RESTORATIVE EFFECTS OF COMBINED SUSTAINABLE PRACTICES ON THE BIOLOGICAL, PHYSICAL AND CHEMICAL PROPERTIES OF A SOIL AND CROP PRODUCTIVITY

by

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Abstract

Unsuitable agricultural practices have exacerbated worldwide soil degradation, thus limiting food production. There is a need to develop and better understand the effect of sustainable production practices that have the potential to increase the fertility of soils and the sustainability of the agricultural systems. Compost incorporations, the use of coordinated fallows and other sustainable technologies are alternatives for soil restoration and increase of crop yields. There is scarce information on the effect of combined organic amendments management over soil properties and their impact in vegetable production. The objective of the present work was to assess the effect of a combination of organic amendments, applied in 67-day cycles, on soil chemical, physical and biological properties and eggplant yield. The soil amendments consisted of incorporating organic matter from coffee pulp compost, planting and incorporation of a mixture of four green manure species, a mycorrhizae culture and compost liquid inoculant. Treatments were the following: CL0, CL1, CL2 and CL3, consisting of 0 (un-amended), 1, 2 and 3 consecutively applied 67-day cycles, respectively. The results showed that CL1 was enough to significantly increase organic matter (OM), P, K and S content in the soil compared with the non-amended soil. Calcium and Mg content were increased by three (CL3) and two (CL2) 67-day cycles of combined practices, respectively. All treatments significantly changed soil pH, buffering it toward neutrality, with increasing cycles when compared with pH 7.9 of un-amended soils. Treatments CL1, CL2 and CL3 increased humic acids content 2.8, 3.8 and 5.9 times respectively, when compared with CL0. Humic acids extracted from un-amended soils exhibited more condensation and more aromaticity when compared with that of amended soil, nevertheless the humic acids of amended soils showed high levels of polymerization due to the use of high quality compost. All amendment treatments significantly increase aggregate stability and reduced bulk density relative to the CL0 treatment. Soil macroporosity, defined as porosity with radius > 38 µm, was significantly increased by treatments CL2 and CL3. All of the organic matter fractions, including total organic matter, humic acids content, microbial biomass C and microbial biomass N were significantly increased by one or more of the amendment treatments. Aggregate stability was highly correlated with all organic matter fractions. The production of stable aggregates appeared to influence all the other physical parameters assessed in this study. The enhancement in soil properties promoted by the combination of practices resulted in an increase in eggplant fruit yield and biomass. This study shows clearly that organic amendments can have positive effects on soil physical, chemical and biological properties and crop productivity.

Resumen

La tendencia mundial de degradación de suelos promovida por prácticas agrícolas inadecuadas impone serias limitaciones a la productividad de los suelos. Existe la necesidad de entender mejor los efectos de prácticas sustentables de producción, las cuales poseen el potencial de aumentar la productividad de los suelos y la sustentabilidad de los sistemas agrícolas. La incorporación de composta, la utilización de barbechos coordinados, así como otras prácticas sustentables surgen como alternativas viables para la restauración de los suelos y el incremento de la productividad de las cosechas. La información de efectos combinados de múltiples prácticas sustentables sobre las propiedades del suelo y la producción de vegetales es limitada. El objetivo de esta investigación fue evaluar el efecto de una combinación de prácticas sustentables aplicadas en ciclos de 67 días sobre las propiedades químicas, físicas y biológicas del suelo y el rendimiento de berenjena. Las enmiendas al suelo consistieron en la incorporación de materia orgánica a través de composta de pulpa de café, siembra e incorporación de una mezcla de cuatro leguminosas, micorrizas e inoculante líquido a base de composta. Los tratamientos fueron CL0, CL1, CL2 y CL3 consistiendo de 0 (sin enmendar), 1, 2 y 3 aplicaciones consecutivas de ciclos de 67 días, respectivamente. Se demostró que un solo ciclo de combinación de practicas (CL1) fue capaz de aumentar significativamente la materia orgánica y el contenido de P, K y S cuando se comparó con los suelos no enmendados (CL0). Los contenidos de Ca y Mg fueron incrementados por los tratamientos CL2 y CL3, respectivamente. Todos los tratamientos afectaron significativamente el pH, amortiguándolo hacia valores neutros conforme se incrementaban los ciclos de 67 días en comparación con el pH de 7.9 del suelo sin enmendar. Los tratamientos CL1, CL2 y CL3 incrementaron el contenido de ácidos húmicos 2.8, 3.8 y 5.9 veces respectivamente comparado con CL0. Los ácidos húmicos extraídos de los suelos sin enmendar mostraron mayor condensación y aromaticidad, sin embargo los ácidos húmicos de los suelos tratados mostraron considerablemente altos niveles de polimerización debido a la utilización de composta de alta calidad. Todos los tratamientos aumentaron significativamente la estabilidad de agregados y disminuyeron la densidad aparente del suelo en comparación con CL0. Los macroporos del suelo, definidos como los poros con un radio > 38 µm, incrementaron significativamente en los tratamientos CL2 y CL3. Todas las fracciones de materia orgánica estudiadas, incluyendo la materia orgánica total, ácidos húmicos, biomasa microbiana de C y biomasa microbiana de N fueron aumentadas por la aplicación de uno o más ciclos de 67 días de la combinación de enmiendas orgánicas. La estabilidad de agregados mostró una alta correlación con todas las fracciones orgánicas. La producción de agregados estables aparentó influenciar todos los demás parámetros físicos estudiados. El mejoramiento general de las propiedades del suelo como resultado de la implementación de ciclos de 67 días de prácticas combinadas resultó en un aumento en la producción y la biomasa de las plantas de berenjena. Este estudio evidenció los

efectos positivos de las enmiendas orgánicas sobre las propiedades químicas, físicas y biológicas y la productividad del suelo.

Dedication

This thesis is dedicated to all the people who fight daily and unconditionally for world justice.

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I would like to thank everybody that collaborated in a way or another to the development of this research from the field, the laboratory to the processing of the data.

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Literature Review

According to the USDA (2012) organic agriculture refers to a production system that "integrates cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity". Organic farming is a relatively modern term developed during the second half of the twentieth century. Although, it has a broad meaning, organic agriculture is still regulated by different agencies and countries around the world. In conjunction to organic agriculture, there are other production systems including biological, biodynamic, ecological and natural agriculture that follow specific criteria. Between these alternative systems of production there are basic common principles like: the prohibition of the use of synthetic agrochemicals, the promotion of sustainable food production with emphasis on energy efficiency, natural resource conservation and the enhancement of biological activity of the agro-ecosystem (FAO and WHO 2001).

Organic practices have being recorded as far back as 800 B.C when Homer in The Odyssey refers to the manure amendment of vineyards. Theophrastus, around 300 B.C recommended the manuring of soils and made a description of the different manures based on their quality (Havlin et al., 1993). Theophrastus also made reference to the use of green manures pointing out the benefits of incorporation of bean residues. In the same manner, ancient civilizations used different organic amendments and coordinated crop rotations to enhance productivity. The formal origin of modern 'organic agriculture' as a production philosophy could be traced back to the 1920's and the teachings of the “Agriculture Course” of the German philosopher Rudolf Steiner (Shi-Ming and Sauerborn, 2006). According to Shi-Ming and Sauerborn (2006) various organic movements emerged during the first half of the 1900’s but was not until the 1960’s that organic agriculture expanded worldwide in part due to the oil crisis and the people awareness toward agro-ecological issues. During the 1970s-1990s appeared the first’s regulatory agencies that standardize the organic production (Shi-Ming and Sauerborn, 2006). In the 1990’s organic production experienced a new stage where the governments got involved and created legislation to regulate organic production. The USDA regulations for organic production were finally published in 2001 (USDA 2000).

According to the United Nations (2008) the world population will reach 9.2 billions in the year 2050. There are serious implications of soil degradation on world food security. Lal (2007) reports that the “long-term use of extractive farming practices has caused severe and widespread problems of soil degradation by erosion and nutrient depletion along with biological degradation”. The “seed based” approach, which promoted new plant varieties highly dependent on agrochemicals, sponsored by the green revolution during the 1960’s with an extreme reliance on chemical fertilizer exacerbated the problems of soil degradation. This approach caused depletion of soil organic matter, nutrient imbalance, accelerated soil erosion, waterlogging and salinity, degradation of soil structure and decline in soil’s water and nutrient retention capacities (Lal, 2009). Unsuitable soil management activities including deforestation, indiscriminate vegetation removal, overgrazing and use of marginal lands for agricultural purposes often precede eventual degradation of soil resources and environmental damage (Henao and Baanante, 2006). It has been repeatedly suggested that soil and natural resource degradation represent a serious threat to world food security (Pimentel et al., 1995; Bumb and Baanate, 1996; Pinstrup and Pandya, 1998; FAO, 1999; Lal, 2000; Henao and Baanante, 2006). It is estimated that land degradation is affecting around one billion people around the world (Eswaran and Reich, 2001) and 1.4 thousand million ha of vegetated land in developing countries are subjected to land degradation (FAO, 1999). A considerable number of peasant farmers around the world, who contributed significantly to the world food production, are located in marginal lands facing different production constraints. This peasant farming is greatly supported by agroecological systems based on traditional knowledge (Altieri et al., 2011). It is estimated that around 1,966 millions hectares around the world are affected by land degradation (Oldeman, 1994). Lal (2000) states that "widespread and severe problems of soil degradation in the tropics are not necessarily caused by soil characteristic per se and may be traced to land misused and soil mismanagement".

It is estimated that the soil carbon pool is around three times larger than the aboveground biomass and around two times larger than the carbon in the atmosphere. Eswaran et al. (1993) estimated the global organic soil carbon pool in 1576×10^{12} Kg and 32 % of this is sequestrated in tropic forest soils. Appropriate soil management technology like no-till farming, cover crops management, manure applications and other agroecology practices can offset the global fossil fuel emissions by 5 to 15 % annually as result of the induced soil carbon sequestration (Lal, 2004). According to Rozanov et al. (1990) in the past century the global loss of humus was 760 million tons per year and nearly 16% of the original stock of organic soil carbon has been lost. Various authors have noted soil organic matter as the most important indicator of soil quality because of its impacts in physical, chemical and biological soil properties (Reeves, 1997; Carter et al., 1999; Lal, 2004).

Van Bruggen and Semenov (2000) define a healthy soil as a “stable system, with resilience to stress, high biological diversity, and high levels of internal cycling of nutrients”. Soil health should consider soil chemical, physical and biological properties (Van Bruggen and Semenov, 2000). Sustainable production practices based on adequate management of organic matter has demonstrated repeatedly to contribute positively to soil restoration as a direct source of essential elements and through the improvement of other soil chemical, physical and biological properties (Babalola et al., 2012). Omotayo and Chukwuka (2009) stated the potential of organic resources utilization for soil fertility and production improvement in degraded lands. In a review of long term experiments Reeves (1997) reports the importance of adequate organic matter management like manuring and crop rotations to maintain long-term crop productivity. When conventional and organic farming were evaluated during 8 years, Clark et al. (1998) reported higher soil organic C, soluble P, exchangeable K and pH on the organic system. In this experiment the organic system consisted of poultry manure applications and vetch as a green manure. The rest of the organic management was made according to the regulations of California Certified Organic Famers. In a similar study between organic and conventional tomato farms Drinkwater et al. (1995) reported a three fold increase in the mineralization potential, one-fourth more inorganic N pools, 28% more organic C and greater K concentrations in organic farms compared with conventional farms. With a similar trend Babalola et al. (2012) reports an increase in organic C, available P, N, and K in compost amended soils. Chuwuka and Omotayo (2008) reported a buildup in N, P, Ca, Mg, Cu, Zn, Mn, Fe, nitrate and nitrite compounds and an increase in pH and organic carbon after 7 weeks of water hyacinth compost and Tithonia green manure incorporation. Van Diepeningen et al. (2006) concluded that organically managed soils are more stable systems due to resilience toward stress events such as drying-rewetting periods. These results showed higher biological diversity and less nitrogen species prone to leaching. Ferreras et al. (2006) reported increased soil organic carbon after application of vermicompost from different feedstocks. Compost additions in a tropical weathered soil have demonstrated to increase nutrient content, cation exchange capacity and pH (Abdel, 2009). With similar results Aggelides and Londra (2000) reported increase in soil organic matter, pH and cation exchange capacity after compost incorporation.

Soil physical properties and its management play a major role in achieving food security despite being extensively ignored with a high cost to the soil health and productivity (Lal, 2000). Soil organic matter management has been directly linked to the enhancement of bulk density, water retention capacity, aggregate stability, infiltration, soil cation exchange capacity and other indicators of soil quality (Aggelides and Londra, 2000; Tejada et al., 2009a). The addition of town waste and sewage sludge compost have demonstrated to enhance hydraulic conductivity, water retention capacity, bulk density, total porosity, pore size distribution, soil resistance to penetration, aggregation and aggregate stability (Aggelides and Londra, 2000). The soil application of beet vinasse and crushed cotton gin compost to soil have demonstrated to increase structural stability and the decrease of bulk density (Tejada et al., 2007). Similarly, composted *Trifolium pretense* green manure and compost made with a mixture of *Trifolium pretense* and beet vinasse also increased structural stability and decreased bulk density when compared with non-amended soils (Tejada et al., 2008). Torres (2009) found greater aggregate stability, enhanced hydraulic conductivity, greater water retention and lower soil bulk density after two years of a legume cover crop establishment in a tropical soil. Spaccini et al. (2004) obtained higher aggregate stability after *Cajanus cajan* fallow and after *Gliricidia sepium* green manure surface incorporation compared with a non amended control. Lal (2000) states that the decline in soil structure triggers the rest of the physical degradative processes like crusting, compaction, low infiltration rate, high runoff, accelerated soil erosion depletion of soil organic matter, and decrease in productivity. Tejada et al. (2009a) demonstrated that structural stability is determined by soil organic matter for its role as a cementing factor and is directly related with humic acid concentration. The application of decomposed stable organic matter have demonstrated to reduce the soil loss due to water erosion as a result of the increase in soil structural stability and organic matter concentration (Tejada et al., 2009b). Oldeman (1994) estimated that from the degradation of land worldwide (1966 Mha), 1094 Mha is caused by water erosion and 549 Mha by wind erosion. Tejada et al. (2009b) also found that the effect of compost application decreases soil bulk density and favors the spontaneous plant cover, able to protect soil against erosion. After two years of a crop residue and poultry manure compost application, Babalola et al. (2012) reported the increase in aggregate stability, total porosity and hydraulic conductivity and the decrease in bulk density. Ferreras et al. (2006) reported enhanced proportion of water stable aggregates after application of vermicompost from household solid waste, from horse and rabbit manure, and chicken manure at 20 Mg/ha.

Soil microbial activity is one of the most altered soil properties by unsuitable human management. It is estimated that 9,000 billion hectares around the world have their biological functions completely destroyed (FAO, 1999). Due to its sensitivity, soil microbial activity is a good indicator of soil quality changes (Tejada et al., 2007). Franchini et al. (2007) reported high responsiveness of soil microbial activity to soil management and crop rotations thus demonstrating its usefulness as indicator of soil quality in the tropics. Soil microbial properties have being directly linked to soil health since it is related to nutrient availability and cycling (Oberson et al., 2006; Zhao et al., 2009), pathogen suppression and pest control (Drinkwater et al., 1995; Altieri and Nicholls, 2003). Soil microbial biomass has being demonstrated to be an important pool of mobile nutrients in soil like N and P (Marumoto et al., 1982). Enhanced microbial biomass by organic soil amendment including green manures has being associated to improved potential nitrogen availability for plants (Tu et al., 2006). Díaz et al. (1993) demonstrated microbial biomass to contribute significantly to the pool and availability of N, P, K and Mg. Some authors report that the most important factor determining the microbial communities is the amount of carbon entering the systems (Drinkwater et al., 1995; Gunapala and Scow, 1998). Multiple studies demonstrate the effects of organic matter incorporation in maintaining and improving levels of organic carbon in the soil (Reeves, 1997; Carter et al., 1999; Tejada et al., 2007; Tejada et al., 2008; Tejada et al., 2009a; Tejada et al., 2009b; Spaccini et al., 2004; Clark et al., 1998; Drinkwater et al., 1995; Garcia et al., 1989; Fließbach et al., 2007; Reeves et al., 1997). Tejada et al. (2009) suggest that the increase in microbial activity due to compost application could be responsible for the observed increase in soil structural stability. Six et al. (2004) extensively review the effect of soil microbial activity in the stabilization of soil aggregates by different means like the binding effect of hyphae and the cementing action of mucilage produced by bacteria and fungi. Tejada et al. (2009b) concluded that the decrease in organic matter in a non-organically amended soil to be responsible for the decrease in microbial biomass. In a comparison study between organically and conventionally managed tomato systems five microbial variables including fumigation extractable carbon and nitrogen, potentially mineralizable N, arginine ammonification and substrate induced respiration where measured 13 times during the growing season (Guanapala and Scow, 1998). On most of the sampling dates evaluated all five microbial biomass variables where significantly higher in the organic than conventional system. The capacity of organic management practices in enhancing microbial activity and biomass has been demonstrated (Gunapala and Scow, 1998; Carpenter et al., 2000; Ros et al., 2003; Diepeningen et al., 2006; Ferreras et al., 2006; Tu et al., 2006; Franchini et al., 2007; Tejada et al., 2007; Arslan et al., 2008; Tejada et al., 2008; Araújo et al., 2009; Tejada et al., 2009a; Tejada et al., 2009b).

The enhancement of crop yield and productivity as a consequence of soil restoration practices has being extensively reported. Ferreras et al. (2006) reported an increase in lettuce yield after the soil was amended with 20 Mg/ha of any of the following organic materials: chicken manure, vermicompost from household solid waste or vermicompost from horse and rabbit manure. Not another amendment was applied to the soil. These treatments also improved aggregate stability, soil organic carbon and soil microbial activity. Atiyeh et al. (2000) reported greater raspberry and tomato plant growth after the soil was amended with pig waste vermicompost. This effect was attributed to pH buffering activity, increased enzymatic activity and beneficial microbial activity since it was compared with a standard horticulture potting media with all essential nutrients supplied. Keeling et al. (2003) reported greater growth of wheat and rape when mature compost was applied in combination with synthetic fertilizers. This growth promotion is attributed to enhancement in N utilization efficiency and direct physiological effect of water-extractable growth promoters present in the compost. Results reported by Tejada and Gonzalez (2006) where higher rice yield, increase of leaf soluble carbohydrates, pigments, grain protein and percentage of full grains was related to the incorporation of different rates of compost after incorporation of inorganic fertilizer. The benefits were greater after the third experimental season due to the accumulation of organic matter. The enhancement in crop production parameters is attributed to enhancement of soil microbial and enzymatic activity since they are related with important nutrient cycles like N, P and S and therefore higher availability to plants. In an experiment with composted cotton gin trash and animal manure amended soil, Tu et al. (2006) found 50 % higher tomato yields in composted cotton gin and 20 %

higher yields in animal manure amended soils when compared with conventional fertilization treatments. These increases in tomato yield are associated with enhanced microbial biomass and activity and higher potential N availability. Mkhabela and Warman (2005) reported no difference in potato and sweet corn yield after the second year of municipal waste compost application when compared with inorganic fertilizer treatment. The compost application rates were based on extractable soil P analysis assuming 100 % compost P availability.

The adequate management of different organic amendments, some of which were previously presented, has demonstrated to positively contribute to soil restoration, enhancing a wide range of soil chemical, physical and biological parameters. Organic amendments have also being capable to support high productivity and contribute to solid waste reduction through composting process. There are few reports of the effects of combined sustainable practices in soils in the tropics.

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1. Chapter 1: Soil physical and biological properties affected by repeated short-term organic amendments applications

1.1. Introduction

Soil degradation seriously threatens world food security (Pimentel et al., 1995; Bumb and Baanate, 1996; Pinstrup and Pandya, 1998; FAO, 1999; Lal, 2000; Henao and Baanante, 2006). Inadequate management of soil has been pointed out as one of the main causes of worldwide soil degradation (Henao and Baanante, 2006; Lal, 2007). Deficient management of soil physical properties has led to deterioration of soil fertility and reduction of productivity (Lal, 2000). Soil organic matter has been pointed out as the most important indicator of soil quality because of its impact on physical, chemical and biological soil properties (Reeves, 1997; Carter et al., 1999; Lal, 2004). It has been extensively reported that physical properties, most notably structure, are largely governed by soil organic matter content and quality (Khaleel et al., 1981). The management of soil physical properties becomes of major concern in the tropics since it has been commonly established that organic matter (OM) decomposes at a faster rate compared to temperate climates (FAO, 1999).

Management of organic carbon has been suggested as a primary practice to enhance and maintain soil structure in the short and long-term (Reeves, 1997). A growing list of practices are being adopted in sustainable agriculture systems that include compost additions, enhanced fallows, compost tea and other organic and biological amendments that intend to improve soil structure and other soil properties such as nutrient status. The incorporation of organic amendments such as animal manures (Annabi et al., 2011), vermicompost (Ferreras et al., 2006), cover crops and other agricultural by-products (Tejada et al., 2007; Tejada et al., 2008b) have been demonstrated to enhance a wide range of physical properties directly linked to crop productivity, such as aggregate stability, water conductivity and water retention. Composted municipal solid waste and sewage sludge can also enhance soil structure; however, a potential hazard of using these materials is high loading of the soil with heavy metals (Angelides and Londra, 2000).

The use of cover crops has been demonstrated to enhance soil physical characteristics (Fischler et al., 1999; Tejada et al., 2008b; Torres, 2009). Torres (2009) found greater aggregate stability, enhanced hydraulic conductivity, greater water retention and lower soil bulk density after two years of an *Arachis spp*. cover crop establishment in the San Antón soil series (Haplustolls). Fischler et al. (1999) reported increased water infiltration and reduced bulk density after incorporation of *Crotalaria sp*. residues. In an experiment evaluating alternatives for restoration of degraded soils Tejada et al. (2008b) found that the incorporation of *Trifolium pratense* L. increased structural stability and reduced bulk density by 5.9% and 6.1%, respectively.

Microbial activity has been pointed out as another important index of soil quality, which is intimately related to the cycling of soil nutrients (Oberson et al., 2006; Zhao et al., 2009), pathogen suppression, and pest control (Drinkwater et al., 1995; Altieri and Nicholls, 2003). It is also related to soil physical properties principally by the action of microbial by-products on stabilization of aggregates (Six et al., 2004). Microbial activity is very sensitive to agricultural management practices such as the level of organic carbon entering the agro-ecosystem (Angelides and Londra, 2000; Drinkwater et al., 1995; Gunapala and Scow, 1998; Tejada et al., 2007). Some important microorganisms, such as mycorrhizae are a primary soil biological functional group associated to the improvement of soil physical properties and nutrient uptake adding considerable sustainability elements to crop production (Cardoso and Kuyper, 2006). The objective of this study was to evaluate the combined effect of coffee pulp compost application, green manure incorporation and addition of compost liquid inoculant and mycorrhizae applied in 67-day cycles on soil physical and biological properties of a Mollisol in the semi-arid southern coastal region of Puerto Rico. Measurable effects of the adoption of a sustainable approach of agricultural production often could become noticeable in the long term (Funes-Monzote et al., 2009). The consecutive application of 67-day cycles were done to concentrate in a short time scale what could be achieved in terms of soil properties with a long-term management of combined organic amendments.

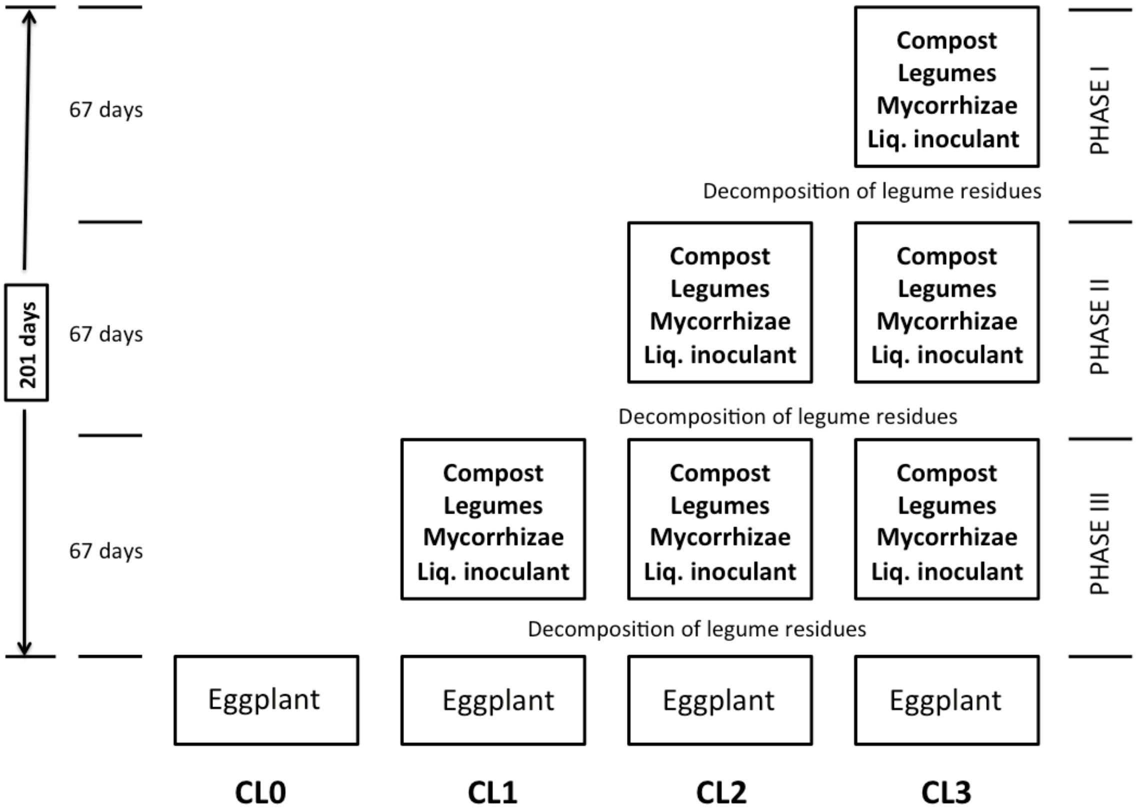
1.2. Materials and Methods

1.2.1. Experimental Design and Treatments

The experiment was conducted at the University of Puerto Rico Agricultural Experiment Station in Juana Díaz, Puerto Rico at a longitude of 66.31 W and latitude of 18.01 N. The soil at this location is a San Antón soil series (Fine-loamy, mixed, superactive, isohyperthermic Cumulic Haplustolls) (Beinroth et al., 2003). The experimental plot had a fallow history of more than three years where spontaneous vegetation was allowed to grow and was periodically plowed down with a disc harrow for its control.

The field experiment consisted of four treatments arranged in a Complete Randomized Block Design with four replications, all of which accounts for 16 experimental units. The experimental unit was a 2.4 x 3.0 m plot. Weeds around the plots were controlled mechanically with a tractor mower. The experimental treatments were labeled CL0, CL1, CL2 and CL3 consisting of no amendments (0), 1, 2 and 3 consecutive 67-day cycles of combined organic amendments application, respectively. Each cycle had the following four management practices: 1) soil incorporation of 5 g/100 g of coffee pulp compost (based on organic matter) followed by 2) planting and incorporation of a cover crop mixture of four legume species and addition of 3) mycorrhizae and 4) liquid inoculant made from compost. At day 0 of each 67-day cycle, 5 g/100 g of coffee pulp compost (based on organic matter) was till-incorporated to 15 cm and the mixture of legumes seeds were broadcasted over the experimental plots. Compost application was based on organic matter percent basis eliminating uncertainties related to moisture and impurities. At day 21 the plots were drench inoculated with 2.5 g inoculant/m^2 of aqueous suspension of commercially available mycorrhizae (MycoApply® Soluble Endo), which contained four endomycorrhizal species. At day 60, the different legume species were cut at the soil line, chopped, weighed and till-incorporated into the top 15 cm soil depth and liquid compost inoculant was applied to ease degradation of green manure residues. After legumes incorporation, seven days were allowed prior to the start of the next cycle to permit partial decomposition of residues. The 67-day cycles corresponding to the CL3, CL2 and CL1 treatments were established in three 67-day phases, such that all treatments were finalized at the same time (Fig. 1-1). During phase I, the first 67-day cycle was implemented in CL3 plots while CL2, CL1 and CL0 plots where kept weed controlled with mechanical methods. During the phase II, 67-day cycles were repeated in CL3 and started in CL2, whereas CL1 and CL0 were kept weed controlled. During phase III additional cycles were repeated in CL3, CL2 and started in CL1 and weeds were controlled in CL0. After the completion of all treatments eggplants were transplanted to evaluate yield response. Eggplant crop results are included in a companion paper (Pagán-Roig et al., 2013). The compost incorporated at each 67-day cycle was obtained from coffee pulp processed with an aerated static pile method. The chemical properties of the compost are shown in Table 1-1. The cover crop mixture consisted of four legume species, *Crotalaria juncea* (sunn hemp), *Canavalia ensiformis* (jack bean), *Vigna unguiculata* (cowpea) and *Mucuna pruriens* (velvet bean). The legume seeds were planted at rates of 42, 14, 13 and 13 kg/ha for *Canavalia ensiformis*, *Crotalaria juncea*, *Mucuna pruriens* and *Vigna unguiculata,* respectively. During the 67-day cycle period the sunn hemp, cowpea and velvet bean bloomed, but none matured their seeds. The jack bean never bloomed during the 67-day period. The compost liquid inoculant was prepared by mixing 11.5 kg of mature wood chip compost obtained from a local distributor and 120 mL of unsulfured molasses in 80 L of water, and was aerated continuously with an aquarium pump for two days.

Figure 1-1 Diagram of treatment establishment. The large squares represent application of compost, legumes, mycorrhizae and compost liquid inoculant under each 67-day cycle. After legumes incorporation and compost liquid inoculant addition, seven days were left to allow decomposition of legumes residues completing an entire 67-day cycle. The implementation of the treatments was done in three phases finishing all treatments at the same time for the establishment of an eggplant crop.



1.2.2. Soil Sampling and Analysis

After all treatments were established, a single sample per experimental unit was obtained by mixing four subsamples collected at a 0-15 cm depth. Twelve weeks after the implementation of all cycles, aggregate stability analysis was performed on disturbed soil samples and water retention curves was obtained from intact soil cores (7.5 cm long and 5 cm in diameter).

Organic matter was determined by the Walkley-Black dichromate oxidation technique as described in Nelson and Sommers (1982). Humic acids (HA) were extracted on the basis of their solubility in acids. Six grams of soil were treated with 0.1 M HCL to extract the fulvic acids. Then the soil was further extracted with 0.1 M NaOH to obtain the HA. Humic acids were precipitated by adding sufficient 6 N HCl solution to reach a pH of 1. After centrifuging and removing the supernatant, the HA precipitates were re-dissolved in a 0.1 N KOH solution. The HA were again re-precipitated with 6 N HCl, centrifuged, and re-dissolved with a solution of 0.1N HCl + 0.3 N HF. Hydrofluoric acid was included in the last dissolution process to remove silicates. The HA were purified with a cation exchange resin (Dowex 50WX8-100), freeze – dried and quantified by weighing on an analytical balance.

Soil microbial biomass carbon (MBC) was quantified with the fumigation-extraction method (Vance et al., 1987) by using the equation MBC= $E_C/0.45$, where E_C is the difference between organic-C extracted by K_2SO_4 from fumigated and un-fumigated soil, respectively (Beck et al., 1997). The analyses were performed by first wetting the soil to field capacity. The wetted samples were split into two equal portions (40 g fresh weight), one of which was fumigated and incubated with ethanol-free chloroform in glass desiccators at room temperature. The other portion was incubated without fumigation for the same period at the same temperature. After 24 h the chloroform was removed by suction, and 15 g of soil was extracted with 40 mL of 0.5 M K_2SO_4. For soil microbial C flush determination, dissolved organic C in the filtered extract was measured with a Rosemount Analytical Dorhmann DC 190 total organic carbon analyzer.

Microbial biomass nitrogen (MBN) and total nitrogen were measured after Kjeldahl digestion of the K_2SO_4 extract according to Brookes et al. (1985), using the equation MBN= F_N/.54, where F_N is the N mineralized from the biomass (Brookes et al., 1985).

Aggregate stability (AS) was determined by the wet sieving method (Kemper and Rosenau, 1986). The soil was air-dried and sieved, and the 1.65 – 4.7 mm sieve fraction was retained. The air-dried aggregate samples were then placed on top of a sieve nest of two sieves of 1.65 and 0.68 mm and agitated in water for 15 minutes at a rate of 30 cycles per minute with a wet sieving machine. The fractions of aggregates retained in the sieves were oven-dried at 105 °C for 24 h and corrected for sand to obtain the real proportion of soil aggregates (Nyamangara et al., 2001).

Water retention curves were determined on the intact soil cores by placing them on a pressure plate. The hanging water column technique was used to measure water retention at hydraulic tension heads of 0, -20, -40, -80, -160 and -320 cm H_2O (Hall, 1991). A five-bar pressure plate extractor (Dane and Hopmans, 2002) was used to measure water retention at -0.5, -0.6 and -1.0 bar (approximately -500, -600 and 1,000 cm H_2O, respectively). To infer pore-size distributions from the moisture release curves, we made use of the capillary rise equation (Kutilek and Nielsen, 1994)

$$r(\mu m) = \frac{1500}{h(cm)} \qquad [1]$$

where r is pore radius in μm and h is the matric potential (expressed in hydraulic head units or cm H_2O) required to drain water out of that pore. By virtue of Eq. [1], the change in volumetric water content between any two matric potentials h_1 and h_2, determined from the moisture release curves, can be interpreted as the fraction of soil volume occupied by pores with radius between r_1 and r_2. By making these determinations for different ranges in *h* and corresponding pore radii *r*, pore size distributions may be constructed which indicate the relative contribution of pores of different sizes to the total porosity of the soil.

Bulk density was determined by measuring the volume and the oven-dry weight of the undisturbed soil cores used for the water retention curve. Saturated hydraulic conductivity was measured in situ by inserting a sharpened cylinder 10 cm in diameter and 15 cm long into the soil at a 5 cm depth. Water was added to the cylinder and allowed to infiltrate the soil, maintaining a constant head of 5 cm inside the core with a Guelph permeameter. Saturated hydraulic conductivity was determined from the steady state water outflow rate from the permeameter (Reynolds and Elrick, 1985).

Table 1-1 Characteristics of coffee pulp compost used in each 67-day cycle.

Coffee pulp compost	
pH	7.13
OM (g/100 g)	60.0
Total N (mg/kg)	17,587.2
Total P (mg/kg)	2,267.4
Total K (mg/kg)	6,700.6
Total Ca (mg/kg)	9,055.2
Total Mg (mg/kg)	1438.2
Total Na (mg/kg)	450.6
Available nutrients	
NH_3-N (mg/kg)	352.6
NO_3-N (mg/kg)	738.8
P	200.6
K	684.6
Ca (mg/kg)	5718.0
Mg (mg/kg)	702.0
Na (mg/kg)	159.9

1.2.3. Statistical Analysis

The data were analyzed by ANOVA according to a Randomized Complete Block Design (RCBD), using Fisher's least significant difference for comparison of means. Correlation analyses were performed using the Pearson Coefficient Analysis. The analyses were performed using Infostat Statistical Software (Di-Riezo et al., 2011).

1.3. Results and Discussion

1.3.1. Organic Matter

The soil organic matter content increased from 1.01 g/100 g in CL0 to 2.50, 4.11 and 4.97 g/100 g for CL1, CL2 and CL3, respectively (Table 1-3). The amount of compost applied every 67-day cycle was equivalent to 5 g/100 g of OM in the top 15 cm layer of soil. This amounted to approximately 5, 10 and 15 g/100 g of soil OM added initially in form of compost to the CL1, CL2 and CL3 treatments, respectively. Comparing these initial values with the final organic matter values in Table 1-3 indicates that between 50 and 67 percent of the added organic matter mineralized during the experimental period. This is consistent with other results (Rivero et al., 2004; Bernal et al., 1998), which showed that even though composting produced a high proportion of stable OM a considerable portion of this OM becomes labile during the first two months after incorporation into the soil. In addition to the compost, the total oven dry biomass of the green manures that was incorporated to the soil at each 67-day cycle ranged from 5,560 to 6,402 kg/ha, which also contributed to the increase of soil OM. These values are considerably larger than those reported for biomass production of individual legume species. Carlo (2009) reported biomass production ranges of 822 - 4175 kg/ha, 821 - 2509 kg/ha, 482 - 2721 kg/ha and 340 - 1461 kg/ha for *Canavalia sp.*, *Crotalaria sp.*, *Mucuna sp.* and *Vigna sp.*, respectively in 20 different trials at five localities in Puerto Rico. This raises the possibility, which should be investigated further, that mixed plantings of these tropical legume cover crops in general produce more biomass and possibly fix more N than individual plantings of the same crops. However, since compost and cover crops did not vary independently in our experiments, it is not possible to infer their relative effects on changes in soil OM with our data. In the present study the HA fraction of OM in amended soils ranged from 23.6% to 30.1% without any specific trend. All organic treatments reported an increased of HA (CL3 > CL2 > CL1 > CL0) over non-amended soil ($p \leqq 0.05$; Table 1-3). The HA content ranged from 2.5 g/kg in the CL0 to 15 g/kg in CL3. Again, it is difficult to establish which of the added organic amendments had the greatest effect on humic acids(HA).

1.3.2. Microbial Biomass

After incorporation of three consecutive cycles (treatment CL3) microbial biomass C (MBC) increased 3.8 times relative to the non-amended soil treatment CL0 ($p \leqq 0.05$; Table 1-2). Treatments CL1 and CL2, though higher than CL0, were not statistically different because of the high variability of the results. The CL2 and CL3 treatments produced significantly ($p \leqq 0.05$) higher levels of microbial biomass N (MBN) than the non-amended soil (CL3 > CL2 > CL1 = CL0). Otherwise, treatments CL2 and CL3 produced an increase in MBN of 7.9 and 11.5 times, respectively, over the non-amended soils. The increase of microbial activity after the application of organic residues has been attributed to the input of easily degradable materials (Blagodatsky et al., 2000; Carpenter et al., 2000; Ros et al., 2003; Tejada and Gonzalez, 2006; Ferreras et al., 2006; Franchini et al., 2007; Annabi et al., 2011). Other authors have seen direct relations between the enhanced soil physical properties like structure and porosity and the increase in biological activity after addition of organic amendments (Marinari et al., 2000; Tejada et al., 2006; Annabi et al., 2011). The microbial population of the compost could partially explain the increase of soil microbial biomass (Beffa et al., 1995). However, the effect of legume and non-legume cover crops in enhancing microbial activity has also been reported (Schulz, 2003; Tilak, 2004; Tejada et al., 2008a). As indicated earlier, it was not possible under our experimental design to separate individual effects of compost and cover crops on changes in microbial biomass.

Table 1-2 Microbial biomass carbon and microbial biomass nitrogen after the implementation of different numbers of 67-day cycles (CL0, CL1, CL2, CL3) of compost application, planting and incorporation of four green manure species, mycorrhizae and compost liquid inoculant.

Identification	Microbial Biomass-C	Microbial Biomass-N
	(µg C/g dry soil)	(µg N/g dry soil)
CL0	96.62b	10.15c
CL1	207.39ab	40.64c
CL2	212.05ab	80.26b
CL3	366.63a	117.08a

Means within columns followed by the same letter are not significantly different at *p*< 0.05 using Fisher's least significant difference (LSD).

1.3.3. Aggregate Stability

Treatments CL1, CL2 and CL3 produced a 4.1, 6.4 and 9.0 fold increase in aggregate stability (AS), respectively, in contrast to the CL0 treatment ($p \leq 0.05$; Table 1-

3). Significant correlations were observed between AS and organic amendments components such as organic matter (r= 0.94, $p \leq 0.05$; Fig. 1-2), humic acid (r= 0.92, $p \leq 0.05$; Fig. 1-3), microbial biomass C (r=0.59, $p \leq 0.05$; Fig. 1-4) and microbial biomass N (r= 0.89, $p \leq 0.05$; Fig. 1-4). The AS increase with increasing OM have been previously reported were it functions as soil particle cementing agents (Aggelides and Londra, 2000; Nyamangaraa et al., 2001; Spaccini et al., 2004; Ferreras et al., 2006, Tejada et al., 2009; Abiven et al., 2009; Zhang et al., 2011; Babalola et al., 2012).

Mature compost has been related with long-term increases in AS principally through the effect of humic substances (Annabi et al., 2011). On the other hand, green manures have been related with a short-term effects on AS associated with the production of microbial exudates (Golchin et al., 1994; Liu et al., 2005; Abiven et al., 2009). Our data, does not permit to separate the effect of compost and cover crops on AS because all organic amendments were combined at each 67-day cycle.

Regressions between AS and OM, HA, MBC and MBN are shown in Figs. 2-3. The regressions in all cases were significant. However, it is difficult to establish which of these parameters had the greatest effect on AS, because OM, HA, MBC and MBN are all strongly correlated to each other (Table 1-4). Also, our experimental design does not allow discriminating between individual effects of compost and cover crops on AS.

Table 1-3 Organic matter, humic acids content, aggregate stability, bulk density and hydraulic conductivity after the implementation of different numbers of 67-day cycles (CL0, CL1, CL2, CL3) of compost application, planting and incorporation of four green manure species, mycorrhizae and compost liquid inoculant.

Identification	Organic matter	Humic Acids	Aggregate stability	Bulk density	Hydraulic conductivity
	(%)	(g/kg)	(%)	(g/cm^3)	(cm/h)
CL0	1.01d	2.5d	2.65d	1.59a	0.463c
CL1	2.50c	7.1c	10.87c	1.34b	3.212b
CL2	4.11b	9.7b	16.93b	1.22c	6.467a
CL3	4.97a	15.0a	24.01a	1.08d	5.576a

Means within columns followed by the same letter are not significantly different at $p < 0.05$ using Fisher's least significant difference (LSD).

Table 1-4 Correlation matrix (r) of soil parameters affecting aggregate stability.

	AS	MO	HA	MBC	MBN
AS	1.00				
OM	0.94	1.00			
HA	0.92	0.96	1.00		
MBC	0.59	0.64	0.68	1.00	
MBN	0.89	0.92	0.89	0.48	1.00

Figure 1-2 Water stable aggregates in relation to organic mater content (r=0.94).

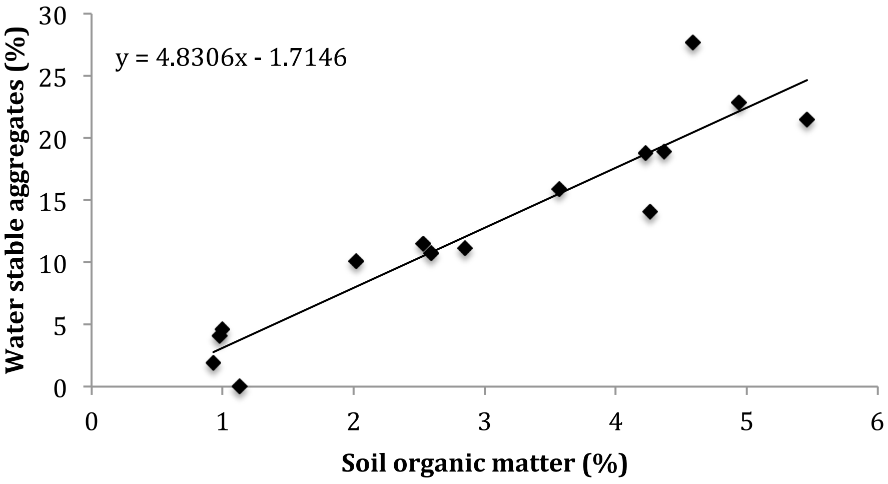


Figure 1-3 Water stable aggregates in relation to humic acids content (r=0.92).

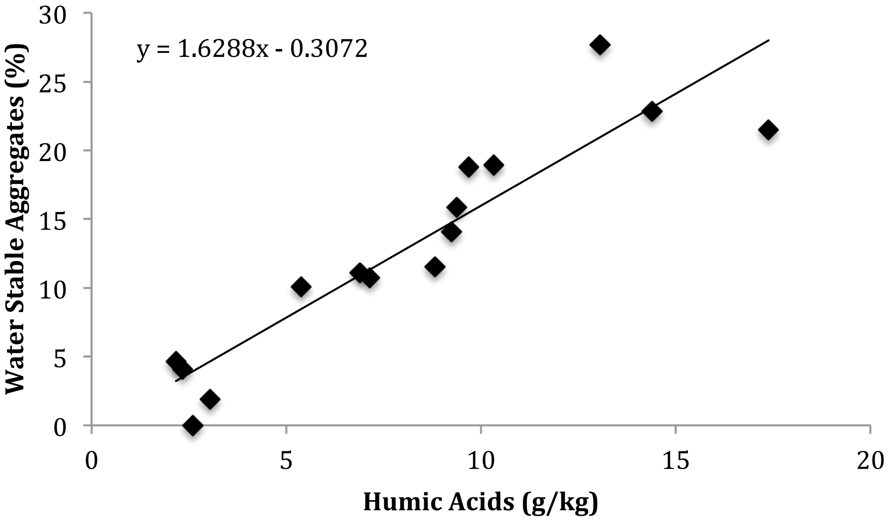
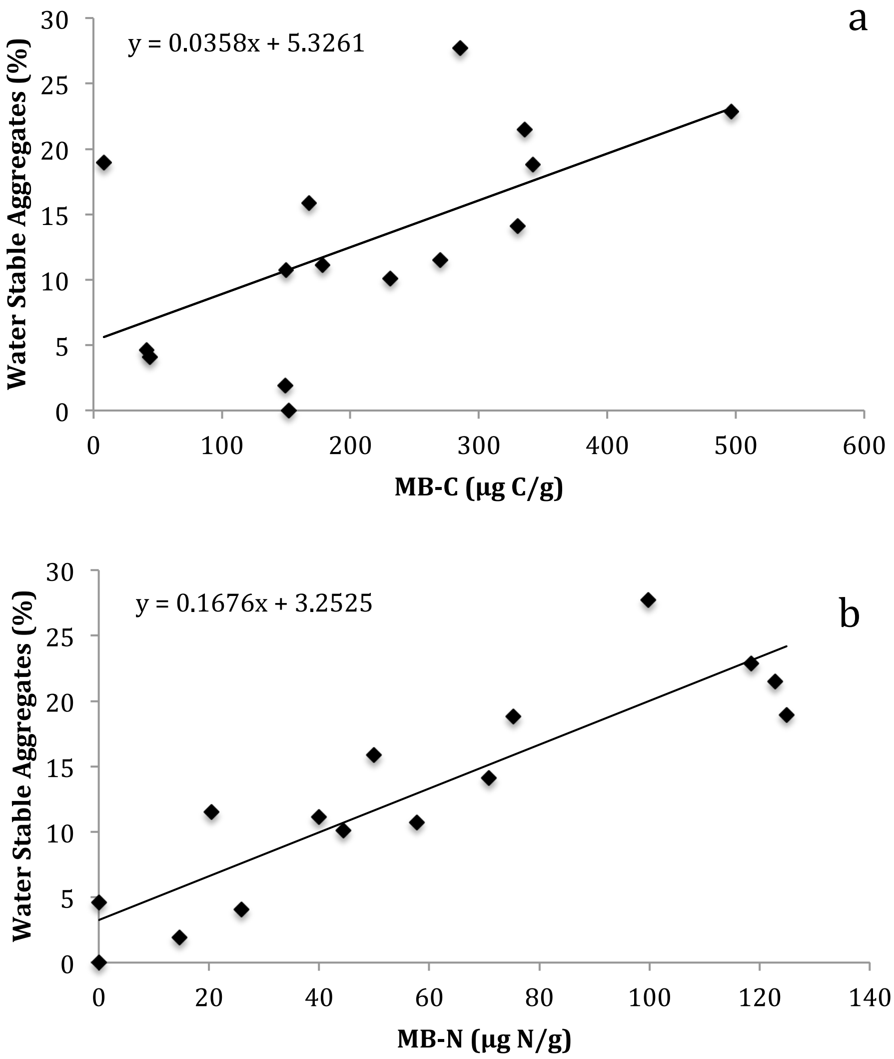


Figure 1-4 Water stable aggregates in relation to microbial biomass estimated after the implementation of different numbers of 67-day cycles (CL0, CL1, CL2, CL3) of compost application, planting and incorporation of four green manure species, mycorrhizae and compost liquid inoculant. (a) Linear correlation between water stable aggregates and MBC (r=0.59), and (b) Linear correlation between water stable aggregates and MBN (r=0.89).



1.3.4. Bulk Density

Table 1-3 shows the effects of combined practices on soil bulk density. Treatments CL1, CL2 and CL3 significantly ($p \leq 0.05$) reduced bulk density in 15.4%, 23.2%, and 31.9% respectively, in comparison with CL0. These results concur with Khaleel et al. (1981), who reviewed experiments of organic residue application and found a constant positive relationship between reduction of bulk density and application of organic residues in 21 soil types and eight different organic residues. The reduction in bulk density after the addition of an organic amendment is attributed to the dilution of denser soil particles and to the increase in porosity carried out by the enhanced structural stability (Tejada et al., 2009).

1.3.5. Water Retention Curves and Pore Size Distribution

Increasing 67-day cycles from CL1 to CL3 increased water retention at all tensions (Fig. 1-5). The CL0 water retention was similar to that of CL1 at any tension. Individual tensions variance analyses show that treatment CL2 and CL3 increased water retention when compared to CL0. Treatment CL3 was more efficient than CL2, increasing water retention capacity at all tensions except for -0.0196 and -0.0392 bar. On average, treatments CL2 and CL3 increased water retention at saturated conditions in about 15.5% and 23.0%, respectively. This finding represents greater potential of rainwater conservation since the water storage of this soil increased with the application of two (CL2) and three (CL3) consecutive 67-day cycles. The observed increase in OM content (Table 1-3) explains the enhancement in water retention capacity. The contribution of water retention of OM itself explains water dynamics retained at high suctions (Rawls et al., 2003), but the structure forming effect of OM is more likely to explain the water retention dynamics between 0 to -1 bar (Snyder et al., 1993; Sharma and Uegara, 1968).

An increase in aggregate stability is expected to be accompanied with an enhancement in water retention since it has been demonstrated that inter-aggregate pores play a major role on the water retention at suctions between 0.0 to -0.3 bars (Sharma and Uehara, 1968). The greatest treatment effect was on porosity associated with pores >38 µm (Table 1-5). Treatments CL2 and CL3 increased >38 µm porosity which were respectively 2.1 and 2.2 times the value for the CL0 treatment. Pore of radius >38 µm are commonly considered macropores associated with inter-aggregate space produced by aggregation (Hillel, 2004). An opposite trend was observed for porosities corresponding to pore-size ranges smaller than 38 µm (Table 1-5). In most of these cases, porosities decreased with increasing number of 67-day cycles. The data suggest that the increase in porosity associated with pores >38 µm occurred at the expense of a decrease in smaller pores (<38 µm).

Figure 1-5 Water retention curves of soils after the implementation of different numbers of 67-day cycles (CL0, CL1, CL2, CL3) of compost application, planting and incorporation of four green manure species, mycorrhizae and compost liquid inoculant.

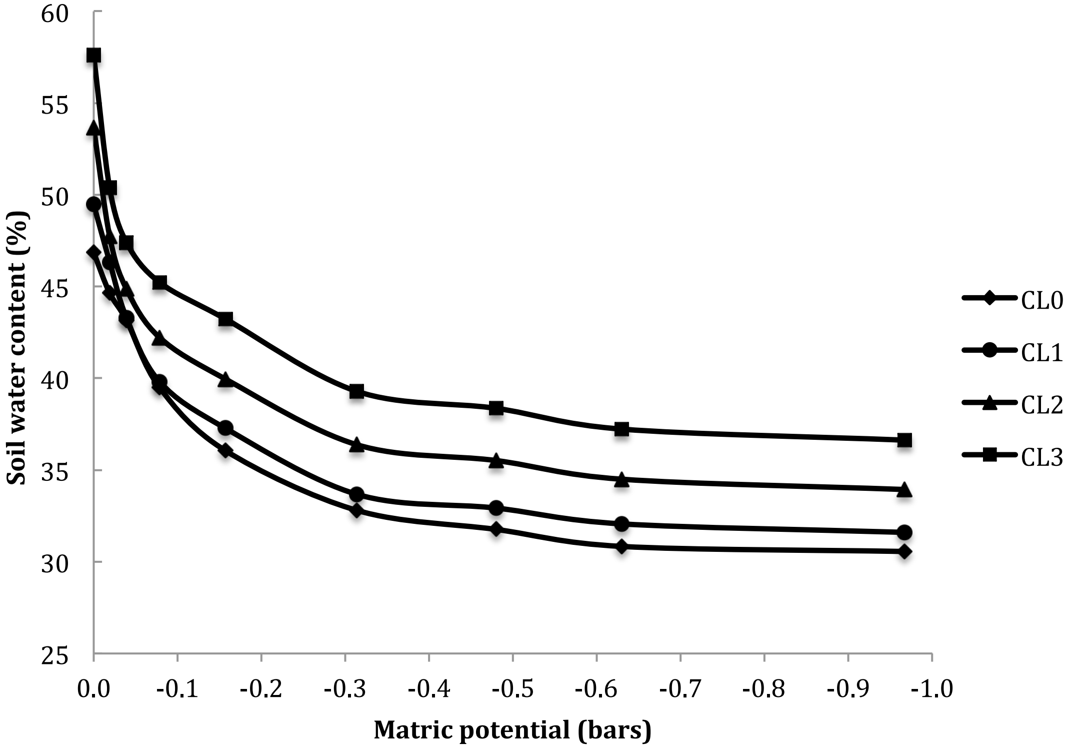


Table 1-5 Pore size distribution as affected by the implementation of different numbers of 67-day cycles (CL0, CL1, CL2, CL3) of compost application, planting and incorporation of four green manure species, mycorrhizae and compost liquid inoculant.

Identification	Pore size (radius)				
	2 to 5 µm	5 to 9 µm	9 to 19 µm	19 to 38 µm	>38 µm
	- percent of total porosity corresponding to pores in the given size range -				
CL0	4.2a	6.9a	7.3a	7.7a	7.9b
CL1	3.3ab	7.3a	5.2b	7.0ab	12.2ab
CL2	3.5ab	6.7a	4.2bc	4.9bc	16.3a
CL3	3.6b	6.9a	3.5c	3.8c	17.5a

Means within columns followed by the same letter are not significantly different at $p \leq 0.10$ using Fisher's least significant difference (LSD).

1.3.6. Hydraulic Conductivity

All treatments showed significantly higher values of hydraulic conductivity compared with that of the non-amended soil (Table 1-3). The CL1 treatment was seven times greater than the CL0 while CL2 and CL3 reported a 14- and 12-fold increase, respectively. Treatments CL2 and CL3 were not significantly different. These results are in accordance with those of various authors (Khaleel et al., 1981; Aggelides and Londra, 2000) who have reported increases in hydraulic conductivity as the result of increased porosity after organic matter additions. As supported by the enhancement in AS (Table 1-3) and the increase in the proportion of macropores (Table 1-5), the enhancement of water conductivity is attributed to greater production and connectivity of macropores due to more stable aggregates (Zhang et al., 2011). As pointed out above, the incorporation of green manures promotes the development of microbial-associated enhancement of AS favoring the development of inter-aggregate pores. Fischler et al. (1999) reported an increase of 51% of water infiltration in a tropical soil associated with an increase in the number of macropores after the incorporation of *Crotalaria sp*. cover crop residues. In our experiment the presence of undecayed legume residues mixed within the soil was still obvious eight weeks after the incorporation of the green manures, which may have contributed to the stabilization of aggregates. Already pointed out is the effect of undecayed cover crop roots and stalks and the biological action protecting macropores (Struddley et al., 2008). These results are transcendent for this soil since efforts have been made in this soil series to increase hydraulic conductivity in order to reduce the incidence of soil-borne diseases during the rainy season (Torres, 2009).

1.4. Conclusion

The 67-day cycles of combined practices promoted a general enhancement of soil properties expressed by a reduction in bulk density, and an increase in aggregate stability, OM content, microbial activity and hydraulic conductivity. Two consecutive 67-day cycles (CL2) were usually sufficient to attain most of the significant effects over soil properties. Even one 67-day cycle was capable to significantly increase organic matter content, hydraulic conductivity, and decrease bulk density. The incorporation of 67-day cycles of combined organic amendments presented in the present study may be considered as tool to reclaim degraded soils since the adoption of these practices had a cumulative positive effect on soil physical parameters. Our experiment was not design to determine the effects of individual amendments on soil properties, but it had the intention to mimic overall effect of a sustainable agricultural system. Similarly, it is difficult to establish which of the organic matter components (total OM, HA, MBC or MBN) had the strongest influence on aggregate stability and associated physical properties. The fraction which best correlated with AS was HA, but HA also co-varied strongly with other indicators such as total organic matter and microbial C and N, which introduces confounding elements. However, regardless of the specific mechanisms involved, this study shows that the combined organic amendments can have strong positive effects on soil physical properties, which influence soil productivity. Effects on yields are documented in a companion paper (Pagán-Roig et al., 2013).

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2. Chapter 2: Combined organic amendments effects on eggplant yield, soil fertility characteristics and humic acid quality

2.1. Introduction

Soil organic matter status has been pointed out as the most important indicator of soil quality because of its impacts in physical, chemical and biological soil properties (Reeves, 1997; Carter et al., 1999; Lal, 2004). The effects of organic amendments on soil properties are determined by their application rate and quality. The humic fraction of soil organic matter plays a major role in the long term fertility of soils influencing slow release of nutrients, pH buffering activity, cation exchange capacity, metal ions and organic molecules interactions and other soil physical properties (Stevenson, 1994; Senesi et al., 1996). The increase in soil humic acid content is considered to enhance the long-term fertility of the agroecosystem because of the durability of this fraction (Senesi et al., 1996).

Composting has been pointed out as an effective method to process organic materials to produce stable organic matter (Bernal et al., 1998; Rivero et al., 2004). The enhancement of native soil humic acids properties through the addition of composted organic residues has been demonstrated to be of considerable potential in the reclamation of degraded soils (García-Gil et al., 2004). The effect of the organic amendment on soil humic acids is determined by the nature of the organic amendment and it’s degree of maturation (Senesi et al., 1996).

There is a growing list of practices being adopted to increase agricultural sustainability, which include compost additions, enhanced fallows, liquid compost inoculants and other organic and biological amendments that intend to improve soil fertility status and sustainability. Sustainable agriculture aims to enhance long-term soil fertility by the efficient use of local resources, increase agroecosystem resilience and stability and reduce dependence on external inputs (Schiere et al., 2002). Compost use has been reported to increase soil organic matter (Aggelides and Londra, 2000; Ferreras et al., 2006), availability of N, P, K, Ca, Mg (Chukwuka and Omotayo, 2008; Bustamante et al., 2011; Babalola et al., 2012) and buffer soil pH (Clark et al., 1998). The quality and type of organic residues and the composting method predominantly determine final compost quality and its effect on soil properties. Compost restorative effects have improved crop productivity and farmers income in undeveloped countries (Ouédraogo et al., 2001). The implementation of leguminous fallows has been proposed to increased soil organic matter and available N in agricultural soils. Leguminous cover crops have been shown to enhance soil organic matter quality by increasing its nitrogen content when compared with non-coordinated fallows (Koutika et al., 2001; Nezomba et al., 2012). *Crotalaria spp*, *Mucuna spp*, *Canavalia spp* and *Vigna spp* are tropical legumes that have being proposed as options for improved fallows in the tropics because of their high biomass production, nitrogen fixation and adaptability (Carlo, 2009). Legumes fallows have been demonstrated to enhance N supply and increased yields of subsequent crops (Fischler et al., 1999; Wortmann et al., 2000; Armecin et al., 2005; Kaizzi et al., 2006) by enhancing soil chemical, physical (Fischler et al., 1999) and biological properties (Tejada et al., 2008). Compost liquid extractions, used as amendments, have been tested on different crops. Compost extractions differ in quality based on the compost used, the preparation time and the oxygenation of the mixture. Compost tea additions have been reported to reduce disease severity and promote plant development (Sidiqqui et al., 2008; Sidiqqui et al., 2009; Pane et al., 2012; Pant et al., 2012). Compost tea mechanisms of action include nutrient efficiency enhancement, nutrient and humic acid source, plant pathogens suppression, beneficial biota promotion and induction of plant inherent resistance (Siddiqui et al., 2008; Siddiqui et al., 2009). Soil microbial communities have multiple functions directly related to soil fertility including the cycling and availability of soil nutrients (Oberson et al., 2006; Zhao et al., 2009), pathogen suppression, and pest control (Drinkwater et al., 1995; Altieri and Nicholls, 2003). Specifically, mycorrhizae are a primary soil biological functional group associated to the improvement of soil physical properties, nutrient uptake and biological quality adding considerable sustainability elements to crop production (Cardoso and Kuyper, 2006). There is a need to better understand the impact of the combination of different sustainable practices on soil properties and crop production since most of the studies have concentrated on the effect of individual practices.

Green manure incorporation, coffee pulp compost application, compost liquid inoculant and mycorrhizae addition were carried out in 67-day cycles. The objective of this study was to evaluate the effects of the combined practices implemented every 67-day on soil chemical fertility parameters and eggplant yield. The changes in soil humic acid quality were measured as an index of long-term soil fertility. The consecutive application of 67-day cycles were done to concentrate, in a short time scale, what could be achieved in terms of soil properties with a long-term management of combined organic amendments.

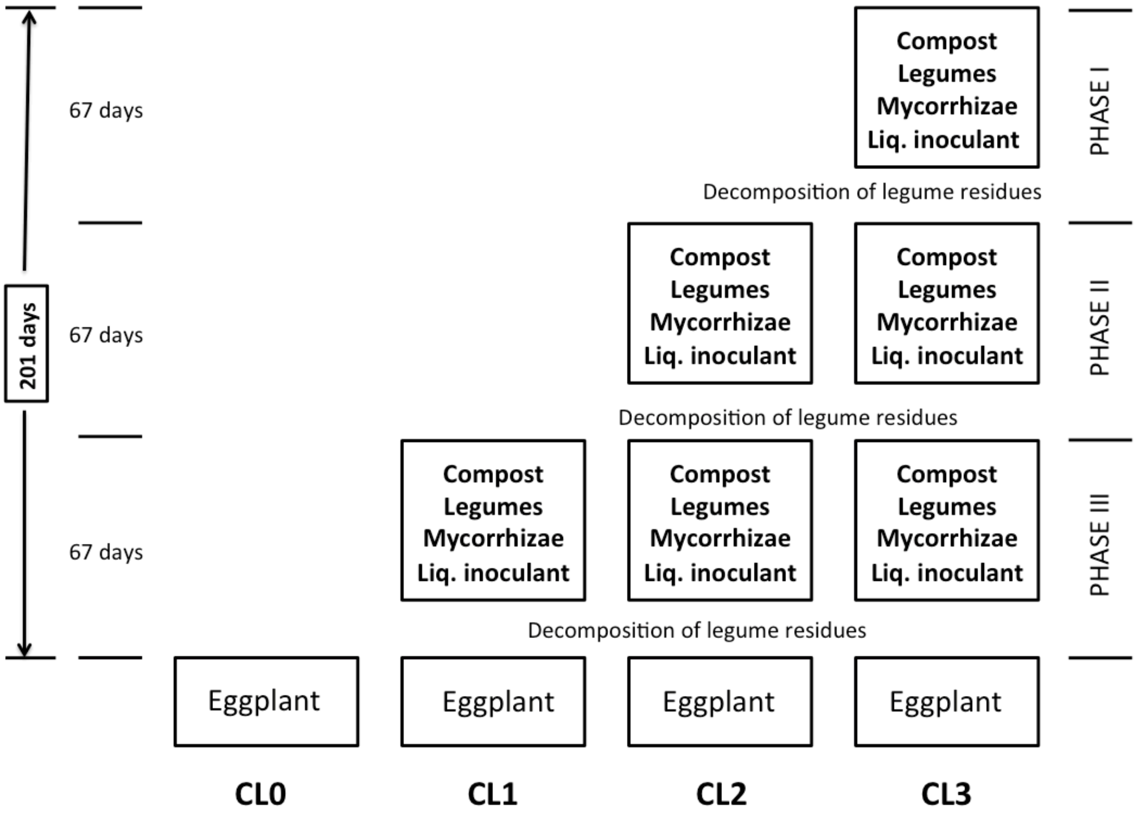
2.2. Materials and Methods

2.2.1. Experimental Design and Treatment Establishment

The experiment was conducted at the University of Puerto Rico Agricultural Experiment Station in Juana Díaz, Puerto Rico. The soil at this location is a San Antón soil series (Fine-loamy, mixed, superactive, isohyperthermic Cumulic Haplustolls). The experimental plot had a fallow history of more than three years where spontaneous vegetation was allowed to grow and was periodically plowed down with a disc harrow for its control.

The field experiment consisted of four treatments arranged in a Complete Randomized Block Design with four replications, all of which accounts for 16 experimental units. The experimental unit was a 2.4 x 3.0 m plot. Weeds around the plots were controlled mechanically with a tractor mower. The experimental treatments were labeled CL0, CL1, CL2 and CL3 consisting of no amendments (0), 1, 2 and 3 consecutive 67-day cycles of combined organic amendments application, respectively. Each cycle had the following four management practices: 1) soil incorporation of 5 g/100 g OM from coffee pulp compost followed by 2) planting and incorporation of a cover crop mixture of four legume species and addition of 3) mycorrhizae and 4) liquid inoculant made from compost. At day 0 of each 67-day cycle, 5 g/100 g OM from coffee pulp compost was till-incorporated to 15 cm and the mixture of legumes seeds were broadcasted over the experimental plots. Compost application was based on organic matter percent basis eliminating uncertainties related to moisture and impurities of the compost. At day 21 the plots were drench inoculated with 2.5 g inoculant/m^2 of aqueous suspension of commercially available mycorrhizae (MycoApply® Soluble Endo), which contained four endomycorrhizal species. At day 60, the different legume species were cut at the soil line, chopped, weighed and till-incorporated into the top 15 cm soil depth and compost liquid inoculant was applied to ease degradation of green manure residues. After legumes incorporation seven days were allowed prior to the start of the next cycle to permit partial decomposition of residues. The 67-day cycles corresponding to the CL3, CL2 and CL1 treatments were established in three 67-day phases, such that all treatments were finalized at the same time (Fig. 2-1). During phase I, the first 67-day cycle was implemented in CL3 plots while CL2, CL1 and CL0 where kept weed controlled with mechanical methods. During the phase II, 67-day cycles were repeated in CL3 and started in CL2, whereas CL1 and CL0 were kept weed controlled. During phase III additional cycles were repeated in CL3, CL2 and started in CL1 and weeds were controlled in CL0. After the completion of all treatments, eggplants were transplanted, grown and yield quantified in each of the experimental plots. The compost incorporated at each 67-day cycle was obtained from coffee pulp processed with an aerated static pile method. The chemical properties of the compost are shown in Table 2-1. The cover crop mixture consisted of four legume species, *Crotalaria juncea* (sunn hemp), *Canavalia ensiformis* (jack bean), *Vigna unguiculata* (cowpea) and *Mucuna pruriens* (velvet bean). The legume seeds were planted at rates of 42, 14, 13 and 13 kg/ha for *Canavalia ensiformis*, *Crotalaria juncea*, *Mucuna pruriens* and *Vigna unguiculata,* respectively. During the 67-day cycle period the sunn hemp, cowpea and velvet bean bloomed, but none matured their seeds. The jack bean never bloomed during the 67-day period. The compost liquid inoculant was prepared by mixing 11.5 kg of mature wood chip compost obtained from a local distributor and 120 mL of unsulfured molasses in 80 L of water, and was aerated continuously with an aquarium pump for two days.

Figure 2-1 Diagram of treatment establishment. The large squares represent application of compost, legumes, mycorrhizae and compost liquid inoculant under each 67-day cycle. After legumes incorporation and compost liquid inoculant addition, seven days were left to allow decomposition of legumes residues completing an entire 67-day cycle. The implementation of the treatments was done in three phases finishing all treatments at the same time for the establishment of an eggplant crop.



2.2.2. Soil Sampling and Analysis

Soils samples were collected at the end of the treatment establishment (day 201). A single sample per experimental unit was obtained by mixing four subsamples collected at a 0-15 cm depth. Soil samples where air-dried, passed through a 6 mm sieve and stored until analysis. Samples for inorganic N where stored at -20 °C until analyzed.

2.2.3. Organic matter and humic acids

Organic matter was determined by the Walkley and Black method described in Nelson and Sommers (1982). A factor of 1.725 was used for conversion of percent organic carbon to percent organic matter. Humic acids (HA) were extracted on the basis of their solubility in acids. Six grams of soil were first treated with 0.1 M HCl to separate the fulvic acid. Then the soil was further extracted with 0.1 M NaOH to obtain the humic acids. Humic acids were precipitated with HCl 6 N solution until a pH of 1 was reached. The precipitated HA were separated by centrifugation and redissolved in a 0.1 N KOH solution. Dissolved humic acids were again reprecipitated with 6 N HCl, centrifuged and redissolved with a solution of 0.1 N HCl + 0.3 N HF to remove silicates. The humic acids were purified with a cation exchange resin (Dowex 50WX8-100), then lyophilized and weighted in an analytical balance.

2.2.4. Humic Acids Characterization

E_4/E_6 ratio - The ratio between the absorbance at 465 and 665 nm were determined by dissolving 2 mg of HA in 25 mL of 0.025 M $NaHCO_3$ (Lguirati et al., 2005). The absorbance at 465 nm and 665 nm were obtained with a Beckman DU 520 UV/Vis spectrophotometer. This ratio is used as a parameter to estimate humic acids humification degree.

Fourier Transformed Infrared - Humic acids infrared analysis was done on a KBr pellet using a Fourier Transformed Infrared Spectrophotometer (FTIR) Perkin Elmer Paragon 1000 from 4000 to 400 cm^{-1} (Lguirati et al., 2005). The pellet was prepared from 100 mg KBr and 1.5 mg humic acids.

Elemental Analysis - For elemental analysis one composite sample of humics acids was used per treatment. Carbon, Hydrogen and Nitrogen elemental analysis was performed on 0.4-1 mg of HA using a 2400 Perkin-Elmer CHN Analyzer. Oxygen was determined by pyrolysis with an oxygen accessory kit fitted to the Perkin-Elmer 2400 Elemental Analyzer that converts oxygen to carbon monoxide. Carbon monoxide was measured as a function of thermal conductivity.

Soil pH was determined by using a glass electrode immersed in the supernatant of 1:2 soil:water mixture after 2 hour shaking period. Phosphorous was determined using the Olsen extraction method (Olsen et al., 1954). Exchangeable bases were determined by atomic absorption spectrometry after a soil extraction with NH_4OAc solution with pH 7.0 (Bower et al., 1952). Nitrate and ammonium were determined by colorimetry with a QuickChem 8500 autoanalyzer after an extraction with 2 N KCl.

2.2.5. Eggplant Assay

After treatment establishment eggplant seedlings of the traditional Rosita variety were transplanted on each plot to evaluate yield and biomass production. A total of four rows of eggplant were planted on each plot leaving 80 cm between rows and 60 cm between plants. The plots were drip irrigated twice a week with well water. Well water analysis is shown in Table 2-1. Weed management within the plots was made by hand. Pest, principally white flies, where controlled by weekly applications of a sesame seed oil-based commercial insecticide. Eggplants were harvested weekly for seven weeks beginning 10 weeks from transplant. Data were collected from the two central plants rows (10 plants). Eggplants were harvested and weighted when the color of fruits started to become opaque. After the last fruit picking, all the plant from the two central rows were cut at ground level and dried at 70 °C to determine dry weight.

Table 2-1 Characteristics of coffee pulp compost and well water used.

Coffee pulp compost	
pH	7.13
OM (g/100 g)	60.0
Total N (mg/kg)	17,587.2
Total P (mg/kg)	2,267.4
Total K (mg/kg)	6,700.6
Total Ca (mg/kg)	9,055.2
Total Mg (mg/kg)	1438.2
Total Na (mg/kg)	450.6
Available nutrients	
NH_3-N (mg/kg)	352.6
NO_3-N (mg/kg)	738.8
P	200.6
K	684.6
Ca (mg/kg)	5718.0
Mg (mg/kg)	702.0
Na (mg/kg)	159.9
Well Water	
pH	7.2
NO_3 (ppm)[*]	44
K (ppm)[**]	3
Ca (ppm)[**]	148
Mg (ppm)[**]	13

[*] Well water nitrate was analysed by colorimetry after reducing all the NO_3 to NO_2.

[**] K, Ca and Mg were determined with an inductively couple plasma (ICP) following EPA-200.7 method.

2.2.6. Statistical Analysis

Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) at a significance level of 0.05 throughout the study were used for comparison of means using Infostat Statistical Software (Di-Riezo et al., 2011). Correlation analysis was performed using Pearson Coefficient Analysis on Infostat.

2.3. Results and Discussion

2.3.1. N and Organic Matter

The 67-day cycles of combined practices resulted in higher concentration of soil organic matter and inorganic N species (Table 2-2). Soil organic matter is considered a major parameter of soil health. Soil organic matter is directly related to physical and biological properties (Pérez-Piqueres et al., 2006) and responsible for the release of plant-available nutrients. The treatments CL1, CL2, and CL3 increased soil organic matter content from 1.01 g/100 g in CL0 to 2.50, 4.11 and 4.97 g/100 g, respectively (Table 2-2). The amount of compost applied every 67-day cycle was equivalent to 5 g/100 g of organic matter (OM) in the top 15 cm layer of soil. This amounted to approximately 5, 10 and 15 g/100 g of soil organic matter added initially as compost to the CL1, CL2 and CL3 treatments, respectively. Additionally, the biomass production of the intercropped green manures incorporated at each 67-day cycle ranged from 5560-6402 kg/ha. Comparing these expected values with the final organic matter values in Table 2-2 indicates that between 50 and 67 percent of the added organic matter mineralized during the experimental period. This is consistent with other results (Rivero et al., 2004; Bernal et al., 1998), which show that even though composting produces a high proportion of stable OM, a considerable portion of this OM becomes labile during the first two months after incorporation into the soil. In the present study the humic acids fraction of soil OM in amended soils ranged from 23.6 to 30.1%. This represents a considerable portion of soil organic matter contributing to its stabilization because of the strong resistance to decomposition of humic acids attributed to their complex chemical structure (Stevenson 1994).

Treatments CL2 and CL3 had the highest values of NO_3-N and NH_4-N at the end of the treatments establishment. Treatment CL1 was not significantly higher than CL0 in both inorganic nitrogen species. The reported increase in inorganic soil nitrogen could be explained by the high nitrate content of the compost (Table 2-1). In addition to the compost, a considerable amount of fresh residues rich in N were incorporated at each 67-day cycle and contributed to the increase of soil organic matter and the release of inorganic N. The total increase of inorganic nitrogen (NO_3-N plus NH_4-N) was 28.2, 185.9 and 314.4 kg N/ha for CL1, CL2 and CL3, respectively, when compared to CL0. The high nitrate content of CL3 (Table 2-2) could have an adverse environmental impact by ground water pollution. It's worth noting that the 67-day cycles were repeated consecutively without intermediate food crops that would extract a considerable portion of the nutrients, which explains the buildup of inorganic nitrogen species.

Table 2-2 Available soil nutrients, pH, organic matter and humic acids content as affected by the implementation of 67-day cycles (CL0, CL1, CL2, CL3) of combined practices.

	pH	OM (g/100 g)	Humic Acids (g/kg)	NO_3-N (mg/kg)	NH_4-N (mg/kg)	P (mg/kg)	SO_4 (mg/kg)	K (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)
CL0	7.9a	1.0d	2.5d	20.3c	6.6c	23.8d	6.0c	0.73d	21.21bc	3.67c	0.60b
CL1	7.6b	2.5c	7.1c	28.6c	10.1bc	38.0c	29.3b	1.95c	20.68c	4.60b	0.69a
CL2	7.2c	4.1b	9.7b	89.8b	15.0ab	45.3b	45.3a	3.04b	22.17b	4.84a	0.73a
CL3	7.0d	5.0a	15.0a	141.2a	17.5a	54.5a	50.0a	3.74a	23.76a	5.18a	0.73a

Means within column followed by the same letter are not significantly different at $p \le 0.05$ using Fisher's least significant difference (LSD)

2.3.2. Humic Acids Characterization

Treatments CL1, CL2 and CL3 increased the humic acids content 2.8, 3.8 and 5.9 times respectively, when compared to CL0 (Table 2-2). This effect is attributed to the compost, which based on its maturity and amount incorporated at each 67-day cycle, had the strongest effect in humic acids quality and quantity. The 67-day cycles produced an increase in H, N, O and O/C ratio in the humic acids of amended soils. Soil humic acids showed lower C, C/H and C/N ratios with increasing 67-day cycles (Table 2-3) and this has been attributed to the incorporation of proteinaceous matter and lower levels of humification when compared with un-amended soil humic acids (Lguirati et al., 2005). There is a trend between increasing E_4/E_6 ratio and increasing cycles of organic amendments (Table 2-3). Since E_4/E_6 ratio is related to humic acids molecular weight and degree of humification (Lguirati et al., 2005), the increasing E_4/E_6 ratio with increasing cycles (Table 2-3) suggest lower levels of humification and condensation in compost humic acids compared to that of native soil humic acids. Despite this, according to the C/H ratio (Table 2-3), humic acids of amended soil show considerable levels of humification. Carbon:hydrogen ratios above 0.90 have been associated with high levels of condensation, polymerization and aromaticity (García et al., 1989; García-Gill et al., 2004; Lguirati et al., 2005). According to Kakezawa et al. (1992), the higher C/H and lower O/C ratio in humic acids from non-amended soils could be attributed to greater degradation of lignin fractions due to higher residence time of soil native humic acids.

Table 2-3 Elemental composition, atomic ratios and E_4/E_6 ratio of soil humic acids after addition of 67-day cycles (CL0, CL1, CL2, CL3) of combined practices.

	C (%)	H (%)	N (%)	O (%)	C/H	O/C	C/N	E4/E6
CL0	53.20	3.42	3.38	34.69	1.31	0.49	18.37	4.47
CL1	50.93	4.41	4.57	34.26	0.97	0.50	13.00	4.98
CL2	50.05	4.69	4.9	35.15	0.90	0.53	11.92	5.42
CL3	49.33	4.57	5.06	35.85	0.91	0.55	11.37	5.87

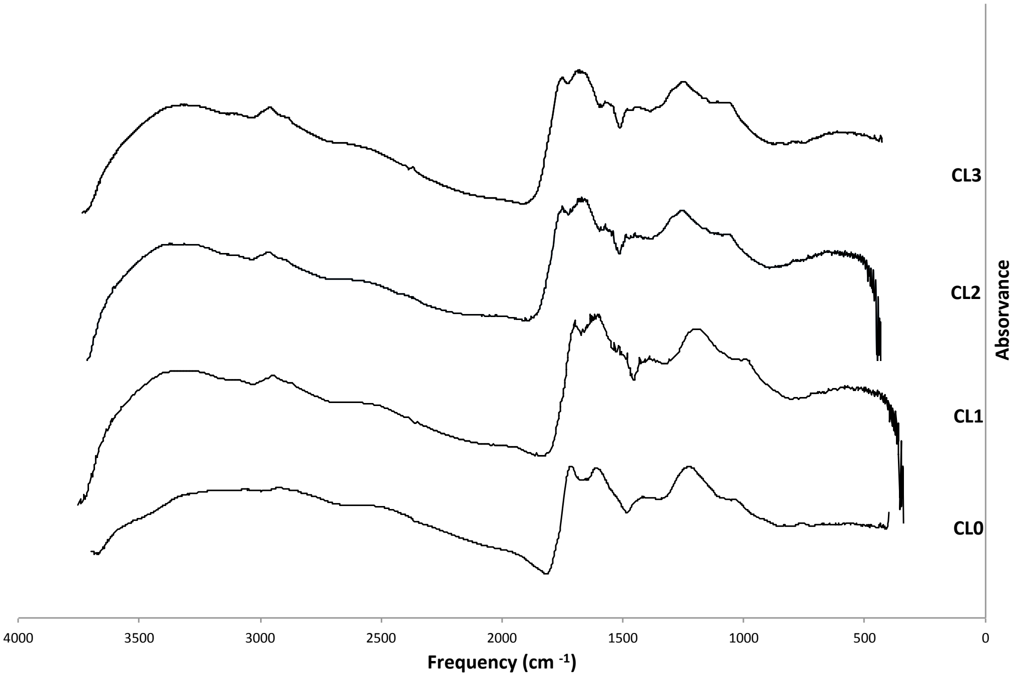
There are contrasting features between FTIR spectra of amended and un-amended soil humic acids to further explain the effect of 67-day cycles. The identified bands on FTIR spectra of humic acids were (Table 2-4): a broad band at about 3400 cm^{-1} commonly attributed to OH stretch, a peak at 2925 cm^{-1} (aliphatic C-H stretching), a band at 1740 cm^{-1} (C=O stretching of COOH and ketones), a band at 1640 cm^{-1} (aromatic C=C), a band in the 1590-1517 cm^{-1} region (C=N stretching), a peak at 1460 cm^{-1} (aliphatic C-H), a band at 1280-1200 cm^{-1} range (C-O Stretching of aryl ethers, C-O and OH of COOH) and a peak in the 1080-1030 range (C-O of polysaccharide-like substances). The spectra obtained (Figure 2-2) from humic acids of amended soils (CL1, CL2 and CL3) show a band at 2925 cm^{-1} that it is weaker in CL0 samples. This is a characteristic of the C-H aliphatic absorption band and is evidence of the more aliphatic character of humic acids from amended soils. In addition, the amended soil humic acids spectra showed an increase in absorbance in the 1080-1030 cm^{-1} range that is attributed to the presence of carbohydrate-like substances. The lower intensity of 2925 cm^{-1} band in CL0 samples could be attributed to long-term aliphatic structures biodegradation of native soil humic acids (Ait Baddi et al., 2004). Treatments CL1, CL2 and CL3 presented a small peak at 1460 cm^{-1} corresponding to C-H of methyl groups that is absent in CL0 spectra. The presence of less methyl groups in un-amended soil humic acids could be attributed to degradation of methyl groups in lignin. Gigliotti et al. (1999) reported similar results in which an annual application of urban waste composts produced a modification in soil humic acids with an increase of aliphatic groups and polysaccharide content. Un-amended samples exhibit a more prominent and sharpest band in the 1280-1200 cm^{-1} range that has been commonly assigned to C-O stretch and OH deformation of aryl ethers and carboxyl groups and it is evidence of more aromaticity and acid functional groups of CL0 HA. Amended and un-amended soil samples presented a distinguishable band at 1640 cm^{-1} corresponding to aromatic C=C, evidencing the presence of aromatic characteristics in all samples. Treatments CL1, CL2 and CL3 samples exhibited a less prominent peak at 1740 cm^{-1} when compared to CL0 which is associated to C=O stretch of carboxyl groups. Again, this demonstrates a higher presence of acidic groups in un-amended soil samples. A distinguishable feature of CL1, CL2 and CL3 samples is a band at the 1590-1517 cm^{-1} region, corresponding to amide structures that becomes more evident with increasing 67-day cycles and it is absent in CL0 samples. This data corroborate the observed increase in elemental content of N in treatment samples (Table 2-3) and agrees with the high nitrogen content of compost and leguminous green manures incorporated in CL1, CL2, and CL3 treatments.

Ouatmane et al. (2000) reported similar results with humic acids extracted from two different composts prepared from materials rich in N compounds. These results concur with García et al. (2004) and Lguirati et al. (2005) in which humic acids extracted from composts exhibited higher N content than un-amended soil humic acids. The organic treatments significantly increased soil humic acids content with a direct impact over long-term soil fertility as previously reported (García-Gil et al., 2004). These authors showed the impact of compost on soil humic acids in terms of molecular size and elemental compositions 9 years after the amendment application.

Table 2-4 Assignment of infrared bands of soil humic acids according to Stevenson (1994).

Wavelength (cm^{-1})	Assignment
3400-3300	O-H stretching, N-H stretching
2940-2900	Aliphatic C-H stretching
1725-1720	C=O stretching of COOH and ketone
1620-1600	C=O stretching in quinones and/or ketonic acids and amides.
1590-1517	COO^- symmetric stretching, N-H deformation and C=N stretching
1460-1450	Aliphatic C-H
1280-1200	C-O Stretching and OH deformation of COOH, C-O stretching or aryl groups
1080-1030	C-O of polysaccharides or polysaccharide-like substance

Figure 2-2 Infrared spectra of soil humic acids from CL0, CL1, CL2 and CL3 treatments



2.3.3. Effect on Soil pH

All amended treatments resulted in a reduction in soil pH as the 67-day cycles increased (CL0>CL1>CL2>CL3). The 67-day cycles were able to decrease soil pH from 7.9 in CL0 to 7.0 in CL3 treatment (Table 2-2). At a pH of 7.9 in the untreated soils it is expected to encounter reduced micronutrients availability (Havlin et al., 2005; Sotomayor and Matínez, 2006) representing a constraint to crop growth and productivity. This phenomenon has been reported in tropical root crops in the semiarid southern coast of Puerto Rico (Sotomayor et al., 2003). The pH buffering capacity of organic amendments effectively adjusting soil pH toward neutrality has been reported after compost (Aggelides and Londra, 2000), animal manure and cover crops incorporation (Clark et al., 1998). The present work demonstrates the pH buffering capacity of organic amendments from alkaline conditions toward neutrality. Three consecutive 67-day cycles (CL3) were able to decrease pH almost an entire unit. The decrease in soil pH as result of the organic amendments is attributed to the action of acid functional groups present humic acids (Jouraiphy et al., 2005). García et al. (2004) reported short and long-term improvement of buffering capacity of humic acids extracted from soils amended with municipal solid waste compost. The buffering effect of humic acids after compost additions have been recorded as far as nine years after addition of organic amendments (García et al., 2004). These results demonstrate a direct effect of humic acids over soil pH buffering capacity enhancing soil fertility status and protecting soil against degradation. The humic acids buffering capacity arises from the acidic functional groups like carboxyl and phenol groups in the humic acids (García et al., 2004). The significant increase in soil humic acids reported in the present study (Table 2-2) supports the pH buffering role of humic acids.

2.3.4. Phosphorus and Sulfate

Treatments CL1, CL2, and CL3 were able to increase Olsen extractable P by 60%, 91% and 129%, respectively, when compared with the CL0. Although this soil series is considered of high fertility and this region is categorized by the United States Natural Resources Conservation Service (NRCS) as a prime farmland, the P fertility levels of CL0 were in the medium phosphorus concentration category of soil fertility (Muñiz-Torres 1992). This suggests that crops grown in these soils at natural conditions will show a response to P fertilization. One 67-day cycle (CL1) increased soil P availability to 38 mg/kg, which is above the 30 mg/kg target level for required conventional fertilization.

However, other authors propose higher Olsen P concentration levels (>40 mg/kg) when growing vegetables (Kelling et al., 1998). According to our findings, two cycles (CL2) were needed to reach this level. These results are in accordance with other studies that have reported an enhancement in soil P availability after additions of mature composts from different sources (Wong et al., 1999; Soumaré et al., 2003; Weber et al., 2007; Courtney and Mullen, 2008; Bustamante et al., 2011). Numerous studies have concluded that soil available P is directly related to amount of organic residues applied to the soil (Wong et al., 1999; Warman et al., 2009; Courtney and Mullen, 2008). In addition to the immediately available P, 53-86% of total P has been reported acting as a low release source after compost incorporation to the soil (Frossard et al., 2002). A portion of the observed increase in P concentration could be explained by means of the reduction of soil alkalinity achieved by the pH buffering activity of the practices done at 67-day cycle. Plant available P shows maximum solubility in the 5.5- 7.0 pH range (Lakhdar et al., 2009). It is expected that at pH 7.9 of CL0, P precipitate with Ca reducing its availability (Hopkins and Ellsworth, 2005). The implementation of 67-day cycles significantly reduced pH, with treatment CL3 achieving pH 7. Despite the significant increase in P as result of organic amendment addition, the levels of soil P were kept within the moderate category (31-70 mg/kg) of pollution potential for the Caribbean area developed by Martínez et al. (2002). Sulfate concentrations increased with 67-day cycles (Table 2-2). At the end of treatment establishment CL1, CL2 and CL3 significantly increase SO_4^{-2} content when compared to CL0. Treatments CL3 and CL2 presented the highest values.

2.3.5. Effect on Soil Cations

At the end of treatments establishment CL1, CL2 and CL3 increased exchangeable potassium 2.7, 4.2 and 5.2 times, respectively, when compared with CL0 (Table 2-2). Treatments CL1 and CL2 did not increase Ca content over CL0. The application of three consecutive cycles significantly increased Ca content when compared with non-amended soils. Treatment CL2 and CL3, had the highest Mg concentration with more than a 30% increase when compared with CL0. Similar results have been reported by numerous authors were the application of different types of compost increase K, Ca and Mg concentration in the soil (Wong et al., 1999; Soumaré et al., 2003; Weber et al., 2007; Courtney and Mullen, 2008; Bustamante et al., 2011). The increase in nutrient content was expected since the coffee pulp compost applied is considered a source of nutrients, characterized by high levels of potassium (Chong and Dumas, 2012). A portion of the observed increase in the concentration of available cations could be attributed to the implementation of green manures as previously reported. The increase in soil nutrients in addition to N has been achieved after the implementation of fallows consisting of tropical legumes (Koutica et al., 2001).

The use of organic amendments increases the effective cation exchange capacity (ECEC) and also could change the cation distribution that could be harmful to crops especially regarding Na concentration. The application of specific agricultural residues has been responsible for the deterioration of chemical, physical and biological properties principally due to the high Na loads of some organic materials (Tejada and Gonzalez 2005; Tejada and Gonzalez, 2006; Tejada et al., 2009a). The use of compost not only changed the concentration of nutrients, but also affected the distribution of cations within the ECEC. As expected, ECEC increased with increasing cycles (Table 2-5). There was a significant increase in ECEC in CL1, CL2 and CL3. There was a larger shift in the saturation values of Ca being reduced and increasing K, and a minor or no significant changes in Mg and Na saturation, respectively (Table 2-5). Although the addition of 67-day cycles increased Na concentration (Table 2-2), the saturation of Na did not significantly increase and did not present a fertility constraint for plant development. The addition of 67-day cycles reduced the saturation of Ca from 81.0% in CL0 to 71.1% in CL3. Treatments CL1, CL2 and CL3 were able to increase K saturation in 2.5, 3.6 and 4.1 times respectively when compared with the control. These results concur with that of Walker and Bernal (2008) in which the incorporation of different types of organic amendments favoured the saturation of Ca, Mg and K over Na.

Table 2-5 Effective cation exchange capacity and base saturation as affected by the implementation of 67-day cycles (CL0, CL1,CL2, CL3) of combined practices.

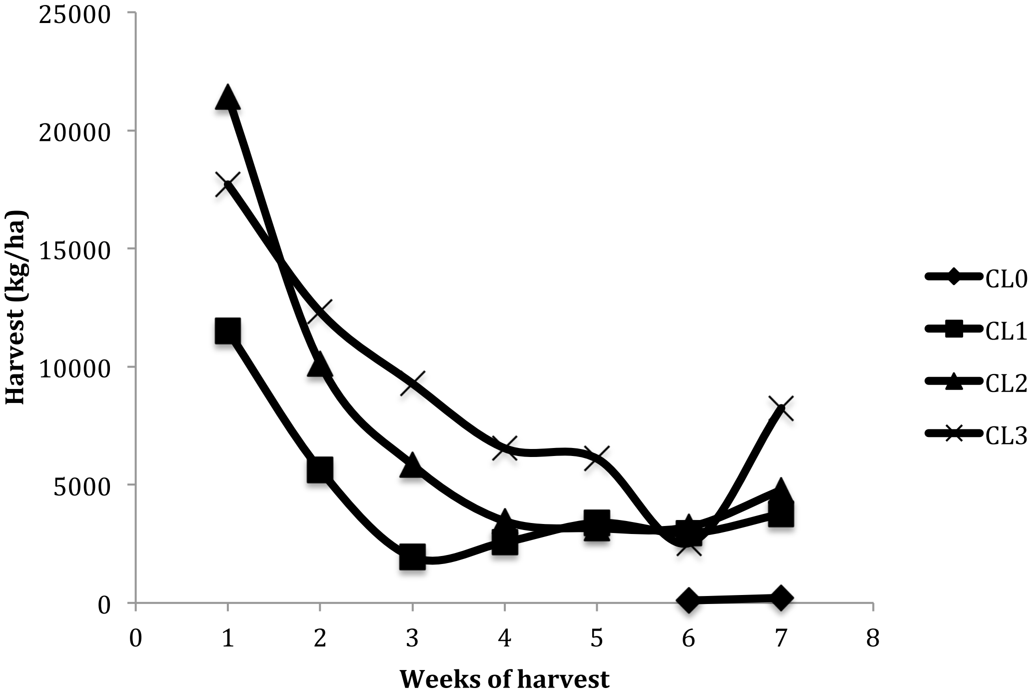
	ECEC (cmol/kg)	Ca saturation (%)	Mg saturation (%)	K saturation (%)	Na saturation (%)
CL0	26.2d	81.0a	14.0b	2.8c	2.3a
CL1	27.9c	74.1b	16.5a	7.0b	2.5a
CL2	30.8b	72.0abc	15.7ab	9.9a	2.4a
CL3	33.4a	71.1c	15.5ab	11.2a	2.2a

Means within column followed by the same letter are not significantly different at $p \leq 0.05$ using Fisher's least significant difference (LSD).

2.3.6. Effect on Crop Yield and Biomass

Un-amended plots did not produce fruits until the last two weeks of picking (Figure 2-3). The implementation of one 67-day cycle (CL1) significantly increased eggplant production (Table 2-6). Treatments CL2 and CL3 produced the highest total eggplant yield and represented a 64 and 97% yield increase over CL1, respectively. These results concur with those of Altieri and Esposito (2010) suggesting the potential of organic amendments in promoting crop productivity comparable to expected yields under conventional production system. The increase in crop yield could be attributed to the overall enhancement in soil status induced by all practices done within each 67-day cycle. The positive effect of the individual application of compost, legume fallows and compost liquid inoculant on crop yield has been previously reported. In this study the combination of practices within each 67-day cycle resulted in a positive response to eggplant productivity. The increase in yield was attributed to the enhanced nutrient availability of essential nutrients previously discussed. These results concur with that of Warman et al. (2009) that reported different rates of municipal waste compost to provide equal or more nutrients levels than a NPK fertilizer applications designed to meet crop demand. Although the nutrient concentration of well water was considerably high, particularly in nitrate, this did not represent a positive response of plants as demonstrated by CL0 yield. Our results are in accordance with other authors who have reported and increase in crop yield due to a nutrient increase induced by organic amendments (Ouédraogo et al., 2001; Soumaré et al., 2003; Courtney and Mullen, 2008). The positive interaction of growth promoters agents present in highly stabilized organic matter with crop productivity whose identity is still unclear but have been related to humic substances has also been suggested (Keeling et al., 2003). Although San Anton soil is recognized to support high crop yields there are limiting conditions in un-amended soils (CL0) that are constraining crop productivity. In addition to the chemical parameters assessed in the present study, the eggplant yield increase should be also attributed to the enhancement of soil physical and biological properties reported in Pagán-Roig et al. (2013).

Figure 2-3 Effect of the implementation of 67-day cycles (CL0, CL1, CL2, CL3) of combined practices on eggplant weekly harvest.



Cycles of combined sustainable practices not only had a positive effect in fruit yield but also in plant biomass. All treatments significantly increased plant biomass compared to CL0 (Table 2-6). Treatments CL2 and CL3 were not significantly different but both reported the highest production in plant biomass. The enhancement of dry matter production due to the application of organic amendments has been reported on crop plants (Wong, 1999; Guerrero et al., 2001; Ouédraogo et al., 2001) and on spontaneous vegetation (Guerrero et al., 2001; Tejada et al., 2009b). The latter have relevant implications in the reclamation of highly degraded land since the promotion of vegetation cover in highly degraded areas is key to conserve soil against erosion and preserve soil from further degradation (Guerrero et al., 2001; Tejada et al., 2009b).

Table 2-6 Effect the implementation of 67-day cycles (CL0, CL1, CL2, CL3) of combined practices on eggplant yield, fruits per plant and dry weight.

	Total eggplant yield (Kg/ha)	Fruits per plant	Dry weight (g/plant)
CL0	311.1c	0.1c	14.7c
CL1	31765.2b	3.8b	124.9b
CL2	52031.3a	6.0a	238.3a
CL3	62761.3a	7.4a	273.6a

Means within column followed by the same letter are not significantly different at $p \leq 0.05$ using Fisher's least significant difference (LSD).

2.4. Conclusion

Our results demonstrate the positive effects of the combination of sustainable agricultural practices in soil properties. The consecutive application of 67-day cycles and the high rates of compost additions were done trying to concentrate in a short time scale what could be achieved with a long-term regime of organic amendments. This was reflected by the reported changes of humic acids characteristics. Organic matter content and quality was increased in amended soils having a positive impact in the long-term soil fertility status expressed in terms of soil humic acids. Humic acids were significantly increased in all amended plots. Soil HA extracted from un-amended plots showed a higher level of condensation and aromaticity compared to that of organic samples, nevertheless the latter showed some characteristics of relatively high degree of humification and aromaticity. When considering soil nutrients, the consecutive application of 67-day cycles produced a general buildup. In this case, is worth noting that the cycles were applied consecutively without intermediate crops that would extract nutrients and maintain a more balanced soil nutrients status. The pH was positively affected with a shift toward neutrality, where most nutrient availability is expected in alkalinic soils. These changes in soil fertility carried out by the implementation of 67-day cycles were reflected in an increase of eggplant biomass production and yields. There is a need to study real agroecosystems to better understand the on-farm dynamics of well-established sustainable production systems.

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