limited research has been done on N fixation by legume cover crops, however there are various estimates on the amount of N they can accumulate. The available estimates of N fixation by different legume cover crops in the tropics indicate they are capable of fixing large amounts

of N, which commonly exceed 100 kg N ha^{-1} within 6 months. Additionally, roots of cover crops also exude organic compounds which can enhance soil microbial activity, mycorrhizal activity, soil structure, and nutrient availability. Furthermore, cover crops with deep roots, such as sunn hemp, are capable of absorbing from deep soil layers and make them available for subsequent crops (Scholberg et al., 2010).

3.6.2 *Soil structure*

Soil structure is an important factor in the functioning of soil and its ability to support plant and animal life. It can be described in terms of degree of structure, shape and size of aggregates, and stability of the aggregates. In general, aggregation is a result of the interaction of many factors including the environment, soil management factors, plant influences and soil properties. Consequently, it can be enhanced through various soil/crop management practices including the use of cover crops (Bronick y Lal, 2005).

When the cover crop is still standing its roots can penetrate compacted soil layers and root channels can enhance soil water infiltration, root penetration, soil water-holding capacity, and thus, crop water use efficiency of subsequent crops. Furthermore, adding plant material to the soil, especially root matter, can also improve water infiltration and holding ability. Cover crops can also maintain or improve soil structure by protecting the soil from rainfall impact and extreme temperature fluctuations, thereby reducing soil crusting and erosion (Scholberg et al., 2010).

3.6.3 Soil organic matter

Soil organic matter (SOM) improves soil quality, and influences greatly its physical properties, which in turn enhances crop productivity. SOM has been linked to improved soil

aggregation, enhanced water infiltration, soil aeration, and reduced erosion. It has been widely acknowledged that the use of cover crops increases SOM, however, this is true only if SOM addition rate exceeds SOM breakdown, and this depends on management and environmental conditions (Cherr et al., 2006).

There are two contradicting views in the value of cover crops. The first value of cover crops is as a source of N to succeeding crops, which occurs when they decompose and organic N is converted to inorganic N (mineralization). The second value is when the cover crop does not decompose and it is accumulated as organic matter. In this case, the N will remain in its organic form and will not be available to the plant. In other words, if a cover crop decomposes rapidly it will be a good source of N for the following crop but the impact on soil organic matter will be minimal since only a small portion of the carbon from the cover crop will be converted to SOM (Bouldin, 1988; Scholberg et al., 2010).

Generally, decomposition of cover crop residues is affected by: (1) the amount applied; (2) the biochemical composition; (3) the crop's developmental stage when incorporated; (4) soil texture, temperature, and moisture conditions; (5) residue particle size; and (6) nutrient availability and fertilizer addition. Due to the various factors that affect decomposition rate, studies have shown that changes in SOM can vary considerably depending on site-specific conditions and the cover crop used. However, changes in SOM often happen over long periods of time (Scholberg et al., 2010).

3.6.4 Soil microbial communities

Soil microbial communities play an important role in agroecosystems because they mediate biogeochemical processes, soil organic matter transformation, and are probably the

earliest indicators of soil quality (Zelles, 1999). Although about 95% of the species in the soil are still unknown, considerable information has been gathered on the most important groups: bacteria, actinomycetes and fungi. Bacteria are unicellular organisms important in organic matter decomposition and biological transformation of nutrients; furthermore, they form symbiotic relationships with plants and other soil microbes. Actinomycetes are a particular form of prokaryote which morphologically resembles fungi; they are important for soil aggregation, production of antibiotic compounds, organic matter turnover, and nitrogen fixation. Fungi are found in many forms in the soil and their role include organic matter decomposition, soil aggregation, plant pathogens, and they also form symbiotic relationships (Nelson and Spaner, 2010).

As a general rule, soil microbes need water, energy (i.e. SOM, or plant and animal residues), and other essential elements (Ilyas and Bano, 2012). Physically, factors that affect soil microbes include: SOM content, composition of the mineral fraction, and air and water proportions. Chemically, microbes are affected by pH, cation exchange capacity, mineral content, nutrient concentration, and concentration of gases, among others. Management practices indirectly affect microbial communities by altering any of the previously mentioned soil properties (Ilyas and Bano, 2012; Nelson and Spaner, 2010). Consequently, research has been focused on determining soil microbial communities on different types of soils and under different management practices.

3.7 Methods available to determine microbial community size and structure

Over the years different methods to study microbial composition have been developed and, due to the high diversity of microorganisms and overwhelming numbers, more efficient methods

than culture media were needed. Methods for assessing microbial community composition that have become increasingly popular are the ones that use fatty acids. Since specific types of fatty are associated with distinct microbial groups such as fungi, gram positive (G+) and gram negative (G-) bacteria, actinomycetes, etc., these methods are useful in gathering information on microbial community composition (Zelles, 1999). Fatty acids can be obtained from soil samples through different extraction methods which have the purpose of producing fatty acid methyl esters (FAME). FAMEs are more volatile than the original fatty acids and therefore can be analyzed using gas chromatography (GC).

The two extraction methods most commonly used are the phospholipid fatty acid (PLFA), and the ester-linked FAME (EL-FAME) methods. The PLFA method extracts FAMEs specifically from phospholipids. Initially the entire lipid content is extracted from the soil and then the phospholipid fraction is separated and then converted to FAMEs (Zelles, 1999; Buyer and Sasser, 2012). The advantage of this method is that it specifically describes the structure of living microbiota, however it is a laborious and time consuming method (Acosta-Martinez et al., 2010). Recently, Buyer and Sasser (2012) adapted the PLFA extraction technique to a 96-well plate format allowing to process 95 samples in 1.5 days, which represents a 5-fold increase in throughput. Additionally the amount of soil needed per sample and, consequently, the amount of chemicals used are also reduced minimizing costs and waste.

On the other hand, EL-FAME is a more simple method where FAMEs are formed from all lipid molecules rather than just from phospholipids. Not having to separate the phospholipid fraction makes EL-FAME an easier and much less time-consuming method, substantially increasing the number of samples that can be processed. A potential limitation is that EL-FAME also extracts fatty acids originating from glycolipids and neutral lipids including those in dead

organic matter, which can potentially interfere with the evaluation of the microbial structure (Steger et al., 2005; Acosta-Martinez et al., 2010). Nonetheless, EL-FAME and the traditional PLFA methods have equally discriminated soil management and environmental effects on microbial communities (Drijber et al., 2000).

FAMEs are named according to the convention *X:Y*ω*Z*, where "*X*" stands for the number of carbon atoms in the chain, "*Y*" stands for the number of double bonds, and "*Z*" is the number of carbon atoms from the methyl end of the molecule to the first double bond. The following prefixes were used: i, *iso* branched; a, *anteiso* branched; *n*Me, methyl branch on the *n* carbon from the carboxylate end; and cy, cyclopropyl. The suffixe "c" stands for the *cis* geometric isomers of unsaturation. FAMES commonly used as biomarkers for specific groups of microorganisms are described in Table 1.

Table 1. Fatty acid methyl esters used as biomarkers for different microbial groups for characterization of soil microbial community structure.

Microbial group	Common FAME used as marker	Reference
Bacteria		
G+ bacteria	*i*14:0, *i*15:0, *a*15:0, *i*16:0, *a*17:0, *i*17:0, *i*18:0	O'Leary and Wilkinson (1988)
G- bacteria	16:1ω7c, 16:1ω7t 16:1ω9c, cy17:0, 18:1ω5c, 18:1ω7c, 18:1ω7t, 18:1ω9c, cy19:0	Wilkinson (1988)
Actinomycetes	10Me16:0, 10Me17:0, 10Me18:0	Kroppenstedt (1992)
Fungi		
Saprophytic fungi	18:1ω9c, 18:2ω6, 18:3ω3	Frostegård and Bååth (1996); Klamer and Bååth (2004)
Arbuscular mycorrhyzal fungi (AMF)	16:1ω5c	Olsson et al. (1995) van Aarle and Olsson (2003)
Microeukaryotes (protozoa)	20:3ω6, 20:4ω6	Lechevalier (1977); Vestal and White (1989)

4 MATERIALS AND METHODS

4.1 Plant research

4.1.1 Site description

Field studies were conducted at the Agricultural Experimental Station of Lajas, University of Puerto Rico (latitude: 18° 01' 55" N, longitude: 67° 04' 18" W, elevation: 26 meters above sea level) in December 2011 and repeated in April 2012. The experimental site was certified organic in 2010 and covers an area of 9.4 acres within a Vertisol clay soil type of the Fraternidad series. This soil is classified as a Fine, smectitic, isohyperthermic Typic Haplusterts, and is described as having a strong medium and coarse granular structure; very firm, sticky, plastic and contains many fine roots. This soil has low permeability and is moderately well drained (NRCS, 2010).

Soil preparation for both the 2011 and 2012 Experiment consisted of deep plowing, followed by two passes with a disc harrow and one pass with a rototiller. The 2012 Experiment was relocated to an adjacent organic plot at the Agricultural Experimental Substation of Lajas, Puerto Rico.

4.1.2 Field methods

To evaluate cover crop and N interactions a 2x4 factorial arrangement of treatments was used. No cover crop (NCC) and cover crop (CC) represent the two levels of one factor, and the N fertilization rates (0, 56, 112 and 168 kg N ha^{-1}) supplied by the commercial fertilizer BioFlora Dry Crumbles™ (6-6-5) represent the four levels of the second factor. The combinations (e.g., NCC0, CC0, NCC56, etc.) denote individual treatment combinations (Table 2).

Table 2. The N fertilization levels and cover crop treatments evaluated at the Agricultural Experimental Station of Lajas, Puerto Rico.

Treatment label	Cover Crop (*Crotalaria juncea*)	Fertilization (kg N ha^{-1})
NCC0	none	0
NCC56	none	56
NCC112	none	112
NCC168	none	168
CC0	seeding rate of 7 kg ha^{-1}	0
CC56	seeding rate of 7 kg ha^{-1}	56
CC112	seeding rate of 7 kg ha^{-1}	112
CC168	seeding rate of 7 kg ha^{-1}	168

2011 Experiment. Treatments were arranged in a split-plot experimental design with four replicates. Each replicate was divided in half and on December 16, 2011 each half was sown with sunn hemp as a cover crop using a mechanical planter, in rows spaced 0.76 m apart and a target density of 7 kg ha^{-1}. Cover crop plots were 6 m x 8 m. After planting, irrigation was applied with an overhead sprinkler once on mid-January to assure proper development during the dry season.

Sunn hemp was incorporated 40 days after sowing (DAS) with a rototiller. Each cover crop plot was split into four sub-plots (3 m x 4 m) and randomly applied a fertilization rate of 0, 56, 112, or 168 kg ha^{-1} of N. Seven days later, on February 1, seedbeds were raised and sweet pepper seedlings previously grown (Appendix 1) were transplanted at a distance of 0.60 m between plants and 0.76 m between rows. Sweet pepper plants were watered using drip line irrigation three times per week (Monday, Wednesday and Friday).

Seedbeds were covered with a layer of hay mulch to suppress weed growth. Insect populations were monitored weekly. Dipel® DF (*Bacillus thuringiensis*, subsp. *kurstaki*; 54% A.I.) and Garlic Barrier AG+ (garlic extract; 99.3% A.I.) were applied at a dose of 0.36 kg ha^{-1} and 9.3 lt ha^{-1} respectively, with a 3 gallon backpack sprayer to control armyworms on 15 February. On 29 February, 23 March, and 23 May Dipel® DF and Trilogy (clarified hydrophobic extract of neem oil; 70% A.I.) were applied at a dose of 0.36 kg ha^{-1} and 1.8lt ha^{-1} respectively, to control the same pest.

2012 Experiment. This experiment was set up as a randomized complete block design with four replicates. Sub-plots (3 m x 4 m) assigned to have the cover crop were planted on April 18, 2012 at a seeding rate equal to the 2011 Experiment. Sunn hemp was incorporated 56 DAS, and a fertilization rate was applied to every subplot. Seedbeds were raised and nine days later sweet pepper seedlings were transplanted as described in the 2011 Experiment. During the first couple of weeks drip irrigation was used to supply water but once the rainy season began irrigation ceased.

Seedbeds were covered with a layer of hay mulch to suppress weed growth and a string trimmer was used every other week for additional control. Pyganic (pyrethrins; 5% A.I.) was applied at a dose of 5.9 lt ha^{-1} with a backpack sprayer to control aphids on 17 August. To control hornworms and pepper weevil Surround® WP (Kaolin; 95% A.I.) was applied once during fructification (31 August) at a dose of 9 kg ha^{-1}.

4.1.3 Sweet pepper yield estimation

2011 Experiment. Fifteen sweet pepper plants were harvested per sub-plot once the fruit had started to ripen or when they were fully ripe (i.e. the fruit had turned red). Sweet peppers were

harvested once a week, from 25 April through 13 June for a total of eight harvests. The number and weight of the total and marketable sweet peppers harvested per sub-plot sample were recorded.

2012 Experiment. About one third of the seedlings died due to an unexpected malfunction of the irrigation system. Consequently, only 14 seedlings per sub-plot were transplanted for one of the blocks and hence one less plant was harvested. Sweet pepper was harvested once a week, from 21 September through 7 December for a total of twelve harvests. Data was collected as in the 2011 Experiment.

4.1.4 Sunn hemp biomass and N content estimation

Sunn hemp's above-ground biomass was sampled right before incorporation by cutting one linear meter per block near the soil surface. The fresh weigh of the samples was recorded to calculate the biomass. Later the samples were air dried, dry weight was recorded and then samples were ground and sent to a private laboratory (Dairy One - Forage Testing Laboratory in Ithaca, New York), to be analyzed for percent N.

4.2 Soil research

4.2.1 Soil analysis

Soil samples (0-20 cm) collected were tested for phosphorus, interchangeable cations (Ca, Mg, Na and K), cationic exchange capacity (CEC), pH, and organic matter in order to evaluate the soil's condition. Since nitrogen is so unstable in the soil it was not tested for at the beginning of the 2011 Experiment, however total nitrogen (Kjeldahl) was later included as a point of

reference. Soil samples were sent to the University of Puerto Rico Central Analytical Laboratory located in Rio Piedras, Puerto Rico.

2011 Experiment. One composite soil sample was taken for the entire plot before starting the experiment on August 2011. When the sweet pepper harvest was over, on June 2012, a soil sample was collected per treatment (eight samples in total).

2012 Experiment. Initially, one soil sample was collected for each of the four blocks on April 2012. Once the sweet pepper crop was harvested a soil sample was taken per treatment on December 2012, however, due to unforeseen reasons samples could not be analyzed. Additionally, enzyme activities important for C cycling (β-glucosidase), and for C and N cycling (β-glucosaminidase) were evaluated using 0.5 g of air-dried soil with their appropriate substrate and incubated for one hour (37 ºC) at their optimal pH. For the complete information of the soil analysis see appendix 2.

4.2.2 Soil microbial analysis

One composite soil sample (0-10 cm) was taken from every experimental unit (32 soil samples total) after sweet pepper harvest for the 2012 Experiment. Field-moist soil samples were passed through a 6.3 mm sieve and maintained at 4 °C. Two analytical replications were done on each soil sample for the two different techniques used for microbial community structure and size determination (PLFA and EL-FAME).

For the analysis of phospholipid fatty acid (PLFA), the samples were extracted following the method described by Buyer and Sasser (2012), which represents a 5 fold increase in throughput over traditional PLFA extraction. Lipids were extracted from 2 g of lyophilized soil by adding 4.0 ml of Bligh-Dyer extractant (80 ml 50 mM K_2HPO_4 in H_2O, 200 ml methanol, and

100 ml chloroform) containing the internal standard (19:0 phosphatidylcholine [Avanti Polar Lipids, Alabaster, AL, USA] dissolved in 1:1 chloroform:methanol). The extracts were separated into neutral lipids, glycolipids, and polar lipids by a solid phase extraction (SPE) using a 96-well plate (Phenomenex, Torrance, CA, USA) and the polar fraction was subsequently transesterified. The transesterification reagent contained 0.112 g of KOH dissolved in 15 ml methanol to which 5 ml toluene was added. The FAMEs were extracted with 75 µl hexane and transferred to GC vials for analysis.

For the EL-FAME method, the extraction procedure described by Schutter and Dick (2000) was performed on 3 g field moist equivalent soil samples. Saponification and methylation of ester linked fatty acids was done by adding to the soil 15 ml of 0.2 M KOH in methanol and incubating in a water bath at 37 °C for 1 hour. When the hour was over, the solution's pH was neutralized by adding 3 ml 1.0 M acetic acid and later the FAMEs were partitioned into the organic phase by adding 5 ml of 1:1 hexane/methyl-tert butyl-ether solution followed by centrifugation. Next, the organic phase was transferred to a glass test tube and evaporated to dryness under a stream of nitrogen. Finally, the FAMEs were redissolved using 100 µl of hexane containing methyl nonadecanoate (19:0) as an internal standard (0.5 mg ml^{-1}). Samples were transferred to GC vials for analysis.

FAME from both methods were analyzed using a 6890 GC Series II (Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector and a fused silica capillary column (2 m x 0.2 mm) using hydrogen as the carrier gas. The temperature program was ramped from 170 °C to 250 °C at 5 °C per minute. A comparison of the major steps of the two extraction methods is shown in Table 3.

Table 3. Comparison of the major steps used for soil sample analysis according to high throughput PLFA (Buyer and Sasser, 2012) and EL-FAME (Schutter and Dick, 2000) procedures.

Stages	PLFA	EL FAME	Comments
Extraction of entire lipid content from soil	Done using Bligh-Dyer extractant containing internal standard		
Lipid separation	Separated into neutral lipids (eluted with chloroform), glycolipids (eluted with acetone) and phospholipids (eluted with methanol)		Neutral and glycolipids are discarded and only phospholipids are used for FAME extraction.
Saponification of lipids and methylation leading to fatty acid release	Mild alkaline methanolysis of phospholipids using the transesterification reagent	Mild alkaline methanolysis at 37 °C by adding directly to the soil 0.2 M KOH in methanol	Saponification is done to liberate fatty acids from the cellular lipids. The mild alkaline methanolysis will extract only ester-linked fatty acids and not free fatty acids.
FAME partitioning into an organic phase/extraction of FAMEs	Acetic acid and chloroform are added and the solution is dried at room temperature in a speed vacuum	Hexane:methyl-tert butyl ether was added. No washing step is necessary	EL-FAMEs are dried/concentrated under a stream of nitrogen. After drying, FAMEs are redissolved with hexane containing an internal standard

4.3 Statistical analysis for plant and soil research

Plant data collected for the 2011 and 2012 Experiments were analyzed separately due to differences in the time of incorporation of the cover crop, number of harvests and overall weather conditions. The data was subjected to analysis of variance (ANOVA) using Infostat. Fisher's Least Significant Difference Tests (LSD) was used for means separation when the F-test indicated significant differences for cover crop treatments and N fertilization rates.

The soil data was also analyzed by ANOVA according to cover crop, N fertilization, and their interaction. Additionally, principal component analyses (PCAs) were performed using R statistical software (ver. 2.15.3) with all FAMEs extracted in order to compare soil microbial community size under the cover crop and N fertilization treatments. Comparisons between FAMEs extraction methods (PLFA and EL-FAME) were done using paired Student's *t*-test analysis.

5 RESULTS

5.1 Plant research

5.1.1 Weather conditions

2011 Experiment. Total precipitation during the cover crop growing period (16 December and 25 January) was 48 mm, while average daily temperature was 21°C. During the sweet pepper growing period (1 February to 24 April), total precipitation was 228 mm and average daily temperature was 23 °C. During the pepper harvest period (25 April to 13 June), total precipitation was 145 mm and the average daily temperature was 28 °C (Figure 1a).

2012 Experiment. Total precipitation during the cover crop growing period (18 April to 13 June) was 146 mm, and the average daily temperature was 27 °C. During the sweet pepper growing period (22 June to 20 September), total precipitation was 474 mm, and the average daily temperature 28 °C. During the harvest period (21 September to 7 December), total precipitation was 504 mm and the temperature was similar to the sweet pepper growing period (Figure 1b).

5.1.2 Sweet pepper yield for the 2011 Experiment

There was no statistically significant interaction between cover crop treatments and N fertilization rates in relation to either marketable number of fruits and weight, or total number of fruits and weight (Table 4). Therefore, the effect of cover crop treatments and N fertilization rates were examined separately. Cover crop had no effect on any of the sweet pepper yield components (total and marketable number of fruits and weight); however, a trend was seen (p= 0.0586-0.0906) toward lower yields when cover crop was used.

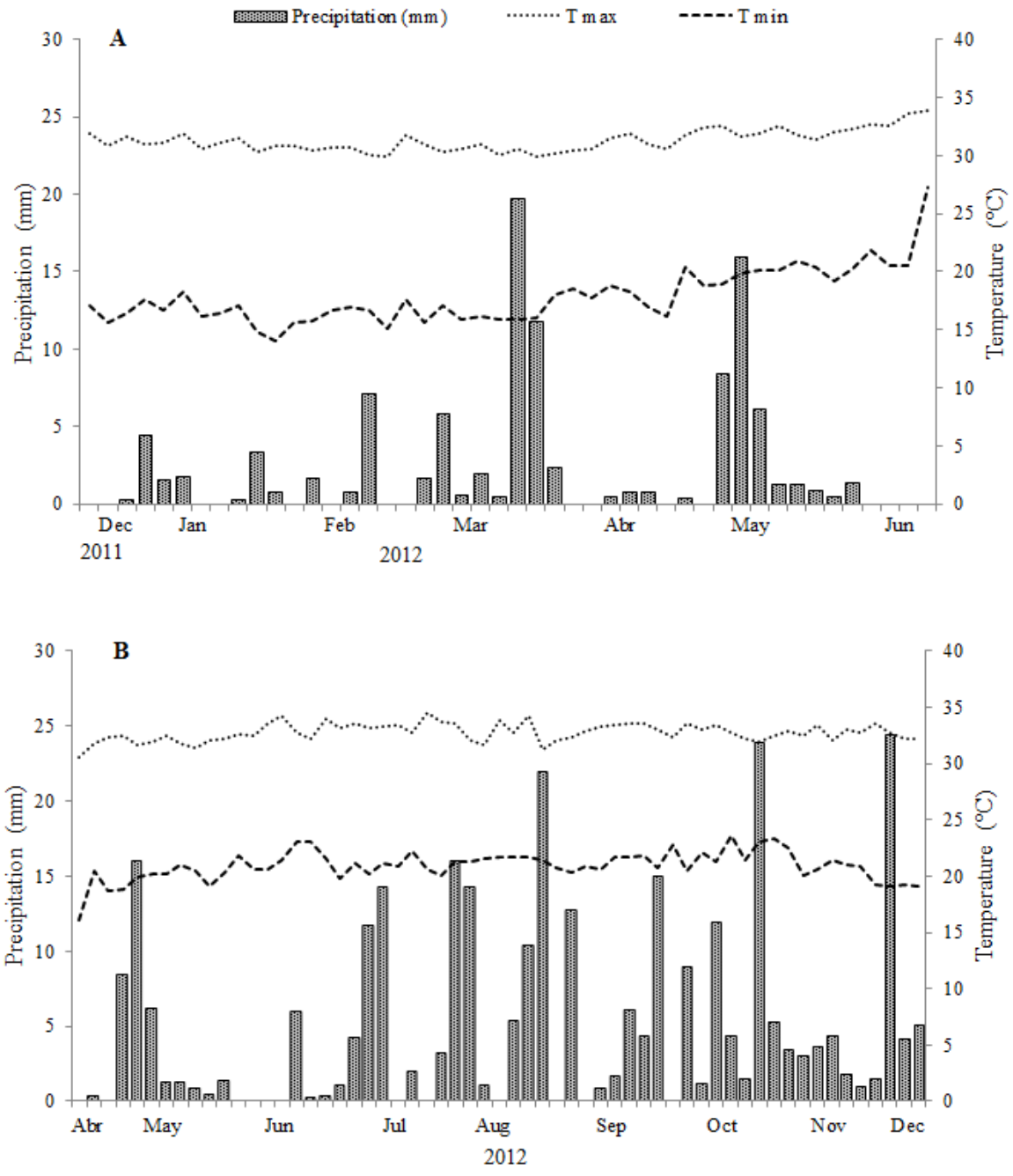

Figure 1. Precipitation and air temperature at 4-day intervals at the Agricultural Experimental Station of Lajas, Puerto Rico during the 2011 Experiment (A) and 2012 Experiment (B).

Regarding N fertilization, analyses of all sweet pepper yield components indicated a significant effect ($p<0.0001$). Overall, there was a significant response ($p<0.05$) with an increase in the fertilization rates 0, 56 and 112 kg N ha^{-1}, however there was no significant difference ($p>0.05$) in these analyses between the two highest N fertilization rates (112 vs. 168 kg N ha^{-1}) (Figure 2).

5.1.3 Sweet pepper yield for the 2012 Experiment

As with the 2011 Experiment, there was no statistically significant interaction between cover crop and N fertilization rates in relation to either marketable number of fruits and weight or total number of fruits and weight (Table 4). Cover crop treatments and N fertilization rates were examined separately and the cover crop had no effect on any of the sweet pepper yield components (total and marketable number of fruits and weight). Nitrogen fertilization rates were significant for all the yield components except for total fruit weight ($p=0.0574$). As opposed to what happened in the 2011 Experiment, there was no significant difference ($p<0.05$) between the lowest three fertilization rates (0, 56 and 112 kg N ha^{-1}). Additionally, the two highest levels (112 and 168 kg N ha^{-1}) were significantly different ($p<0.05$) from the rest (Figure 3).

Table 4. Summary of analyses of variance showing the sources of variation on sweet pepper yield for 2011 and 2012 Experiments.

		Fruit weight (t ha^{-1})		Number of fruits	
		Total	Marketable	Total	Marketable
Year	Sources of variation	P>F			
2011	Cover crop	0.0586	0.0553	0.0906	0.0892
	N fert	<0.0001	<0.0001	<0.0001	<0.0001
	Cover crop x N fert.	0.4992	0.5352	0.5179	0.6117
2012	Cover crop	0.5934	0.3648	0.5800	0.6292
	N fert	0.0574	0.0322	0.0310	0.0325
	Cover crop x N fert.	0.2925	0.3517	0.3300	0.2956

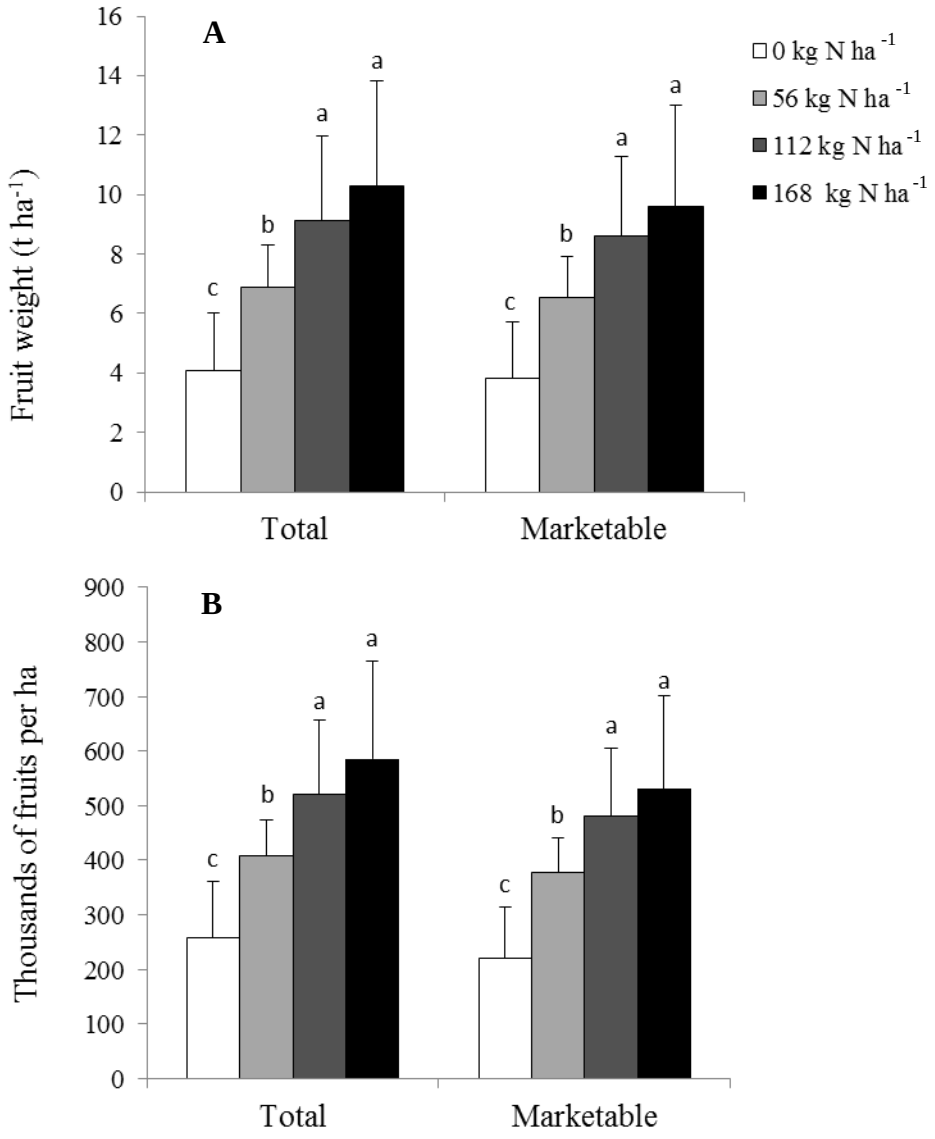

Figure 2. Effects of N fertilization rates on sweet pepper fruit weight (A) and number of fruits (B) for the 2011 Experiment. Treatment means with a different letter indicate a significant difference between treatments according to LSD tests ($p<0.05$). Error bars represent standard deviation.

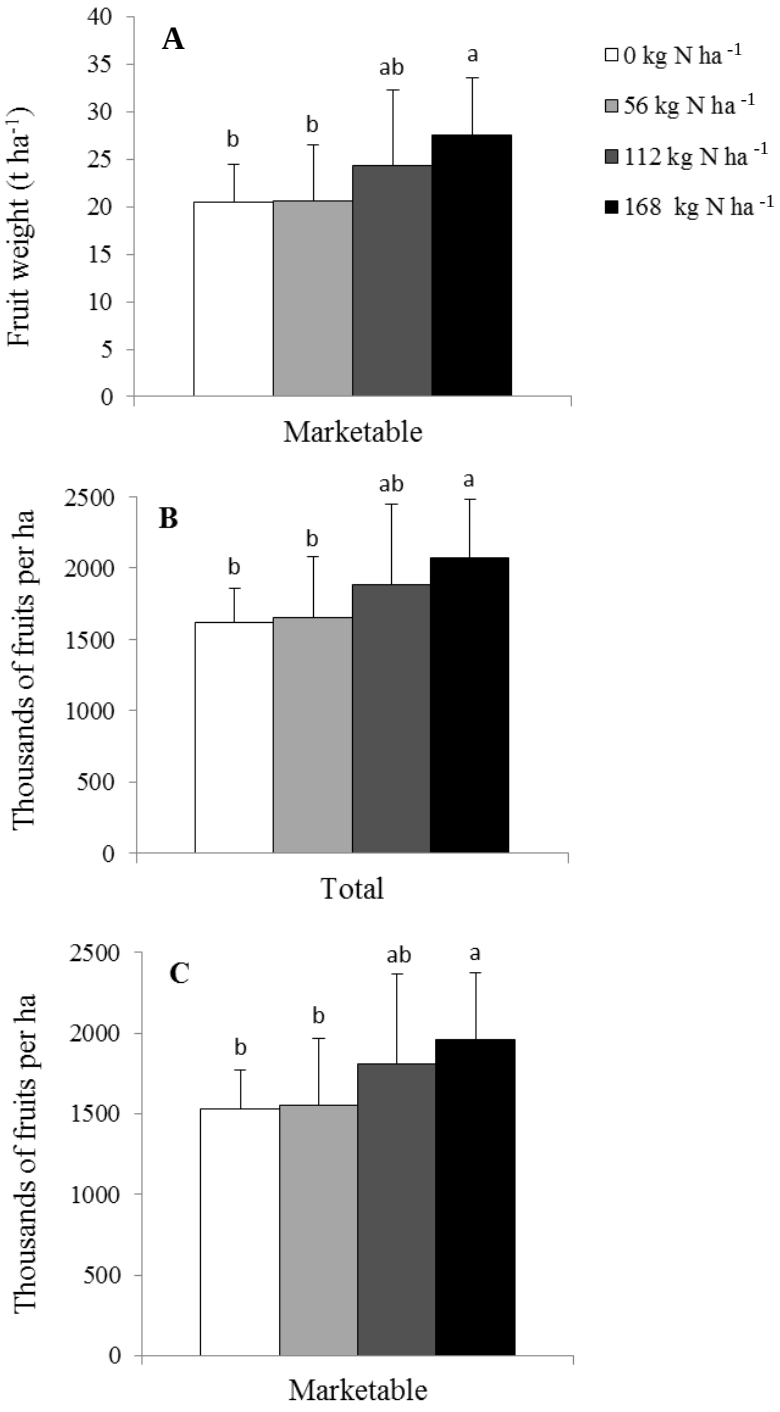

Figure 3. Effects of N fertilization rates on sweet pepper marketable fruit weight (A), total (B) and marketable (C) number of fruits for the 2012 Experiment. Treatment means with a different letter indicate a significant difference between treatments according to LSD tests ($p<0.05$). Error bars represent standard deviation.

5.1.4 Sunn hemp biomass and N content estimation

For the 2011 Experiment, sunn hemp was in flowering stage at the time of incorporation (40 DAP) and the mean biomass based on dry matter was 1173 kg ha^{-1}. For the 2012 Experiment, sunn hemp had not yet reached flowering stage at time of incorporation (56 DAP) but more biomass was produced (1913 kg ha^{-1}). Regarding N content, mean N concentration was 45 g kg^{-1} in 2011 and 33 g kg^{-1} in 2012, and total N incorporated based on dry biomass was 52.8 kg ha^{-1} and 63.1 kg ha^{-1}, in 2011 and 2012, respectively.

5.2 Soil Research

5.2.1 Soil microbial community characterization

In this Vertisol, the sum of total FAMEs ranged from 190.38 nmol g^{-1} to 229.47 nmol g^{-1} for EL-FAME, which can provide an indication of the soil microbial community size (Table 5). According to ANOVA, the microbial community size was not affected by cover crop, N fertilization or their interaction.

The ANOVA for individual FAMEs (15 FAMEs) showed no significant differentiation on the soil microbial community structure due to cover crop, N fertilization treatments or their interaction. PCAs comparing the 15 FAMEs together supported these results (data not shown). PCAs comparing all FAMEs (total of 45 extracted from the soil) together, to better represent the microbial community structure, showed no separation due to N fertilization (Figure 4a), but a trend toward separation in the soil microbial community structure due to cover crop was evident (Figure 4b).

Table 5. Selected FAMEs (nmol g^{-1} soil) as affected by the use of sunn hemp as a cover crop and different N fertilization rates using the high throughput PLFA (Buyer and Sasser, 2012) and EL-FAME (Schutter and Dick, 2000) methods.

	Bacteria (B)								Fungi (F)							
	G+		G-		Actinomycetes		B sum		Saprophytic		AMF[b]		F:B ratio[c]		Total FAME	
Treatment[a]	PLFA	EL	PLFA	EL	PLFA	EL	PLFA	EL	PLFA	EL	PLFA	EL	PLFA	EL	PLFA	EL
NCC0	31.82	57.70	23.81	22.94	16.55	23.92	72.17	104.56	6.98	20.41	3.66	6.73	0.10(0.15)	0.20(0.26)	111.98	216.35
NCC56	28.30	49.29	21.71	19.04	14.49	20.86	64.51	89.20	5.88	18.73	2.91	6.25	0.09(0.14)	0.21(0.28)	99.04	190.38
NCC112	31.60	53.44	22.84	21.86	16.41	22.85	70.85	98.14	6.77	20.37	3.16	6.12	0.10(0.14)	0.21(0.27)	107.42	205.06
NCC168	27.56	54.15	23.34	21.67	15.59	22.62	66.48	98.44	7.24	19.99	3.12	6.28	0.11(0.16)	0.20(0.27)	103.04	207.08
CC0	30.65	54.90	24.26	22.07	16.62	23.16	71.52	100.13	6.90	18.66	3.57	6.70	0.10(0.15)	0.19(0.25)	109.73	206.27
CC56	34.58	58.86	26.08	22.83	17.83	25.15	78.49	106.84	7.38	20.86	3.69	7.51	0.09(0.14)	0.20(0.27)	119.67	221.15
CC112	30.82	52.68	23.36	20.64	15.59	22.22	69.77	95.54	6.64	19.62	3.02	6.33	0.10(0.14)	0.21(0.27)	105.82	197.82
CC168	33.74	60.31	25.91	24.04	17.95	25.78	77.60	110.12	9.00	22.96	3.49	7.34	0.12(0.16)	0.21(0.28)	120.50	229.47

[a]NCC = with out cover crop; CC = with cover crop; and 0, 56, 112, 168 are the fertilization rates (Kg N ha^{-1})

[b]Arbuscular mycorrhizal fungi (AMF)

[c]AMF is not included in the F:B ratio, while included in the values in parenthesis

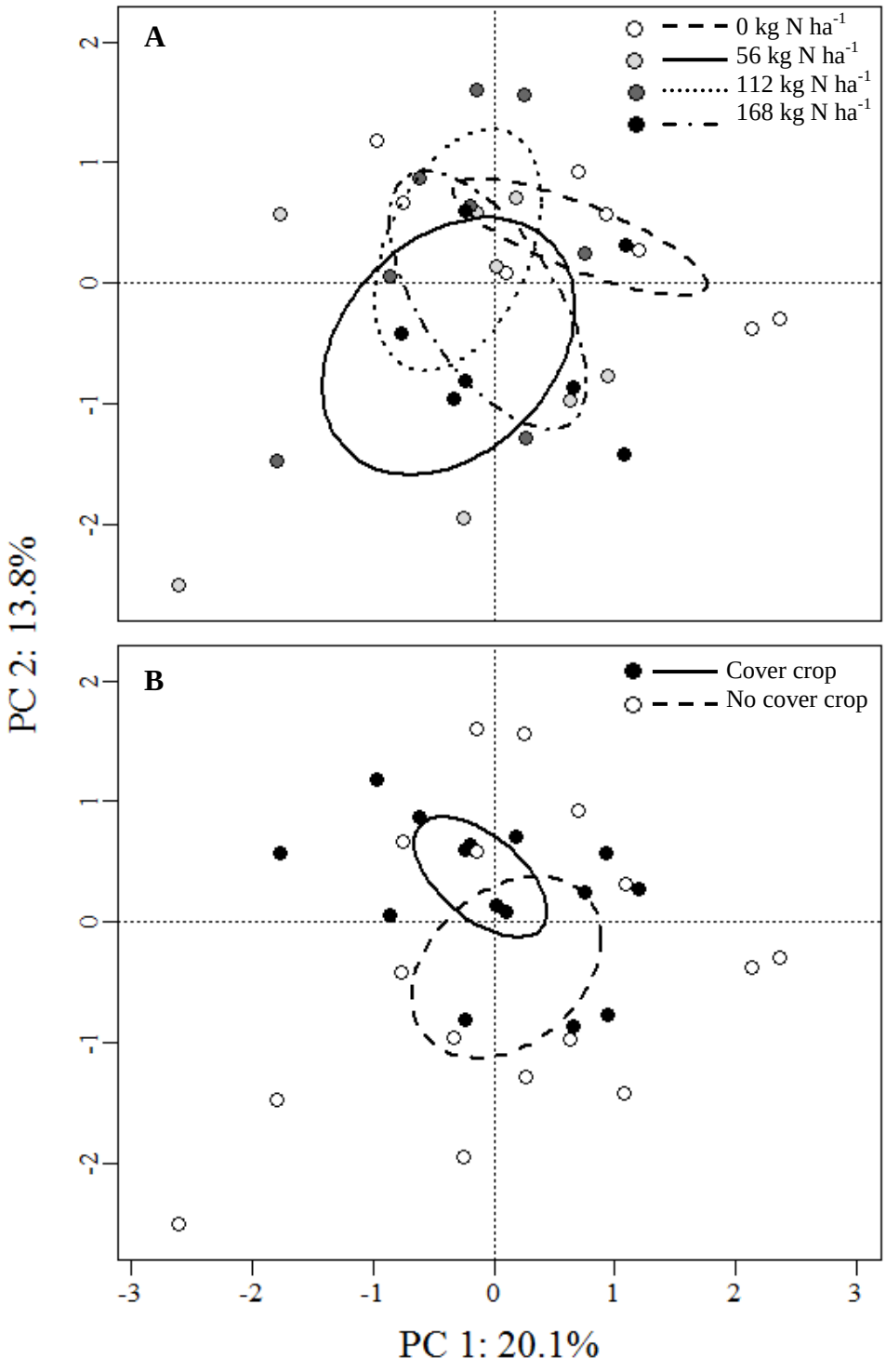

Figure 4. Principal component analysis (PCA) of the soil microbial community structure as affected by the use of N fertilization rate (A) and cover crop treatment (B) according to the EL-FAME method using 45 FAMEs.

There was a significant difference between extraction methods, with the average concentration of total FAMEs for the PLFA method (109.65 nmol g^{-1} soil) substantially lower than that using the EL-FAME method (209.20 nmol g^{-1} soil). According to the PLFA procedure, a total of 34 different FAMEs were identified in this soil, of which 23 were consistently present in the samples and were used for data analysis. On the other hand, the EL-FAME procedure identified 58 FAMEs and 45 were used for data analysis. Twenty two fatty acids were common between both methods.

Regarding microbial groups abundance 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 by the sum of G- bacteria, G+ bacteria and actinomycetes, corresponded to 65% and 48% of total PLFA and EL-FAME concentrations, respectively (Table 6). Fungal markers constituted 10% and 13% of the total FAMEs for the PLFA and El-FAME methods, respectively. FAMEs that correspond to saprophytic fungi were most abundant compared to 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. Consequently, the fungal sum presented in table 6 includes only 18:1ω9c and 18:2ω6c which are saprophytic fungi indicators. The F:B ratio obtained was ~0.1, which indicates bacterial dominance over fungal dominance in this soil.

In relation to the extraction methods used, the PLFAs showed higher proportions of all G- bacteria indicators (mol %) and lower proportions for all fungi indicators, however, there were variations on the trends for other FAMEs (Table 6). It was interesting and perhaps an important trend the fact that the AMF indicator (16:1ω5) was extracted similarly (similar relative abundance) with both methods. It is also of ecological significance that there was no significant difference in the F:B ratio obtained from both methods.

Table 6. Comparison of relative abundance (mol %) of selected FAMEs obtained with the high throughput PLFA (Buyer and Sasser, 2012) and EL-FAME (Schutter and Dick, 2000) protocols.

FAME	PLFA	EL	Mean difference	Significance
Bacteria				
G+ bacteria				
*i*14:0	0.72	0.88	-0.17	***
*i*15:0	11.32	9.26	2.06	***
*a*15:0	5.55	3.76	1.79	***
*i*16:0	4.34	5.74	-1.39	***
*i*17:0	3.72	3.81	-0.09	ns
*a*17:0	2.77	2.91	-0.15	***
G-bacteria				
cy17:0	2.39	1.41	0.98	***
18:1ω7c	6.82	3.69	3.14	***
cy19:0ω8c	12.62	5.34	7.27	***
Actinomycetes				
10Me16:0	11.63	6.91	4.73	***
10Me17:0	0.52	1.17	-0.65	***
10Me18:0	3.3	3.07	0.23	***
Fungi				
Saprophytic fungi				
18:1ω9c	5.75	7.01	-1.26	***
18:2ω6c	0.7	2.68	-1.98	***
AMF				
16:1ω5c	3.02	3.17	-0.15	ns
Bacterial sum	65.2	47.97	17.23	***
Fungal sum	6.45	9.68	-3.24	***
Fungal:Bacterial ratio	0.09	0.1	-0.01	ns

Values are significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns = not significant

5.2.2 Soil properties

Additional characteristics determined in this soil showed that the soil pH in both sites remained close to neutral, organic matter (OM) was slightly lower in site 1 than in site 2, and both the cation exchange capacity (CEC) and N content remained approximately the same in both sites (Table 7). Clay content for this soil series (Fraternidad clay) ranges from 40 to 60%.

Table 7. Selected soil properties before cover crop establishment (initial) and after sweet pepper harvest (final) for the 2011 and 2012 Experiments.

	2011 Experiment (site 1)		2012 Experiment (site 2)	
Soil Properties	Initial	Final	Initial	Final
pH	7.40	7.04	6.88	-
OM (%)	1.71	2.02	2.60	-
CEC (meq/100g)	31	28	31	-
N (%)	-	0.20	0.18	-
Enzyme activities (mg p-nitrophenol kg^{-1} soil h^{-1})				
β-Glucosaminidase	-	-	-	33.40
β-Glucosidase	-	-	-	95.76

(-) Information not available

6 DISCUSSION

6.1 Plant research

Even though studies have shown sunn hemp to increase subsequent crop yields (Jeranyama, 2000; Wang et al., 2003; Balkcom and Reeves, 2005), it had no significant effect on sweet pepper yields in this experiment. This could be due to a lack of synchrony between N availability from cover crops and sweet pepper N demand. Meeting sweet pepper N demand through organic N sources such as cover crops depends on mineralization by soil microorganisms. In tropical climates, sunn hemp residues decompose in a short period of time, and the peak N mineralization rate would also occur around this time period (Wang et al., 2004).

Tillage also affects decomposition rates, which was done to incorporate sunn hemp into the soil. Previous studies have reported 65 to 70% of the residues can decompose within approximately two weeks (Wang et al., 2004). Reeves et al. (1996) indicated that sunn hemp residues left on the soil surface as organic mulch would decompose at a much slower rate over a longer period of time, and they can contribute to higher SOM accumulation compared to tilled counterparts. Additionally, a strip-till cover crop system, where the remaining sunn hemp is cut and left on the soil surface, allows partial residues to be incorporated in the soil and release N promptly while the portion left as mulch will release nutrients over a longer period of time.

Chemical characteristics of cover crop residues, especially C:N ratio, also plays an important role in residue decomposition and consequently in the supply of nutrients to the

subsequent crops. Decades of research have shown that when organic amendments with C:N ratios below 20:1 are added to soils N is readily mineralized (Sylvia et al., 2005). Sunn hemp residues have reported to have a C:N ratio of 18.9:1 (Marshall, 2002), and most likely N was mineralized before the sweet pepper had a high demand for it. For sweet pepper, nutrient needs increase during flowering which, for both the 2011 and 2012 Experiments, took place approximately 8 weeks after transplanting and 9 weeks after sunn hemp incorporation.

Although there was no significant difference among cover crop treatments, the 2011 Experiment indicated a trend toward lower yields when sunn hemp was used as a cover crop ($p=0.0586$-0.0906), which could have been caused by sunn hemp's allelopathic properties. Allelopathy is the inhibitory or stimulatory effect of a plant on another species as a result of the release of chemicals into the environment. Previous work on sunn hemp demonstrated allelopathic properties of ground dried residues and aqueous leaf extracts on certain weeds and vegetable crops which inhibited germination and seedling growth (Adler and Chase, 2007; Skinner et al., 2012). Allelopathic effects on bell pepper germination have been conflicting, with sunn hemp leaf extract strongly inhibiting germination on one study (Skinner et al., 2012) and having no effect on the other (Adler and Chase, 2007). Contrasting results were attributed to differences in the stage of development, suggesting that the concentration of allelochemicals in leaves was higher during the reproductive stage. This would also explain the trend in the 2011 Experiment, as sunn hemp was incorporated at the reproductive (flowering) stage. However, other environmental conditions that affect allelopathic activity such as photoperiod, temperature stress, water stress, low nutrient

availability, and disease pressures could have also been an issue (Reigosa et al., 1999). Overall, longer cover crop cycles are needed to confirm or refute this trend.

As opposed to the cover crop treatment, N fertilization did have a significant effect on sweet pepper yields in both experiments. In the 2011 Experiment, the fertilization rates which produced the highest yields were 112 and 168 kg N ha^{-1} and there was no significant difference among these two yields. This indicates that the application rate of 112 kg N ha^{-1}, which is the application rate for conventional sweet pepper production recommended by the University of Puerto Rico's "Conjunto Tecnológico", avoids wasting fertilizer with yields that go up to 12.7 t ha^{-1}.

In the 2012 Experiment, there was no difference in marketable yields of sweet pepper plots fertilized at a rate of 0, 56, and 112 kg N ha^{-1}. However, the 168 kg N ha^{-1} produced significantly higher yields than all the other fertilization rates. Consequently, the fertilization requirement was higher than the recommended for conventional sweet pepper production and total yields went as high as 25.6 t ha^{-1}.

It is difficult to explain the lower yields during the 2011 Experiment as they could be attributed to a combination of factors such as weather conditions and insect pest infestation levels. Studies have shown that sweet pepper development (including flower and fruit set) is very sensitive to the environmental conditions. Higher yields are favored by warmer temperatures and inhibited when air temperatures fall below 18 °C for extended periods (Juroszek and Tsai, 2009). Regarding pest infestation, fewer applications of products for insect control were done during the 2012 Experiment, which can demonstrate there was a

lower pest incidence; however this variable was not measured and their effect on fruit yields for both experiments is not clear.

6.2 Soil research

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-Martinez et al., 2010). However, studies determining changes in soil microbial communities within the first years of management implementation can aid on management redirection and decision making. This information is especially important since organic farming is a recent management practice in Puerto Rico. Also soils in the tropics are more susceptible to SOM oxidation and mineralization due to the high temperatures that they are exposed to most of the year. Cover crop and N fertilizer rates in this experiment were tested within the first year of establishment, and it revealed a trend of microbial community shifts with the cover crop only, while no differences were found due to the fertilization levels. These early results for the Vertisol 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. The results not only emphasize the importance of cover crop's aboveground biomass for sustaining the microbial community of soil, but also the importance of greater root exudate substrate's composition in soil with cover crops.

Regarding the use of the commercial organic fertilizer (BioFlora 6-6-5), no effects were found in soil microbial community abundance and structure. Other studies have found that amendment with manure or compost significantly affect soil microbial community

structure and the effects these amendments might have vary depending on the type of compost and soil type (Pérez-Piqueres et al., 2006). 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 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 3 years of application compared to the non-treated soil (i.e., Acosta-Martinez and Harmel, 2006). The lack of an early response of the soil microbial communities to the poultry based fertilizer used can be due to its manufacturing process, which includes composting and heating to temperatures over 83 °C for pelletization. This process may reduce the microbial and enzyme load of the fertilizer, however, its use as pellets can extend its efficacy as a long-term nutrient supply. It is possible that the effects on the microbial communities from the fertilizer will be observed over time if the sweet pepper biomass and root exudates experience more significant increases that improve soil conditions for microbial communities (i.e., increases in nutrients and substrates, improvements in SOM and soil aggregation).

Results showed that one cover crop cycle was too early to detect significant changes in microbial community composition according to differences in microbial groups using both EL-FAME and PLFA analyses. However, perhaps changes in microbial community composition could have been detected by methods that characterize microbial diversity at the species level such as pyrosequencing. 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 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 trends of early shifts in microbial community structure due to cover crop are ecological significant for the implications in improved biogeochemical cycling and SOM dynamics in this Vertisol soil under organic farming.

Since the management practices were just implemented, two methods for the characterization of the soil microbial community structure were used in order to attempt detecting early changes due to management, if present. Although, it was intended to compare the trends in microbial community structure shifts from both methods (PFLA and EL-FAME), this was not possible due to the lack of significant effects of cover crop and N fertilization rates on the FAMEs evaluated. However, this study elucidated three important findings: 1) the AMF indicator (16:1ω5c) showed similar abundance with both methods; (2) the F:B ratios were also similar with both methods, and (3) the PCAs from both methods showed the trend of separation in the microbial community structure of the cover crop vs non-cover crop soil.

The quantitative differences in FAME indicators 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 is an advantage when it comes to 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 phospholipids is an issue (Zelles, 1999; Schutter and Dick, 2000), and as seen in this experiment, EL-FAME extracted 45 different fatty acids while 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 acid that are not part of the microbial community. Given 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 methods are suitable to continue evaluating the microbial community of soils from Puerto Rico under organic farming practices.

This study addressed selected chemical and biological properties of the Vertisol evaluated. Soil pH was near neutral which is optimal for decomposition of plant residue and other important processes such as nitrification. The SOM content of this Vertisol is relatively low (1.71 - 2.60 %) (Sotomayor-Ramirez et al., 2009) probably because decomposition of organic materials in the tropics is higher due to the warm and humid weather. This can increase microbial activity and limit SOM accumulation (Powlson et al., 2001). Regarding the F:B ratio, it is known that disturbed soils (e.g. conventional tillage) are dominated by bacteria and hence have a low F:B ratio, as was the case in this soil. The bacterial dominance may be due to the soil type and its low SOM content. The levels of β-glucosaminidase activity (33.40 mg p-nitrophenol kg^{-1} soil h^{-1}) found in this soil were two times higher than another Vertisol in Texas under a three year rotation of corn-wheat-corn

under conservation tillage (Acosta-Martinez and Harmel, 2006). This enzyme activity was also two times higher than a Vertisol sampled at the Agricultural Experimental Station of Lajas, where this study took place, with a field history of over 20 years of vegetable crop rotations under conventional tillage (Sotomayor-Ramirez et al., 2009). On the other hand, β-glucosidase activity (95.76 mg p-nitrophenol kg^{-1} soil h^{-1}) was approximately the same as the Vertisol in Texas (Acosta-Martinez and Harmel, 2006); however this enzyme activity was also higher (more than two times) than the activity obtained in this same location in Lajas under vegetable production (Sotomayor-Ramirez et al., 2009). Even though no significant effects were found among treatments, the soil data obtained in this study provides background information about this soil to establish a soil quality index over time.

7 CONCLUSIONS AND RECOMMENDATIONS

The overall trends found in this research did not demonstrate an early positive effect of the use of sunn hemp as a cover crop for organic sweet pepper production. There is a possibility that sunn hemp's alellopathic properties may have exerted a negative effect on the sweet pepper yields, but this assessment was not performed in this study. At this point, further cycles should be tested to confirm the effects sunn hemp could have. Further time may show more definite trends for making recommendations for using this cover crop in organic sweet pepper production in this area. On the other hand, the use of the BioFlora (6-6-5) fertilizer at different rates demonstrated an increase in sweet pepper yields. Since the fertilization rate that resulted in higher yields was different for the 2011 and 2012 Experiments (112 kg N ha^{-1} and 168 kg N ha^{-1}, respectively), it is possible to suggest that fertilizer rates higher than 112 kg N ha^{-1} are suitable to improve sweet pepper production in this location. The variation could be attributed to different reasons including changes in temperature, precipitation or insect infestation.

As opposed to the plant research, results for the soil microbial component evaluation indicated an initial shift in the microbial community structure after one cover crop cycle and no differences were detected due to the fertilization rates. Although there was an early response of the microbial communities to this cover crop, further evaluation is needed to determine the long-term effect of sum hemp and possibly other cover crops on soil microbial communities. It is important that this study continues under replicated long-term plots in order to confirm the information gathered in relation to sunn hemp and N fertilization

treatments on the microbial community size and structure due to their importance in soil quality and metabolic functioning.

Vertisols occupy a significant role in agricultural production (i.e., ~2.4% of the total soil area), but they also have the lowest soil organic carbon pool and density among all orders (Coulombe et al., 1996; Sotomayor-Ramirez et al., 2009). Studies have reported that Vertisols are susceptible to physical degradation, which can be higher in the tropics, and thus, conservation practices are necessary to maintain a healthy soil (Martens et al., 2003). Thus, organic farming may improve this soil quality. Studies to make management decisions for organic farming in Puerto Rico will depend on assessments of both crop yields and the soil microbial component as sensitive indicators of soil quality.

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Appendix 1: Seedling Growing Procedure

Sweet peppers were seeded in ten 72 cell propagation trays. The growing media was one third compost, one third peatmoss and one third perlite. When sweet pepper seeds germinated they were fertilized once a week with DynaMega™ (2-1-1) in a 1 to 10 dilution with water until seedlings reached the adequate size for transplanting to the field (at around 45 days).

Appendix 2: Soil Sampling Results

						Interchangeable ions (pmm)				Enzyme Activity (mg p-nitrophenol kg-1 soil h-1)	
	pH	OM %	P (ppm)	CEC	N (%)	Ca	Mg	K	Na	Enzyme 1[a]	Enzyme 2[b]
2011 Experiment											
Initial analysis	7.4	1.71	3	31	-	3794	1412	118	84	-	-
Final analysis											
NCC0	7.18	1.74	7	28	0.16	3511	1227	174	58	-	-
NCC56	6.9	1.81	5	27	0.16	3278	1222	130	55	-	-
NCC112	7.17	1.95	10	28	0.16	3495	1225	177	52	-	-
NCC168	6.9	1.81	9	27	0.16	3298	1207	161	60	-	-
CC0	7.27	2.17	9	28	0.17	3415	1212	203	51	-	-
CC56	7.06	1.9	9	29	0.43	3478	1334	213	62	-	-
CC112	6.92	2.55	13	28	0.19	3422	1186	241	61	-	-
CC168	6.96	2.21	12	28	0.18	3415	1157	215	56	-	-
2012 Experiment											
Initial analysis											
Block 1	6.95	2.26	44	31	0.18	3780	1319	287	50	-	-
Block 2	6.69	2.83	25	32	0.17	3876	1456	280	51	-	-
Block 3	6.88	2.63	43	30	0.20	3737	1244	300	47	-	-
Block 4	6.99	2.67	32	32	0.16	4011	1352	311	53	-	-
Final analysis											
NCC0	-	-	-	-	-	-	-	-	-	33.78	106.05
NCC56	-	-	-	-	-	-	-	-	-	30.15	86.59
NCC112	-	-	-	-	-	-	-	-	-	31.96	90.47
NCC168	-	-	-	-	-	-	-	-	-	34.82	99.28
CC0	-	-	-	-	-	-	-	-	-	30.08	97.58
CC56	-	-	-	-	-	-	-	-	-	34.04	90.74
CC112	-	-	-	-	-	-	-	-	-	34.18	91.92
CC168	-	-	-	-	-	-	-	-	-	38.18	103.45

[a] β-Glucosaminidase

[b] β-Glucosidase

(-) Information not available