Evaluation of potential living cover crops under a banana canopy and their short-term effects on soil microbial activity

by

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Abstract

The use of cover crops has been widely promoted as a strategy to enhance soil quality and health. The main objective of this research was to evaluate three common plant species in Puerto Rico (*Heterotis rotundifolia*, *Spagneticola trilobata*, *Tradescantia zebrina* and *Geophila repens* as reference plant) as potential cover crops for banana (*Musa acuminata* AAA), and their short-term effects on soil microbial activity. The study was conducted following a randomized complete block design (with four replicates) at the Agricultural Experimental Station of Gurabo. Plant growth analysis was conducted with measures of aboveground biomass (AGB), leaf area index (LAI), relative growth rate (RGR), net assimilation rate (NAR) and crop growth rate (CGR). Soil coverage by plant species, weed biomass and labor time were measured among treatments. In addition, soil organic carbon (SOC), Dehydrogenase (DHA), β-Glucosidase enzyme activity, phospholipid fatty acids (PLFA) among cover crops to determine size and composition of the microbial community and functional groups of soil organic matter with mid-infrared spectroscopy were evaluated. Results showed that AGB of cover crops species after 229 days after planting (DAP) were significantly different from each other, with a dry weight of 367 g m^{-2} for *S. trilobata*, 244 g m^{-2} for *H. rotundifolia*, and 149 g m^{-2} for *G. repens*. After 229 DAP, *S. trilobata* had a significantly higher LAI and CGR. The LAI and AGB were correlated, suggesting that plant species with higher LAI generated more AGB. After 257 DAP, *S. trilobata* and *H. rotundifolia* had the highest soil coverage, with 91 % and 84 % respectively, and control plots without a plant species had the highest average weed biomass with 65 g m^{-2}. Labor time was reduced significantly in plots with cover crops species. After 289 DAP, results showed a significant difference among treatment, where *G. repens* and control plots reflected higher SOC. Overall enzyme activity (DHA and β-Glucosidase) increased significantly among sampling dates. The plant species with the greatest concentrations of microbial biomass were *S. trilobata* and *G. repens*, reflecting the highest trophic levels with the presence of predators (protozoan groups). *H. rotundifolia* showed the highest value and variance for the stress and community ratio. Soil organic functional groups did not reflect a significant difference among treatments. A non-metric multidimensional scaling analysis showed a positive association of PLFA with aliphatic type-C bonds. This study concluded that *S. trilobata* and *H. rotundifolia* were the two species with the highest potential as living cover crops for banana fields, according to their functional growth traits, soil cover and competition with weeds. In addition, living cover crops enhance soil quality, increasing SOC and enzyme activity after 254 DAP. Results of microbial community size and composition indicated *S. trilobata* and *G. repens* as suitable cover crops to enhance soil health.

Resumen

El uso de plantas de cobertura se ha promovido ampliamente como una estrategia para mejorar la calidad y salud del suelo. El objetivo principal de esta investigación fue evaluar el potencial de tres plantas comunes en Puerto Rico (*Heterotis rotundifolia*, *Spagneticola trilobata* y *Tradescantia zebrina* con *Geophila repens* de planta referencia) como plantas de cobertura para cultivos de guineo (*Musa acuminata*, AAA), y sus efectos a corto plazo en la actividad microbiana. La investigación se hizo con un diseño experimental de bloques completos aleatorizados (con cuatro replicas) en la Estación Experimental Agrícola de Gurabo. El análisis de crecimiento de las plantas se hizo con medidas de biomasa (AGB), índice de área foliar (IAF), tasa de crecimiento relativo (TCR), tasa de asimilación neta (NAR), y tasa de crecimiento del cultivo (TCC). La cobertura del suelo por las plantas de cobertura, la biomasa de plantas arvenses y el tiempo de labor fueron medidos entre tratamientos. Además, se evalúo el carbón orgánico del suelo (COS), actividad enzimática (Deshidrogenasa [DHA] y *β*-Glucosidasa), ácidos fosfolípidos grasos (PLFA) para determinar el tamaño y composición de la comunidad microbiana y grupos funcionales de la materia orgánica con espectroscopia de infrarrojo. Los resultados indicaron que la biomasa de las plantas de cobertura después de 229 días de su siembra (DDS) fueron significativamente diferentes entre sí, con un peso seco de 366.6 ± 42.38 g m^{-2} para *S. trilobata*, 244 g m^{-2} para *H. rotundifolia*, y 149 g m^{-2} para *G. repens*. Luego de 229 DDS, *S. trilobata* tuvo un IAF y TCR significativamente mayor que las otras especies. El IAF y AGB resultaron con una correlación fuerte, sugiriendo que las especies de planta con mayor IAF, generaron mayor AGB. Luego de 257 DDS, *S. trilobata* y *H. rotundifolia* tuvieron la mayor área de cobertura de suelo, con 91 % y 84 % respectivamente, mientras las parcelas control sin planta de cobertura tuvieron un promedio de plantas arvenses de 65 g m^{-2}. El tiempo de labor se redujo significativamente en aquellas parcelas con plantas de cobertura. Luego de 289 DDS, los resultados demostraron que hubo diferencia significativa entre los tratamientos en COS, donde *G. repens* y parcelas control reflejaron valores más altos. La actividad enzimática (DHA y *β*-Glucosidasa) incremento significativamente entre las fechas de muestreo. Las especies de plantas con concentraciones mayores de biomasa microbiana fueron *S. trilobata* y *G. repens*, reflejando un nivel trófico mayor con la presencia de depredadores (grupos protozoarios). *H. rotundifolia* mostró el mayor valor y varianza para las tasas de estrés en la comunidad microbiana. Los grupos funcionales orgánicos no reflejaron una diferencia significativa entre las plantas de cobertura. Un análisis multidimencional no-métrico reflejó una asociación positiva entre PLFA con enlaces de carbono alifáticos. Este estudio concluyó que las especies *S. trilobata* y *H. rotundifolia* fueron las dos especies con mayor potencial como plantas de cobertura viva para cultivos de guineo según el análisis de las características de crecimiento funcional, cobertura de suelo y supresión de plantas arvenses. Además, los cultivos de cobertura permanentes mejoran la calidad del suelo, incrementando la actividad enzimática y COS luego de 254 DDS. Los resultados del tamaño y composición de comunidad microbiana indican que *S. trilobata* y *G. repens* son cultivos de cobertura adecuados.

Dedication

To my aunts, who has been an inspiration through their wisdom and strength.

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List of Abbreviations

Abbreviation	Meaning
DAP	days after planting
LAI	leaf area index
CGR	crop growth rate
RGR	relative growth rate
NAR	net assimilation rate
AGB	aboveground biomass
HW	hand weeding without any tool
SOC	soil organic carbon
DHA	dehydrogenase enzyme
β-Glu	β -glucosidase enzyme
PLFA	phospholipid fatty acid
DRIFTS	diffuse reflectance Fourier transform mid-infrared spectroscopy

General Introduction

The integration of cover crops in agricultural systems has been widely promoted as a strategy for soil and water conservation (Carlo-Acosta, 2009; Fongod et al., 2010). There is substantial agreement in the literature demonstrating the relationship and the benefits among the use of cover crops, weed suppression and effects on soil chemical, physical and biological properties (Brennan and Smith, 2005; Finney et al., 2017). Cover crops have been encouraged as an alternative to reduce the use of herbicides due to the concern of its environmental pollution and as a means towards achieving sustainable agriculture (Abdin et al., 2000). In the Caribbean, specifically St. Croix, St. Thomas and Puerto Rico, the effects of cover crops in improving soil properties has been evaluated throughout years. It has been reported by National Resources Conservation Services (NRCS) (2014) that on-farm applications have been increasing in the last decades.

Banana (*Musa acuminata* AAA) is the third largest crop in Puerto Rico. Banana and plantain (*Musa acuminata x M. balbissiana* AAB) combined bring an economic contribution between $55.2 to $78.5 million in 2008 (Cortes y Villagómez, 2005; Senado de Puerto Rico, 2010). According to a census by the National Agricultural Statistics Services (NASS) a branch of U.S. Department of Agriculture (USDA) (2012), Puerto Rico has a total banana production of 6,559 acres composed by 1,828 farms. The Senate of Agriculture Commission in Puerto Rico has identified some significant production problems in the banana industry, including the effects of the fungal disease Black Sigatoka (*Mycosphaerella fijiensis*), lack of adequate equipment and field personnel, and the increasing costs in production inputs such as fertilizers and herbicides (Senado de Puerto Rico, 2010). Thus, banana farmers in Puerto Rico need to adopt alternative strategies to protect the soil while improving its fertility. A potential solution is to intercrop banana plantations with perennial cover crops.

Cover crops can reduce labor time and the use of external inputs in cash crops (Baumman et al., 2000; Isaac et al., 2007). Additionally, cover crops can provide agroecosystem services, protecting soil from degradation, while improving soil quality and biological activities such as microbial populations (Finney et al., 2017). In Puerto Rico, there is only one record published on

the use of cover crops in Musa spp. farms (Ortiz et al., 2014). Cover crops have been used in plantain farms in Puerto Rico, to improve soil biological activity and suppress weeds (Parreño, 2014; Colón, 2016). Colón used *Canavalia enfisormis* and *Crotalaria juncea* as cover crops in a small plantain farm, and after two years, fungicide and herbicide applications were reduced by 78% and 72%, respectively (Colón, 2016). However, there are no specific studies on the use of cover crops in banana for Puerto Rico.

Evaluation of cover crops to improve soil properties and to control weeds in banana plantations is needed. Currently, there is no consensus on identifying the characteristics and basic functions of the best cover crop plant species as applied to banana production in Puerto Rico. Despite the scientific literature available about the benefits of cover crops (Hartwig and Ammon, 2002; Brainard et al., 2004; Six et al., 2006; Berg et al., 2009; Djigal et al., 2012; Liu et al., 2014) evaluations of how specific cover crops species affect and influence soil microbial populations remains limited (Finney et al., 2017). The latter is especially limited for crops grown in tropical environments (Lienhard et al., 2013; de Oliveira et al., 2016).

For efficient and effective management practices, its critical to identify potential cover crops and evaluate how cover crop selection shapes microbial populations (Lehman et al., 2015). The main objective of this research is to assess three potential plants species as cover crops for banana fields in Puerto Rico and their short-term effects on the soil's biological properties. Knowledge on how to properly select cover crops will enable farmers and land managers to design efficient banana plantations.

References (General Introduction)

Abdin, O.A., X.M. Zhou, D. Cloutier, D.C. Coulman, M.A. Faris, and D.L. Smith. 2000. Cover crops and interrow tillage for weed control in short season maize (*Zea mays*). *European Journal of Agronomy*. 12:93−102 DOI:10.1016/S1161-0301(99)00049-0

Baumann, D.T., M.J. Kropff, and L. Bastiaans. 2000. Intercropping leeks to suppress weeds. *Weed Res.* 40:359−374

Berg, G. and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS. *Microbial Ecol.* 68:1−13

Brainard D.C., R.R. Bellinder, and A.J. Miller. 2004. Cultivation and interseeding for weed control in transplanted cabbage. *Weed Technol*. 18:704−710

Brennan, E.B., and R.F. Smith. 2005. Winter cover crop growth and weed suppression on the central coast of California. *Weed Technol*. 19:1017−1024

Carlo-Acosta, S. 2009. Promoting the use of tropical legumes as cover crops in Puerto Rico. Master's in sciences in Agronomy. University of Puerto Rico, Mayagüez.

Colón, D. 2016. Cover crops for improving recalcitrant soil organic matter and soil biota management in plantain production systems in Puerto Rico. FS13-271 Project Sustainable Agriculture Research and Education. [unpublished] URL https://projects.sare.org/sare_project/fs13-271/

Cortés, M. and R. Villagómez. 2005. Estimados de la elasticidad de la oferta para el cultivo de guineo en Puerto Rico. *J. Agr. U. Puerto Rico*. 89(3):181−192

De Oliveira, P., A.S., Nascente, E.P. de Brito Ferreira, J. Kluthcouski, and M. Lobo Junior. 2016. Response of soil fungi and biological processes to crop residues in no-tillage system. *Pesq. Agropec. Trop. 46* (1):57-64

Djigal, D., C. Chabrier, P. Duyck, R. Achard, P. Queneherve, and P. Tixier. 2012. Cover crops alter the soil nematode food web in banana agroecosystems. *Soil Biol. Biochem*. 48−150

Finney, D.M., and J.P. Kaye. 2017. Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. *J. Appl. Ecol*. 54(2):509−517

Fongod, A.G.N., D.A. Focho, A.M. Mih, B.A. Fonge, and P. Sama-Lang. 2010. Weed management in banana production: The use of *Nelsonia cenescens* (Lam.) Spreng as a non-leguminous cover crop. *Afr. J. Environ. Sci. Technol*. 4(3):167−173

Hartwig, N. and H. Ammon. 2002. Cover crops and living mulches. *Weed Science*. 50: 688−699

Isaac, W.A., R. Brathwaite, J. Cohen, I. Bekele. 2007. Effects of alternative weed management strategies on *Commelina diffusa* Burm. Infestations in Fairtrade banana (Musa spp.) in St. Vicent and the Grenadines. Crop Protection. 26(8)1219−1225

Lehman, R.M., V. Acosta-Martinez, J.S. Buyer, C.A. Cambardella, H.P. Collins, T.F. Ducey, J.J. Halvorson, V.L. Jin, J.M.F. Johnson, R.J. Kremer, D.K. Lundgren, J.E. Manter, J.L. Maul, and D.E. Smith. 2015. Soil biology for resilient, healthy soil. *J. Soil Water Conserv.* 70:12−18

Lienhard, P., F. Tivet, A. Chabanne, S. Dequiedt, M. Lelievre, S. Sayphoummie, B. Leudphanane, N. C. Prévost-Bouré, L. Séguy, P.A. Maron, and L. Ranjard. 2013. No-till and cover crops shift soil microbial abundance and diversity in Laos tropical grasslands. *Agronomy for Sustainable Development.* 33 (2): 275-384

Liu, Y., J. Wang, D. Liu, Z. Li, G. Zhang, Y. Tao, J. Xie, J. Pan, F. Chen. 2014. Straw mulching reduces the harmful effects of extreme hydrological and temperature conditions in citrus orchards. *Plos One* 9(1) e87094

Parreño, J.G. 2014. Sistema integrado de leguminosas y su efecto en el establecimiento de plátano (*Musa acuminata* x *balbisiana*, AAB) en dos agroecosistemas de Puerto Rico. Tesis M.S. University of Puerto Rico, Mayagüez Campus.

Ortiz, C., E. Más, J. Valentín. 2014. Validación de cultivos de cobertura para la región del Caribe. Sociedad Puertorriqueña de Ciencias Agrícolas (SOPCA). Puerto Rico Abstract.

Senado de Puerto Rico. 2010. Segundo Informe Parcial sobre la R. del S. 59. 16ta Asamblea Legislativa. Tercera Sesión Ordinaria. Gobierno de Puerto Rico.

Six, J., D. Frey, R.K. Thiet, and K.M. Battern. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70(2):555−569

USDA-NASS. 2012. Census of Agriculture. Puerto Rico Island and Municipio Data. Vol 1. Geographic Area Series Part 52

Objectives

General Objective Part I

Evaluate the potential of three common plant species in Puerto Rico: *Heterotis rotundifolia*, *Tradescabtua zebrina* and *Spagneticola trilobata* as cover crops in a field of banana (*Musa acuminata* AAA) var. Gran Nain as compared to the standard cover crop *Geophila repens.*

Specific Objectives

1 – Study the development and percent of soil cover by the plant species when used as cover crops.

2 – Determine the effectiveness of the four species interfering with other plant species naturally present in the banana field.

3 – Examine if all or any of the plant species used as cover crop could be a potential pest host.

General Objective Part II

Evaluate and characterize the short-term effects of selected plant species to improve soil biological characteristics through microbial activity.

Specific Objectives

1 – Assess the short-term effects of the plant species in the soil organic carbon content.

2 – Measure and compare microbial biomass carbon among plant species when used as cover crops.

3 – Assess the short-term effects of the four-plant species as cover crops on soil enzyme activity through β-Glucosidase and Dehydrogenase measurements.

4 – Evaluate the effects of four-plant species as cover crops on the size and composition of microbial community in soil trough phospholipid fatty acids (PLFA) analysis

5 – Characterize in a qualitative and quantitative analysis the soil organic matter functional groups among cover crops with mid-infrared spectroscopy.

Chapter 1: Literature Review

1.1. Cover crops (Living mulch)

Definition of cover crops

Cover crops are a living ground cover planted into or after cash crops (Hartwig and Ammon, 2002). The main purpose of cover crops is to protect the soil's surface from unfavorable factors while also enhancing its physical, chemical and biological properties (Lu et al., 2000). Cover crops also improve growing conditions for the main crop by reducing weeds and problems related to insect pests (Lu et al., 2000; Brainard et al., 2004; Kolota and Adamezewska-Sowinska, 2013). Traditionally, cover crops are destroyed and incorporated into the soil before the next crop is planted (Wang et al., 2008). However, according to the interest and the main crop, the use and practices of cover crops can vary (Wallace and Bellinder, 1992; Borowy, 2012; Kolota and Adamezewska-Sowinska, 2013) In the United States (US), the main plant families utilized as cover crops are: Fabaceae (e.g. beans and peas), Brassicacea (e.g. mustards), and Poceae (e.g. grasses) (Hartwig and Ammon, 2002).

Classification of cover crops

Ground cover crops could be classified according to their use and time of growth as fallow, short-term and intercropping crops. Farmers use fallow cover crops during the off-season while rotating crops to cut pest cycles and supply nutrient demands (Snapp et al., 2005; Kolota and Adamczewska-Sowinska, 2013). Biomass of fallow crops is incorporated into the soil at the end of their cycle (Kolota and Adamczewska-Sowinska, 2013). Short-term rotation of cover crops is limited to a season, mainly winter or summer (SAN, 1998; Creamer et al., 2000; Belfry et al. 2017). Intercropped or living mulch cover crops are species that can grow together with the main crop. When living mulch is a perennial plant or self-seeding annuals, reseeding is not necessary (Hartwig and Ammon, 2002; Kolota and Adamczewska-Sowinska, 2013). NRCS makes a distinction

between permanent and temporary cover crops, where permanent cover crops are known as conservation covers (code 327) and temporary cover crops are known as cover crops (code 340).

Selection of cover crops and effects on yields

Commonly, the most important feature a cover crop should have is tolerance to drought conditions (Petter et al., 2013; Liu et al., 2014; Mwangi et al., 2015; Bashe et al., 2016). However, the main characteristics required for plants to be used as cover crops are short germination periods, quick soil covering, short height, low management, and low water and nutrient demands (Kolota and Adamczewska-Sowinska, 2013). To reduce costs related to crop production, conservation tillage and cover crops are efficient management strategies while improving soil properties (Price and Norsworthy, 2013). Though, the selection of cover crop species should be addressed to meet a specific purpose and taking into consideration how using cover crops will fit in the crop rotation. Liu et al., (2014) showed that total yield could be increased by covering and mulching the soil to control thermal and moisture conditions in the soil. Thus, cover crops can be used to increase profits, reduce cost production and maintain desirable soil properties.

Limitations with cover crops

Besides the substantial literature showing positive results in total yields and soil properties using cover crops, certain studies have demonstrated decrease in crop yields (Kolota and Adamczewska-Sowinska, 2013). This may be attributed, however, to inappropriate selection of a cover crop (Khatri et al., 2014). A proper matching of cover crop will depend on the main crop, local climate, soil conditions and the agricultural techniques implemented (Hartwig and Ammon, 2002). Competition for water between cover crops and cash crops is one of the major concerns for cover cropping success (Alonso-Ayuso et al., 2014). If cover crops compete with cash crops for resources, they could reduce the yield (Nielsen et al., 2016).

Cover crops may affect soil microclimatic conditions that could adversely affect yields. A study conducted by Jędrszczyk and Poniedzialek (2007), with rye (*Secale cereal* L.), lucerne (*Medicago lupulina* L.) and clover (*Trifolium repens* L.) as cover crops, concluded that these living mulches decrease the yield of corn (*Zea mays*). However, the starch content and total sugar in kernels was higher in corn crops intercropped. Those effects appeared to be related to local climate conditions, high humidity and lower soil temperatures that can delay the maturity of some vegetables (Wallace and Bellinder, 1992). For example, in a study of Bowory (2012), soil covered with living mulch lowered temperatures and delayed tomato fruit maturity.

Weed control with cover crops

Currently, integrated weed management aims to find non-chemical weed control strategies with ecological benefits and low environmental impact. The list of herbicide-resistant weeds has increased over time (Heap, 1997; Heap, 2014). A system based on living cover crops can suppress weeds (Altieri, 2012). By reducing weeds, the positive effects of permanent cover crops can be achieved through reducing weed high competition for water, nutrient, light and space (Ekeleme et al., 2003). However, a proper selection of ground cover crops should suppress weeds without stressing the cash crop.

The main source of weed infestation in crops is the weed seedbank and annual recruitment. Weed seedbank characteristics will influence weed population and their success in crops (Ekeleme et al., 2003). According to Brainard et al. (2012), living mulches suppress weeds effectively, but can result in higher weed density if is compared to practices with use of herbicides. Complementing cultural weed-control practices with living cover crops have been successful in preventing accumulation of weed seeds (Brainard et al., 2012; Price and Norsworthy, 2013). In a study conducted by Schmidt et al. (2019), the use of cover crops and compost were significant strategies to prevent weed seed bank accumulation in herbicide-free crops with conservation tillage. Teasdale et al. (1991) showed that if a cover crop produced a biomass of more than 200g m^{-2} and had a ground cover greater than 90 percent, weed infestation could be reduced by 78 percent compared to treatments without cover crops. Mehring et al. (2016) found similar results

reducing 90 percent of weed infestation in potato crops (*Solanum tuberosum* L.) with the incorporation of cover crops at 29 days after planting.

Ecological benefits of cover crops

Cover crops influence microbial communities by providing resources such as food and habitat (Six et al., 2006). In turn, microbial communities benefit plants by maintaining soil fertility and through their influence in carbon and nutrient fluxes (Nikiema et al., 2012; Agrawal and Goshal, 2016). Soils without vegetation and those deeper than rhizosphere are stressful environments for most bacteria (Coleman et al., 2004; Finney et al., 2017). Cover crops can promote higher microbiological activity and arbuscular fungi colonization by providing carbon to the soil with the development of root biomass (Xu et al., 2007; Kong and Six, 2012; Kong et al., 2012).

Nikiema et al. (2012) evaluated changes in biological activity according to changes in microbial communities using ryegrass (*Lolium perenne*), alfalfa (*Medicago sativa)* and Dutch white clover (*T. repens*) as cover crops. The control treatment was a plot without cover crops and managed conventionally by suppressing weeds with glyphosate. Results showed that microbial biomass was related to the amount of biomass generated by each cover crop; higher microbial biomass were in cover crops with higher biomass inputs.

1.2 Banana crop

The banana plant is a tropical herbaceous evergreen with more than 1000 varieties around the world (Food and Agriculture Organization of the United Nations [FAO], 2017). Banana is the fruit with second highest production in the world (FAO, 2017). According to the statistics for the FAO in 2016, the global banana industry generates approximately 8 billion dollars (USD) per year. Banana production cost are mainly in labor, fertilizers, phytosanitary control and pesticides (FAO,

2017). The leading exporter is Ecuador, followed by Philippines, Costa Rica, Colombia and Guatemala.

Banana crop in Puerto Rico

Banana are one of the most important crops in Puerto Rico, representing a critical agricultural business on the island. Puerto Rico does not import banana, except for a brief period after hurricane María made landfall on the island (September 2017). Banana and plantain production in Puerto Rico are for local consumption, generating a direct contribution to the economy. In 1993, Puerto Rico had near 1,745 ha exclusively for banana crops or intercropped with coffee and citrus (Cortés and Villagómez, 2005). Irizarry et al. (1989) reported that most of the production intercropped with coffee was without adequate management, resulting in low yield or poor quality. Although banana industry has been growing in the last decades, research in Puerto Rico for banana production has been scarce.

Banana plants require fertile soils with good drainage, Table 1.1 (Appendix 1) shown the recommendations in the 'Conjunto Tecnológico' (CT) by the Agricultural Experimental Station (1995) for farmers in Puerto Rico. It's widely known that Puerto Rico's climate, soils and infrastructure make affordable an increase in the banana production to maintain the local needs but also to export (Irizarry and Vicente, 1984). However, at present Puerto Rico still supplies the local needs, but the banana market has not been expanded to export. According to the census of by NASS of the U.S. Department of Agriculture (2012), Puerto Rico has 2,559 acres of banana production, composed by 1,828 farms. Currently, banana production in Puerto Rico is concentrated in the southern coastal valleys with the main production in approximately seven farms in 1,000 ha (Ortiz, personal communication, 2018).

In Puerto Rico, it has been shown that banana produces well with minimum tillage practices (Agricultural Experimental Station, 2005). Banana plants have a high total leaf area (25 m^2). Primary roots are about 5-8 mm in diameter and adventitious root system commonly expand 1 to 2 m. The vertical rootzone is about 40% of the root volume and is in the top 10 cm of soil, while

85% of the roots are in the top 30 cm (Robinson, 2010). They require irrigation during dry periods to avoid reductions in yield and quality of fruits (Robinson and Alberts, 1986). It's widely recommended that weeds are controlled mechanically or with herbicides. An important production area, however, is in the mountainous zone with prominent slopes susceptible to erosion, a factor that limits the use of heavy machinery. Conventional banana and plantain production rely upon conventional full tillage and chemical control for arthropods and weeds (Weiss, 2016).

Besides weed control, the banana industry faces challenges due to diseases such as the fungus Black Sigatoka. This disease was detected early in 2004 on the west side of Puerto Rico (Irish, 2006; Cortéz and Díaz, 2012). Control of Black Sigatoka follows agronomic management to reduce the favorable microenvironment for the fungus development and to increase the strength and vigor of the banana plants. Orozco-Santos (2008) explains that this management should consider irrigations and drainage methods, weed control, the density of the crop, fertilization, and nematode control. The manual for soil and water best management practices for banana growers in Australia recommend the use of cover crops to enhance the physical properties of soil such as drainage and to avoid erosion (Akehurst et al., 2008).

Cover crops in banana fields

Cover crops in banana farms have been shown to provide several benefits including: the reduction of weeds (Marín and Veloz et al., 1999; Rodas-Cazal and Godoy-Mendez, 2003; Fongod et al., 2010; Tixier et al., 2010; Ramos et al., 2011; Quintero-Pertúz and Carbonó-Delahoz, 2015; Achard et al., 2018) decreasing the effect of raindrop impact on soil, minimizing soil erosion and runoff by slowing the water's movement through the surface (Abbasi and Jamal, 1999), and improving water infiltration and air movement within the root channels penetrating the soil (Ouma, 2009; Akehurst et al., 2018). In addition, cover crops in banana farms have been shown to provide benefits improving nutrient cycles (Baligar et al., 2006; Lavigne et al., 2012; Barbosa et al., 2016; Quaresma et al., 2017) and biological trophic groups (Djigal et al., 2012). Also, with low-growing cover crops, easy access of machinery into the plantation is allowed (Weiss, 2016; Akehurst et al., 2018). The conventional agriculture of banana recommends keeping the crop without weeds or

another plant between inner roads. In tropical wet areas, weed management is critical because environmental conditions are favorable for the fast establishment of weeds (Lavigne et al., 2012). Moreover, weeds in banana crops reduce yields (Acuña, 1993; Rodríguez and Agüero, 2000).

As a sustainable alternative, cover crops are intercropped with banana to reduce weeds and the cost of herbicide application (Quintero-Pertúz and Carbonó-Delahoz, 2015). In a study conducted by Barbosa et al. (2016), management with leguminous cover crops showed to be economically rentable, because profits returned in half of the years considered in the investment. No inter-row weed control should be needed when cover crops are established for at least six months (Akehurst et al., 2018). Cover crops for banana fields should be established quickly after the soil have been tillage or prepared for the main crop, to reduce erosion and avoid the proliferation of weeds. In Australia, the Department of Primary Industries recommended growing cover crops before the removal of old bananas plants (Akehurst et al., 2018).

1.3 Potential cover crops for banana in Puerto Rico

Geophila repens

Geophila repens (L.) Johnst is a small herbaceous and perennial creeper that belongs to the Rubiaceae family. Although *G. repens* is widely distributed in the tropics, it has been listed as endangered in its native precedence, Singapore (Teo et al. 2010). It is commonly known as mouse-ear plant, snake pennyworth, pegaga tekukur, and corrida yerba guava. *Geophila repens* can be propagated vegetative and by seeds, although the later can be difficult (Fongod et al., 2010). It can grow in different climatic conditions but does not tolerate direct sunlight (Marin and Veloz, 1999).

Usually, *G. repens* is confused with *G. macropoda*; a plant species also used as a cover crop in shaded fields. However, *G. macropoda* has been reported as host for banana nematodes (*Helicotylenchus multicinctus* and *Rotylenchulus reniformis*), limiting its use as a cover crop (Waele et al., 2006). In Puerto Rico, a banana farmer used *G. macropoda* and other plant species, intercropped with banana (Ortiz et al., 2014). *G. repens* has been used as a cover crop on banana

plantations in Ecuador due to it being a poor host of banana nematodes (Waele et al., 2006). It has also been used in Panama as ground cover in the shade of banana farms.

According to Fongod et al. (2010), *G. repens* was the first non-leguminous species evaluated for use as a ground cover in banana farms, and their results encourage its use because it doesn't climb up the banana plants, reduces soil erosion and it does not host any banana pests. However, disadvantages of using *G. repens* as cover crops could be the high cost required to be vegetatively propagated and its slow growth rate (Wielemaker et al., 1997; Fongod et al., 2010). In Costa Rica, *G. repens* and *Arachis pintoi* have been evaluated as cover crops for banana (Wielemaker et al., 1997; Marin and Veloz, 1999; Agüero-Alvarado et al., 2018). Although *G. repens* has acceptable performance controlling weeds, the cost for establishing it as a cover crop is a limitation.

Heterotis rotundifolia

Heterotis rotundifolia (Sm.) Jacq., is a fast-growing and climbing perennial plant. The species belongs to the Melastomatoideae family. *H. rotundifolia* is native to tropical Africa and has been naturalized in Australia, Puerto Rico, Costa Rica, and the Pacific. The previous scientific name of *H. rotundifolia* was *Dissotis rotundifolia*. The common names for this species are the pink lady, chickweed, Spanish shawl (mantilla) and trailing dissotis.

This species has been evaluated as a potential ground cover for South Florida (Meerow and Black, 1993). In Puerto Rico, it was assessed in coffee farms as a cover crop (Ramos et al., 2014). In this evaluation, *H. rotundifolia* had a significant effect on weed suppression, requiring less labor time (Ramos et al., 2014). In addition, has been reported used by a citrus farm in Ciales, Puerto Rico (Ortiz, C., personal communication, 2017). Although the species shows some benefits, farmers had concerns of it becoming a weed while using it as a cover crop. However, in a citrus fruit farm in Ciales, Puerto Rico has been using *Heterotis rotundifolias* as living cover crops without any concern or problem (Ortiz, personal communication, 2018). Adeleke (2018) suggested this species as a cover crop in coffee farms in Nigeria.

Sphagneticola trilobata

Sphagneticola trilobata (L.) Pruski is a creeping perennial clonal herb, mat-forming with fast-growing stems from the Asteraceae family (Si et al., 2014). The species is native to the tropics of Central America, Mexico and the Caribbean (Xie et al., 2010; Macanawai, 2013). The species is widely known as wedelia, creeping-oxeye, creeping daisy, Singapore daisy, and wild marigold. In the following descriptions, the common name wedelia will be used for *S. trilobata*.

Wedelia is an invasive species in many areas of the tropics and subtropics (Xie et al., 2010). At present, reports indicate it being very invasive in the Pacific islands and has been listed as one of the 100 worst invasive alien species in the world (Lowe et al., 2000). It has been listed as one of the worst weeds by the International Union for Conservation of Nature and Natural Resources (IUCN). In China, wedelia was introduced in the 1970's as an ornamental groundcover, but escaped from gardens to roadsides and plantations, overgrowing native plants (Thaman, 1999).

Studies with *S. trilobata* have focused on the control of this species in Pacific islands, gas exchange characteristics and allelopathic effects (Cabrera-Asensio and Bastidas-Lopez, 2000). In a study by Si et al. (2014) in China, wedelia's adaptation and phenotypic plasticity in different habitat conditions was evaluated: full sunlight, shady conditions, and in soils of different moisture capacity. Their results showed that *S. trilobata* has two major mechanisms that work together and allow it to colonize large environmental gradients: local adaptation and phenotypic plasticity. The phenotypic plasticity index (PI) shows the ability of a genotype to produce different phenotypes as an adaptation mechanism in response to variable environmental conditions. Wedelia shows a PI of 0.64, approximately 30% higher in comparison with other invasive species as *Leucaena leucocephala*, *Psidium catteianum*, *Paspalum urvillei* and *Rubus ellipticus* (Si et al., 2014; Funk, 2008).

On the other hand, according Wang et al. (2015) a study by Ke et al. (2013) shown that long-term effects of wedelia could alter soil physical and chemical properties, increasing organic matter and the available plant nutrient content such as total N and K. Studies have found that some plants have successful mechanisms of colonization because they can accelerate the succession of soil microbial communities in their rhizosphere. Strengthening soil microorganisms' metabolic

activities is one of the mechanisms that may facilitate wedelia's invasion (Si et al., 2013). A study evaluating the effects of *S. trilobata* in soil microbial community found that *S. trilobata* can promote changes in soil fungal community; however, changes in soil bacterial community were not significant (Si et al., 2014; Dai et al., 2016).

Although considering *S. trilobata* as a very invasive species; it's important to recognize that it can be controlled by repeated physical removal of the plants and with the use of herbicides (Macanawai, 2013). In addition, studies have shown how the presence of *S. trilobata* could benefit crop growers, by promoting the dynamics in the nitrogen cycle and attracting beneficial insects (Prasad et al., 2013; Si et al., 2013). Currently, this species has been evaluated in another research project also as cover crop (Dumas, J., personal communication, 2018). However, results have been not published. In a study by Ramos-Rodríguez (2011) in Puerto Rico, aqueous extracts of *S. trilobata* were evaluated to control *Cosmopolites sordidus*, a pest that commonly affect *Musa* plants. In this study, Ramos-Rodríguez (2011) found that extracts of *S. trilobata* repelled adults of *C. sordicus*. Thus, taking into consideration its 'nativeness' in the Caribbean, this study aims to evaluate the potential as a cover crop under the canopy of banana plants in Puerto Rico.

Tradescantia zebrina

Tradescantia zebrina Heynh. Ex. Bosse is widely known as *Zebrina pendula.* This plant species is native to Mexico, Costa Rica, Guatemala, Nicaragua, Panama, and Belize (USDA-ARS, 2012; Govaerts, 2013). It belongs to the Commelinaceae family. *T. zebrina* is an herbaceous succulent and perennial plant, commonly grown as a groundcover. The common names of *T. zebrina* are inch plant, zebra-striped, cockroach grass, wandering Jew, silver inch, and cohitre morado in Spanish. The plant has been widely introduced as an ornamental plant, registered in Australia, Pacific islands, the Caribbean, southern USA and Asia (Oppenheimer and Bartlett, 2000; Acevedo-Rodríguez and Strong, 2012). This species has been enlisted as an invasive species in the Canary Islands, Singapore, Taiwan and South America (Chong et al., 2009; Forzza et al., 2012; Daisie, 2013).

The use of Commelinaceae plants as cover crops for weed suppression and green fertilizer has been documented for coffee farms in Mexico (Ramos et al., 1983). In the middle of 1990s, the use of Commelinas was promoted to protect the soil from erosion (Wellman, 1961). According to Anaya (2003), some species of the Commelinaceae family release a toxic substance which contributes to their dominance over weed species. Ramos et al. (1983) evaluated the effects of Commelina species on the germination and growth of other plant species. Their results indicate that *T. zebrina* was the species with more dominance, highest weed inhibition and coverage during both the rainy and dry season. A study evaluating the allelopathic effects of *T. zebrina* on *Coffea arabica*, found that using *T. zebrina* stimulated the development of *C. arabica* plants, suggesting that it could be used intercropped in coffee agroecosystems (Navarro et al., 2013).

1.4 Soil quality

What is soil quality?

The soil is the basis of natural plant communities and agricultural systems (Doran and Zeiss, 2000). Further, soil has been recognized as a 'nonrenewable resource' (Abawi and Wildmer, 2000). As soil is critical for agricultural productions, management programs have been developed to archive a sustainable agriculture and enhance soil properties. Scientists have been assessing different criteria and parameters to evaluate soil under different management practices and determine if those effects are sustainable (Doran and Zeiss, 2000). The assessment of soil quality/health has been useful to monitor changes related to agricultural practices.

The Soil Science Society of America Ad Hoc Committee defined soil quality as 'the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen et al., 1997). A standardized term by Johnson et al. (1997) defined soil quality as 'a measure of the condition of soil relative to the requirements of one or more biological species and/or to any human purpose'. On the other hand, the term soil health is more specific to recognize the soil as a living and dynamic system, where the biodiversity is

essential to execute functions in soil and promote environmental quality (Doran and Zeiss, 2000). Soil quality has been conceptually attached as the parameter with strategies for conservationist management practices and sustainable agriculture (Acton and Gregorich, 1995).

Biological parameters

Soil organisms have been commonly used as an indicator of soil quality and health due to their sensitivity to respond to management practices (García-González, 2017). A suitable parameter should respond and reflect the long-term effects of management and/or climate but should be stable with short-term effects of weather patterns (Doran and Parkin, 1996). Commonly, proxies well correlated with the soil function are efficient strategies to evaluate soil quality (Pankhurst et al., 1997; van Bruggen and Semenov, 2000; Lazcanao et al., 2013).

Werner and Dindal (1990) conducted a study comparing organic management versus conventional practice to evaluate soil biological parameters. They showed that chemical and physical indicators didn't show a significant difference, while biological parameters were significantly different. In this study, organic management used animal and green manures, while in the conventional management used inorganic fertilizers and pesticides. They evaluated soil respiration, faunal populations, and infiltration rates. Their results indicate that under organic management those biological parameters were higher than in a conventional management. Visser and Parkinson (1992) proposed that indicators like soil organic matter (SOM), microbial biomass, decomposition rates, N cycling, and soil enzymes could reflect changes in soil quality. Soil enzymatic activities has been used as early indicators to soil management due its relationship with soil microbial communities, environmental effects, plants and biogeochemical cycles (Dick and Tabatabai, 1993; Acosta-Martínez, 2011). Although biological parameters are widely used, it is necessary to emphasize that biological, chemical and physical indicators of soil quality are not independent of each other.

1.5 References

Abawi G.S. and T.L. Widmer. 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology*. 15:37−47

Abbasi, M.A., and T. Jamal. 1999. Soil loss and runoff measurement from banana-pineapple intercropping system. 2 (3): 689−692

Acosta-Martínez, V., Lascano, R., Calderón, F., Booker, J.D., Zobeck, T.M., Upchurch, D.R. 2011. Dryland cropping systems influence the microbial biomass and enzyme activities in semiarid sandy soil. *Biol Fert Soils*. 47: 655−667

Acevedo-Rodríguez, P. and M.T. Strong. 2012. Catalogue of the seed plants of the West Indies. Smithsonian Contributions to Bontany. 98:1192 Washington DC, USA. https://naturalhistory2.si.edu/botany/WestIndies/

Achard, R., P. Tixier, M. Dorel, and J.R. Estrade. 2018. Intercropping of grass cover crops in banana plantations: impacts on banana growth and yield. *Tropical Agriculture and Development*. 62(1):1−8

Acton, D.F. and L.J. Gregorich. 1995. The health of our soils: toward sustainable agriculture in Canada. *Agriculture and Agri-food Canada*. Publication 1906/E

Acuña, G. 1993. Control de malezas en el cultivo del banano. Compañía Bananera de Costa Rica. División Golfito. Mimeografiado. 15

Adeleke, S.A., M. Idrisu, and I.F. Abdul-karim. 2018. Status of weed control in coffee farms in Nigeria: A need for improved technologies. *International Journal of Advance Agricultural Research*. 59-68

Agrawal, A., and N. Ghoshal. 2016. Soil microbial biomass dynamics in grassland and agroecosystems receiving varying resource quality soil inputs in dry tropics. *International Journal of Agriculture, Environment and Biotechnology* 9(5) 787−900

Agricultural Experimental Station, 1995. Conjunto Tecnológico para la producción de plátanos y guineos. Univ. P.R., Río Piedras, PR. Publ. 97

Altieri, M.A. 2012. Insect pest management in the agroecosystems of the future. *Atti Academia Nazionale Italiana di Entomologia*. 137−144

Alonso-Ayuso, M., J. L. Gabriel, and M. Quemada. 2014. The kill date as a management tool for cover cropping success. *Plos one*. https://doi.org/10.1371/journal.pone.0109587

Akehurst, A., P. Newley and M. Hickey. 2008. Soil and water best management practices for NSW banana growers. Department of Primary Industries. State of New South Wales, Australia.

Anaya, A.L. 2003. Ecología química. Chapter 12: Antecedentes y perspectivas de la investigación en ecología química en México. San Rafel, México, D.F. 316−318

Baligar, V.C., N.K. Fageria, A.Q. Paiva, A. Silvera, A.W.V. Pomella, and R.C.R. Machado. 2006. Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. *Journal of Plant Nutrition*. 29 (11): 1959−1974

Barbosa, F.E.L., C.F. de Lacerda, A.V. Amorim, R.N.T. Costa, J.A. da Silva, and F.F.F. Hernández. 2016. Production and economic viability of banana managed with cover crops. *Revista Brasilera de Engenharia Agrícola e Ambiental*. 20(12)1078−1082

Basche, A.D., T.K. Kaspar, S.A. Archontoulis, D.B. Jaynes, T.B. Sauer, T.S. Parkin, and F.E. Miguez. 2016. Soil water improvements with the long-term use of a cover crop. *Agricultural Water Management*. 172:40−52

Borowy, A. 2012. Growth and yield of stake tomato under no tillage cultivation using hairy vetch as living mulch. *Acta Sci Pol*. 11(2):229−252

Brainard D.C., R.R. Bellinder, and A.J. Miller. 2004. Cultivation and interseeding for weed control in transplanted cabbage. *Weed Technol*. 18:704−710

Brainard, D.C., J. Bakker, and D.C. Noyes, A.J. Myers. 2012. Rye living mulch effect on soil moisture and weeds in asparagus. *Hort Sci*. 47(1):58−63

Belfry K.D., c. Trueman, R.J. Vyn, S.A. Loewen, and L.L. Van Eerd. 2017. Winter cover crops on processing tomato yield, quality, pest, pressure, nitrogen availability, and profit margings. *Plos One.* 12(7) e0180500

Cabrera-Asensio, I. and H. Bastidas-López. 2000. Effect of *Wedelia trilobata* L. (Asterales:Asteraceae) on the whitefly *Bemisia tabaci* G. (Homoptera:Aleyrodidae). *J. Agric. Univ. P.R.* 84(3-4):181−184

Chong, K., T.W. Hung, and R.T. Corlett. 2009. A checklist of the total vascular plant of Singapore: native, naturalized and cultivated species. Singapore: Raffles Museum of Biodiversity Research, National University of Singapore. 273

Coleman, D., D.A. Crossley, and P. Hendrix. 2004. Fundamental of Soil Ecology. Second Edition. Georgia, US

Cortés, M. and R. Villagómez. 2005. Estimados de la elasticidad de la oferta para el cultivo de guineo en Puerto Rico. *J. Agr. U. Puerto Rico*. 89(3):181−192

Cortés, M., M. Díaz, and L. Gayol. 2012. Cambio en las preferencias del consumidor de plátano en Puerto Rico, 2003-3008. *J. Agri. Univ. P.R.* 93(3-4): 269−272.

Creamer, N.G. and Baldwin, K.R. 2000. An evaluation of summer cover crops for use in vegetable production systems in North Carolina. *Hort Science.* 35(4):600−603

Dai, Z.C., W. Fu, L.Y. Wan, H.H. Cai, N. Wang, S.S. Qi, and D.L. Du. 2016. Different growth promoting effects of endophytic bacteria on invasive and native clonal plants. *Front. Plant Sci*. 7:706

Daisie (Delivering Alien Invasive Species Inventories for Europe. 2013 Species Factsheet: *Zebrine pendula*. http://www.europe-aliens.org/speciesFactsheet.do?speciesId=5456#

Dick, W. and M.A. Tabatabai. 1993. Significance and potential uses of soil enzymes. Soil microbial ecology: application in agricultural and environmental management. Marcel Dekker, New York. 95−125

Djigal, D., C. Chabrier, P. Duyck, R. Achard, P. Queneherve, and P. Tixier. 2012. Cover crops alter the soil nematode food web in banana agroecosystems. *Soil Biol. Biochem*. 48−150

Doran J.W. and T.B. Parkin. 1996. Quantitative Indicators of Soil Quality: A minimum data set. Methods for Assessing Soil Quality, Special Publication. Soil Science Society of America. 49:25−37

Doran, J.W. and M.R. Zeiss. 2000. Soil health and sustainability:managing the biotic component of soil quality. Applied Soil Ecology 15:3−11

Dorn, B., M. Stadler, M.V.D. Heijden, and B. Streit. 2013. Regulation of cover crops and weeds using a roll-chopper for herbicide reduction in no-tillage winter wheat. Soil and Till Research. 123:121−132 https://doi.org/10.1016/j.still.2013.07.014

Ekeleme, F., I.O. Akobundu, O. Isichei, and D. Chikoye. 2003. Cover crops reduce weed seedbank in maize-cassava systems in southwestern Nigeria. *Weed Science*. 51:774−780

Finney, D.M., and J.P. Kaye. 2017. Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. *J. Appl. Ecol.* 54(2):509–517

Food and Agriculture Organization of the United Nations (FAO). 2017. Banana Market Review and Banana Statistics *2015-16.* Rome. http://www.fao.org/3/a-i7410e.pdf

Fongod, A.G.N., D.A. Focho, A.M. Mih, B.A. Fonge, and P. Sama-Lang. 2010. Weed management in banana production: The use of *Nelsonia cenescens* (Lam.) Spreng as a non-leguminous cover crop. *Afr. J. Environ. Sci. Technol*. 4(3):167−173

Forzza, R.C., p.m. Leitman, A.F. Costa, and Jr A.A. Carvalho. 2012. List of species of the Flora of Brazil (Lista de especies Flora do Brasil). Rio de Janeiro, Brazil. Rio de Janeiro Botanic Garden.

Funk J.L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. *J. Ecol.* 96(6):1162−1173

García-González, I., C. Hontoria, J.L. Gabriel, M. Alonso-Ayuso, and M. Quemada. 2018. Cover crops to mitigate soil degradation and enhance soil functionality in irrigated land. *Geoderma*. 322 81−88

Govaerts, R. 2013. World Checklist of Commelinaceae. Richmond, UK: Royal Botanic Gardens.

Hartwig, N. and H. Ammon. 2002. Cover crops and living mulches. *Weed Science*. 50: 688−699.

Heap, I. 1997. The occurrence of herbicide-resistant weeds worldwide. *Pest Management Science*. 51(3):235−243

Heap, I. 2014. Global perspective of herbicide-resistant weeds. *Pest Management Science*. 70(9):1306−1315

Irish, B. M., R. J. Goenaga and R. O Ploetz, 2006. *Myeosphaerella fijiensis*, causal agent of black Sigatoka of Musa spp. found in Puerto Rico and identified by Polymerase Chain Reaction. *Plant Disease*. 90:684

Irizarry, H., E. Rivera, I. Beauchamp de Caloni, and R. Guadalupe. 1989. Performance of elite banana (*Musa acuminata*, AAA) cultivars in four locations of Puerto Rico. *J. Agric. Univ. P.R.* 73(3):209−221

Jędrszczyk, E. and M. Poniedziałek. 2007. The impact of the living mulch on plant growth and selected featured of sweet corn yield. *Folia Horticulurae*. 19(1):3−13

Johnson, D.L., S.H. Ambrose, T.J. Bassett, M.L. Bowen, D.E. Crummey, J.S. Isaacson, D.N. Johnson, P. Lamb, M. Saul and A.E. Winter-Nelson. 1997. Meanings of Environmental Terms. *Journal of Environmental Quality* Abstract. 26(3): 581-589

Karlen, D.L., M.J. Mausbach, J.W. Doran, R.G. Cline, R.F. Harris, and G.E. Schuman. 1997. Soil quality: a concept, definition and framework for evaluation (a guest editorial). *Soil Sci. Soc. Am. J.* 61:4−10

Ke, Z.H, P.X. Qiu, D.X. Hu, H. Zhu, and L.Y. Song. 2013. Effects of *Wedelia trilobata* invasion on soil enzyme activities and physical-chemical properties. *Ecology and Environmental Sciences* 22: 432−436

Kolota, E. and K. Adamczewska-Sowinska. 2013. Living mulches in vegetable crops production: perspectives and limitations. *Acta Sci. Pol.* 12:127−142

Kong, A.Y. and J. Six. 2012. Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems. *Plant and Soil*. 356(1-2):315−330

Kong, A., K. Scow, A.L. Cordova, W. Holmes, and J. Six. 2012. Microbial community composition and carbon cycling within soil microenvironments of conventional, low input, and organic cropping systems. *Soil Biol Biochem*. 43(1):20−30

Lavigne, C., R. Achard, P. Tixier, and M. Lesueur-Jannoyer. 2012. How to integrate cover crops to enhance sustainability in banana and citrus cropping systems. *Acta Hort*. 351−358

Lazcano, C., M. Gómez-Brandón, P. Revilla, and J. Domínguez. 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. Biology and Fertility of Soils. 49 (6): 723−733

Liu, Y., J. Wang, D. Liu, Z. Li, G. Zhang, Y. Tao, J. Xie, J. Pan, and F. Chen. 2014. Straw mulching reduces the harmful effects of extreme hydrological and temperature conditions in citrus orchards. *Plos One* 9(1) e87094

Lowe, S.M., M. Browne, S. Boudjelas and M.D. Pooter. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database

Lu, Y., K. Watkins, J.R. Teasdale, and A.A. Abdul-Baki. 2000. Cover crops in sustainable food production. *Food reviews International* 16:121−157

Navarro, G.B., L.C. Pelie,and M.M. Favier. 2013. Efectos alelopáticos de dos coberturas vivas *Commelina difusa* Burm. F. Y *Tradescantia zebrina* Shunltz sobre *Coffea arabica*. *Revista Centro Agricola, Barcelona.* 75−78

Nielsen, D.C., D.J. Lyon, R.K. Higgings, G.W. Hergert, J.D. Holman, and M.F. Vigil. 2016. Cover crop effect on subsequent wheat yield in the central great plains. Agronomy Journal. 108 (1):243−256

Nikiema, P., P. Nzokou, D. Rothstein and M. Ngouajio. 2012. Soil microbial biomass as affected by groundcover management in a Fraser fir plantation after one year. *Biol Fertil Soils*. 48:727−733

Macanawai, A.R. 2013. Impact of Sphagneticola trilobata on Plant Diversity in Soils in South-East Viti Levu, Fiji. *Journal of Life Sciences*. 7(6)634−642

Marin, E. and Veloz, D. 1999. Establecimiento de la especie silvestre Geophyla repens como cobertura viva en el cultivo de banano. Universidad Earth. Guácimo, Costa Rica

Meerow, A.W. and Black, R.J. 1993. Enviroscaping to conserve energy: ground covers for south Florida. Circular EES-39 Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida.

Mehring, G.H., Stenger J.E. and Hatterman-Valenti, H.M. 2016. Weed control with cover crops in irrigated potatoes. Agronomy. 6(1) 3 doi:10.3390/agronomy6010003

Mwangi, H.W., A.W. Kihurani, J.M. Wesonga, E.E. Ariga, and F. Kanampiu. 2015. Effect of *Lablab purpureus* L. cover crop and imidazolinone resistant (IR) maize on weeds in drought prone areas, Kenya. *Crop Protection*. 72:36−40

Orozco-Santos, M., J. Orozco-Romero, O. Pérez-Zamora, G. Manzo-Sánchez, J. Farias-Larios, and W. Moraes. 2008. Prácticas culturales para el manejo de la Sigatoka negra en bananos y plátanos. *Tropical Plant Pathology.* 33(3)189−196

Ortiz, C., E. Más, J. Valentín. 2014. Validación de cultivos de cobertura para la región del Caribe. Sociedad Puertorriqueña de Ciencias Agrícolas (SOPCA). Puerto Rico. Abstract.

Oppenheimer, H.L. and R.T. Bartlett. 2000. New plant records from Maui, O'ahu, and the Hawai'I Islands. Records of the Hawaii Biological Survey for 1999. *BishopMus.Occas.*64:110

Ouma, G. 2009. Intercropping and its application to banana production in East Africa: A review. Journal of Plant Breeding and Crop Science. 1(2): 013−015

Pankhurst, C., B.M. Doube, and V.V.S.R. Gupta. 1997. Biological indicators of soil health. CAB International. Washington DC, USA. Pag. 1−23

Parreño, J.G. 2014. Sistema integrado de leguminosas y su efecto en el establecimiento de plátano (*Musa acuminata* x *balbisiana*, AAB) en dos agroecosistemas de Puerto Rico. Tesis M.S. University of Puerto Rico, Mayagüez Campus

Petter, F.A., L.P. Pacheco, A.M. Zuffo, A.C. Piaulino, Z. Fernandes, J. Moraes dos Santos, and J. Miranda. 2013. Performance of cover crop under the water deficit. *Semina: Ciencias Agrarias*. 34:3307−3320

Prasad, A.V. and Hodge, S. 2013. Factors influencing the foraging activity of the allodapine bee Braunsapis puangensis on creeping daisy (*Sphagneticola trilobata*) in Fiji. Journal of *Hymenoptera Research*. 35:59−69

Price, A., and J. Norsworthy. 2013. Cover crop for weed management in southern reduced-tillage vegetable cropping systems. *Weed Technology.* 27:212−217

Quaresma, M.A.L., F.L. Oliveira, and D.M.N. Silva. 2017. Leguminous cover crops for banana plantations in semi-arid regions. Rev. *Caatinga* 30(3)614−621

Quintero-Pertúz and Carbonó-Delahoz. 2015. Outlook of weeds management in banana crops in the Magdalena department, Colombia. *Revista Colombiana de Ciencias Hortícolas*. 9(2):329−340

Ramos, L., Anaya, A.L., and de Pascual, J.N. 1983. Evaluation of allelopathic potential of dominant herbaceous species in a coffee plantation. *Journal of Chemical Ecology*. 9(8):1079−1097

Ramos M., E. Alvarado, M. Davila, O.R. Rodriquez and Y. Sanchez. 2014. Weed management alternatives for organic coffee agroforestry systems of Puerto Rico. Available at htpps://projects.sare.org/project-reports/Is10-231/".

Ramos, O., R. Vaquero, R. León, and F. Ayuso. 2011. Efecto de tres especies de cobertura viva en el control de malezas en una plantación de banano orgánico (Musa AAA cv. Williams). *Tierra Tropical*. 7(1):1−10

Robinson, J.C. and A.J. Alberts. 1986. Growth and yield responses of banana cultivar 'Williams' to drip irrigation under drought and normal rainfall conditions in the subtropics. *Scientia Horticulturae* 30(3):187−202

Robinson, J.C. and V. Galán Saúco. 2010. Chapter 3: Morphological characteristic and plant development. *Bananas and Plantains.* 2nd Edition. 51−66

Rodas-Cazal, L.C. and Godoy-Mendez, N.E. 2003. Efecto de las prácticas culturales sostenibles en el manejo de malezas del cultivo de banano (Musa AAA) de la Universidad Earth. Guácimo, Costa Rica

Rodríguez, A.M. and R. Agüero. 2000. Identificación de malezas trepadoras del banano (Musa sp.) en la zona caribe de Costa Rica. *Agronomía mesoamericana*. 11(1):123−125

Schmidt, J.H., J. Stephan, M.R. Finckh. 2019. Cover crops and compost prevent weed seed bank buildup in herbicide-free wheat-potato rotations under conservation tillage. Ecology and Evolution. 9 (5): https://doi.org/10.1002/ece3.4942

Si, C. X. Liu, C. Wang, L. Wang, Z. Dai, S. Qi., and D. Du. 2013. Different degrees of plant invasion significantly affect the richness of the soil fungal community. Plos One. 8(12) e85490

Si, C.C., Z.C. Dai, Y. Lin, S.S. Qi, P. Huang, S.L. Miao, and D.L. Du. 2014. Local adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical island. *Annals of Botany* 114:425–433

Six, J., D. Frey, R.K. Thiet, and K.M. Battern. 2006. Bacterial and Fungal Contributions to Carbon Sequatration in Agroecosystems. *Soil Science Society of American Journal*. 70 (2) 555−569

Sustainable Agriculture Network (SAN). 1998. Managing Cover Crops Profitability. Second Edition. Hill Building, UVM, Burlington, VT

Snapp, S., S. Swinton, R. Labarta, D. Mutch, J.R. Black, R. Leep, J. Nyraneza, and K. O'Neil. 2005. Review and Interpretation: Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agron. J.* 97:322−332

Thaman, R. 1999. Wedelia trilobata: Daisy invader of the Pacific Islands. IAS Technical Report. 99(2)

Tixier, P., C. Lavigne, S. Alvarez, A. Gauquier, M. Blanchard, A. Ripoche, and R. Achard. 2010. Model evaluation of cover crops, application to eleven species for banana cropping systems. *Europ. J. Agronomy*. 34:53−61

Teasdale, J.R., C.E. Beste, W.E. Potts. 1991. Response of weeds to tillage and cover crops residue. *Weed Sci*. 39:195−199

Teo, S., B.R. Kurukulasuriya, H.T.W. Tan. 2010. The distribution and status in Singapore of the snake pennyworth, Geophila repens (L.) I.M. Johnst. (Rubiaceae) Nature in Singapore. 3:183−186

USDA-ARS, 2012. Germplasm Rsources Information Network. Online Database. Beltsville, Maryland, USA:National Germplasm Resources Laboratory.

USDA-NASS. 2012. Census of Agriculture. Puerto Rico Island and Municipio Data. Vol 1. Geographic Area Series Part 52

van Brugger, A.H.C. and A.M. Semenov. 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology. 15: 13−24

Visser, S. and D. Parkinson. 1992. Soil biological criteria as indicators of soil quality: Soil microorganisms. *Amer. J. Altern. Agric.* 7:33−37

Waele, D., R. Stoffelen, and J. Kestemont. 2006. Effects of associated plant species on banana nematodes. *InfoMusa*. 15:1−2

Wallace, R.W. and R.R. Bellinder. 1992. Alternative tillage and herbicide options for successful weed control in vegetables. *Hort. Sci.* 27(7):745−748

Wang, R., T. Dai, G. Quan, and J. Zhang. 2015. Changes in soil physico-chemical properties, enzyme activities and soil microbial communities under *Mimosa pudica* invasion. *Allelopathy Journal*. 36(1) 15−24

Wang, K.H., R. McSorley, N. Gallaher, and N. Kokalis-Burelle. 2008. Cover crops and organic mulches for nematode, weed and plant health management. *Nematology*. 10: 231−242

Weiss, S. 2006. Developing Sustainable Tropical Leguminous Cover Crop and Green Manure Mulch Systems for Low-External-Input crop Production in the U.S. Virgin Island, Puerto Rico, and Florida. Final Report for LS12-252. SARE https://projects.sare.org/project-reports/ls12-252/

Wellman, F.L.1961. Coffee. Botany, Cultivation and utilization. World Crop Series. Wiley-Interscience, New York.

Werner, M.R. and D.L. Dindal. 1990. Effects of conversion to organic agricultural practices on soil biota. American Journal of Alternative Agriculture. 5:24−32

Wielmaker, F. 1997. Banana production with “Pinto’s Peanut” (*Arahis pintoi* cv. Amarillo) and “Oreja de raton” (Geophila repens) as cover crops’. H. Yglesias Luconi. Memoria primer taller internacional sobre control biológico y producción integrada en el cultivo de banano. Universidad EARTH, Costa Rica. 84−89

Xie, L.J., R.S. Zeng, H.H. Bi, Y.Y. Song, R.L. Wang, Y.J. Su, M. Chen, S. Chen, and Y.H. Liu. 2010. Allelochemical mediated invasión of exotic plants in China. *Alleopathy J.* 25(1):31−50

Xu, Y., W. Chen, and Q. Shen. 2007. Soil organic carbon and nitrogen pools impacted by long-term tillage and fertilization practices. *Communications in Soil Science and Plant Analysis*, 38:347−357

Chapter 2: Growth analysis of three plants species intercropped in a banana plantation

2.1. Introduction

The assessment of plant growth is fundamental in a wide range of scientific fields (Tessmer et al., 2013). Growth has been defined as the result of the division, expansion, and differentiation of cells, resulting in an increase in dry mass, length, volume or area (Lambers et al., 2008). For the evaluation of new potential cover crops, plant growth analysis is a key to understand how the plants species respond to the agricultural systems. Although plant growth analysis uses simple primary data such as biomass weight, areas, and volumes, the plant growth rate could reflect how biotic and abiotic conditions affect the photosynthesis activity or energy distribution of the plant (Hunt et al., 2002; Tessmer et al., 2013).

Plant growth analysis have been widely used as a standard method to assess plant development and productivity (Hunt et al., 2002; Ozalkan et al., 2010; Tessmer et al., 2013). Taking in consideration the productivity, some cover crops have multiples benefits, including weed suppression, and others mentioned before, but also could be a second cash crop (Altieri, 2012). Whichever the purpose using a cover crops in a farm, integrating cropping system requires an initial investment. The costs of cover crops are led by the costs of cropping system establishment, including seed price, machinery, labor and sometimes supplementary irrigation systems (Snapp et al., 2005). To reduce the cost of establishment, is necessary to select the best cover crops for a specific agricultural system. Following this, the 'best' cover crop should be able to cover the soil area in a minimum lapse of time, compete with other plants reducing seedling emergence of weeds, and other critical benefits such as low competition with the cash crop (Blanco-Canqui, 2015; Wittwer et al., 2017).

The main objective in this study is to evaluate the potential of three common plant species in Puerto Rico: *Heterotis rotundifolia*, *Tradescantia zebrina* and *Spagneticola trilobata*-as cover crops in a perennial field of banana *Musa acuminata* var. Gran Nain. as compared to the standard

cover crop *Geophila repens*. The specific objectives were: (1) study the development of soil coverage by plant species; (2) determine the effectiveness of the three species interfering with other plant species naturally present in the banana field and; (3) examine if the plant species used as cover crops could be potential pest host.

The hypothesis for the respective specific objectives were (1) After a period of five months, at least one of the three plant species will have greater percent of soil cover than the reference plant (*G. repens*). *T. zebrina* will adapt better than *H. roundifolia* and *S. trilobata* due to shade tolerance variability among cover crops species; (2) at least one of the three plants under study will have a significant difference in the weed suppressive effects and; (3) at least one of the three living cover crops will have a lower mean of infestation with insects than the reference plant, *G. repens*.

2.2. Materials and Methods

Study site

The study was performed at the Gurabo Agricultural Experiment Substation of the University of Puerto Rico (18.2534 N, -65.9896 W). The area is located at the agricultural coastal plains zone in eastern of Puerto Rico, where the mean annual temperature is 25.2^{o} C and annual precipitation is 1869 nm (National Weather Service, 2014). The experimental field used to be planted with sugarcane (*Sacchararum officinarum* L.) beginning in the 1970's (Alexander et al., 1979). However, the field has been planted in the last 10 years with banana (*Musa acuminata*) and other farinaceous crops such as plantain (*Musa* spp AAB), taro (*Colocasia escualenta*), tannier (*Xanthosoma* spp.), cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batata*) (Ortiz, C. Ortiz, personal communication, 2018).

The soil in the experimental field is a Vertisol classified as Mabi series (Very-fine, mixed, active, isohyperthermic Aquic Hapluderts) (Muñoz et al., 2018; Soil Survey Staff, 2017). Soils of this series are mainly found on alluvial fans or terraces of the Humid Coastal Plains and are characterized as very deep clay soils with slow permeability and poor drainage capacity (Soil Survey Staff, 2006). Chemical properties of soil from 0-20 cm depth are summarized in Table 2.1. This analysis was conducted by the Central Analytical Laboratory in the Río Piedras Agricultural Experimental Station of the University of Puerto Rico before the experiment started.

Agricultural system and cover crops

The study required an established semi-perennial crop to evaluate the growth response of new cover crops to a shaded field. For this purpose, a crop of banana (*Musa acuminata,* AAA*)* var. Gran Nain was planted on February 11, 2016. Planting distance was conventional, 3.04 m between rows and 1.83 m between plants with the row. The experimental field was managed under conventional practices, with fertilization and fungicides applications (Table 2.2). Tillage practices were applied before planting with a rotary tiller Bush Hog ® RTS 40-04.

As the banana crop grew, a pilot test (Appendix 2) was conducted to evaluate the most efficient techniques to propagate different plants species. For this study, three new plants species (treatments) were evaluated as intercropping for banana plantations. The plants under study as cover crops were: pink lady (*Heterotis rotundifolia*), wedelia (*Spagneticola trilobata*) and zebra plant (*Tradescantia zebrina*). In addition, *Geophila repens* was used as a reference plant, due its widely known effectiveness as a cover crop in banana and shaded fields (Waele et al., 2006). However, it's important to emphasize that in Puerto Rico, *G. repens* has been not evaluated in a research as cover crop. Although *G. repens* will be used as a reference plant, the potential as living cover crop under a banana canopy for Puerto Rico climate conditions will be evaluated for first time. Plant specimens were obtained from the nursery in the Experimental Substation of Gurabo.

Experimental design

The field had five roads between the six rows of *M. acuminata*. Between plants with the row, 34 banana ramets ("suckers") were planted every 1.83 m. However, for this study the three innermost spaces roads were used to prevent border effects caused by solar radiation (Figure 2.1). The experimental design was arranged in a randomized complete block design with four replicates. Each block was divided into five experimental plots (4 m x 2.15 m each), four of them with the plants under study as cover crops, and one without a cover crop as a control. Between each plot, border rows of 1 m were designed to avoid border effects from the adjacent cover crops (treatments). Each experimental plot had plants of a single species, which were cut at a standardized size (12 cm) and planted every 20 cm. Study plants were supplemented with water during the first two weeks after they were planted into the field. Cover crops were planted in July 26th of 2017, 17 months' after the banana plants were planted.

Plant growth analysis

A functional approach to growth analysis was used to evaluate growth development in plant species under the shaded canopy of the banana plants. The methodology was performed with modifications of the methodology described by Hunt (1990). Sampling started two weeks after planting the cover crops. Plant samples were randomly selected while avoiding the borders of the plot. In each plot, three plants were harvested by cutting each 1 cm above of the soil level. Although sampling was originally designed in biweekly intervals for a period of five months or until the ground was completely covered, after hurricane María, limitations changed the design and samples were taken at 16 days, 48 days, 175 days and 229 days after planting (DAP). Hurricane María made landfall 56 DAP cover crops. For the analysis, plants were separated into leaf lamina from the base of the petiole and stem. The leaf lamina was used to determine the leaf area with an electronic planimeter, model LICOR LI300[1]. Samples were stored in paper bags, dried at 70 ± 1°C for 48 hours and weighted. An average of the three subsamples per plot was used in the calculations of growth analysis. Growth analysis was determined by calculating the Leaf Area Index (LAI), Crop Growth Rate (CGR), Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) with the following equations:

$$\text{(Eq. 2.1) Leaf Area Index} = \frac{\text{Total leaf area}}{\text{unit ground area}}$$

$$\text{(Eq. 2.2) Crop Growth Rate} = \frac{\mathrm{W2 - W1}}{\mathrm{P\ (t2 - t1)}}$$

$$\text{(Eq. 2.3) Relative Growth Rate} = \frac{\mathrm{lnW2 - lnW1}}{\mathrm{t2\text{-}t1}}$$

$$\text{(Eq. 2.4) Net Assimilation Rate} = \frac{(\mathrm{W2 - W1})(\mathrm{Log\ L2 - Log\ L1})}{(\mathrm{t2 - t1})(\mathrm{L2 - L1})}$$

[1] Trades names are mentioned to provide detailed information and do not constitute an endorsement by the University of Puerto Rico or affiliated institutions.

Where, W1= Dry weight of the plant/m^2 at time t1, W2= Dry weight of the plant/m^2 at time t2, t1 and t2 were intervals of time between samplings of evaluation, P= ground area, ln= Natural logarithm, L2 = Leaf area of the plant at time 2 and L1 = leaf area of the plant at time 1. CGR is expressed as g/m^2/day, RGR is expressed as g/g/day and NAR as g/m^2/day.

Soil surface coverage and weed suppression

To determine the effectiveness of the three species in interfering or suppressing other plant species naturally present in the banana field, a combination of visual estimate assessment and dry biomass collection was performed. For the visual assessment, a randomly placed quadrant (30.5 x 30.5 cm) was used to evaluate with a percent scale where ground area without any plant, cover crop area and weed area was calculated (Figure 2.2). Two replicates per plot were conducted and its average was used in the statistical analysis. After visual assessment was completed, weeds were hand dug, stored in paper bags without roots, dried at 70 ± 1 °C for 48 hours and weighted. Hand weeding (HW) without any tool, was performed over four periods: August 30th, 2017 (35 DAP), November 14th, 2017 (after 76 days of the first HW), January 22nd, 2018 (after 69 days of the latter HW) and April 9th, 2018 (after 77 days of the last HW). Data for the third period, January 22nd, was not submitted to statistical analysis due to missing data. In the last HW, cover crops had grown for eight months and one week. In this evaluation, shaded conditions were greater than in November 14th due to the side shoots of banana plants which had been growing for 7 months.

Common weed species in each plot were identified according to the book of Common Weeds in Puerto Rico and U.S. Virgin Island by Más and Lugo-Torres (2013). In addition, the labor time required to remove the weeds by HW per plot was measured. To reduce the effect of human-caused variability, this procedure was conducted in a 5x5 Latin Square Design (Richarson, 2018). After hurricane María and the loss of one cover crop species, the procedure was modified to a 4x4 Latin Square Design. The labor time was measured in minutes.

Potential host of pest evaluation

Insect damage was evaluated on the potential cover crops. The sampling was *in situ* with a non-destructive method. A visual assessment was completed in a randomly placed quadrant (30.5 x 30.5cm) by recording the presence or absence of insects on leaves and stems. If in a sample, insects were present, the level of infestation was estimated with the Modified Cobb scale (Carlo-Acosta, 2009). The categories were: none (1-5%), extreme light infestation (6-10%), light infestation (11-25%), medium infestation (26-40%), heavily infestation (41-64%) and (65-100%) severe infestation (Carlo-Acosta, 2009). Three replicates per plot were conducted and its average was used in statistical analysis. This evaluation was performed 48 and 229 DAP cover crops. In addition, two yellow strips were placed on each plot in April 2018 to assess the presence of wing insects in the plots.

Statistical Analysis

For the growth analysis, an analysis of variance (ANOVA) was performed to determine differences in AGB, LAI, RGR and NAR. AGB data were log-transformed for statistical analysis to meet the assumptions of homoscedastic and normal distribution. For the presentation in text and figures, data were back-transformed. AGB statistical analysis was performed with a Poisson distribution and a link function logarithm. Generalized linear mixed models (PROC GLIMMIX) was used to analyze data in a randomized block split-plot in time design with fixed effects of cover crops species, days and their interactions (cover crops x days), and a random effect of block and the interaction block x cover crops. Separation of least square means was performed using Least significant difference (LSD) by Fisher at $\alpha = 0.05$. Regression analysis was performed between variables to evaluate the relationship. Statistical analyses for growth analyses were performed using SAS version 9.1 and the extension JMP ® version 14 (SAS Institute, Cary, NC). Soil surface coverage and weed suppression was analyzed with an ANOVA for each period ($\alpha = 0.05$) using InfoStat program (DiRienzo et al., 2018). Contrast analysis was performed to compare the effect of all cover crops instead of control plots without any coverage planted.

2.3 Results

Weather conditions

In 2017, the months after the planting of cover crops were followed by an active hurricane season. Figure 2.3 and Figure 2.4 show the weather conditions (precipitation, temperature and solar radiance) for the period of January 2016 to mid-September 2017. This data was collected with a Weather Station Kit (Onset ®) using a Hobo U30-WIF data logger with S-THB-M002 Temperature/Relative Humidity sensor and a S-RGA-M002 Rain Gauge. Data was provided by E. Jimenez (personal communication). However, the weather station was lost due to the strong winds of hurricane María, which made landfall on the east of Puerto Rico on September 20th, 2017. Rain patterns changed after the hurricane, causing a drought-stress in cover crops between December 2017 and early February 2018 (personal observation).

Plant growth analysis

Aboveground biomass (AGB) production of cover crops plant species (Figure 2.5) were significant different (F = 13.81 *p*-value < 0.0001). Difference among sampling dates also result significant different (F = 235.93 *p*-value < 0.0001) and the interaction cover crops × days was significant (F = 17.32 *p*-value < 0.0001). In the early development (16 and 48 DAP), *G. repens* was the highest in AGB, with a mean of 13.84 ± 11.42 g m^{-2} and 24.11 ± 9.24 g m^{-2} respectively. Besides, *S. trilobata* reflect an AGB of 3.77 ± 1.11 g m^{-2} at 16 DAP and 14.89 ± 7.72 g m^{-2} at 48 DAP. Similarly, *H. rotundifolia* did not reflect a significative difference from *S. trilobata* and had an AGB mean of 4.13 ± 1.67 g m^{-2} at 16 DAP and 14.11 ± 1.01 g m^{-2} at 48 DAP. The measured AGB dry weight at 175 DAP, was after the effects of the hurricane María. In this evaluation, cover crop species had similar dry weights and were not significantly different from each other. AGB of cover crop species after 229 days of establishment were significantly different from each other, with a dry weight of 366.6 ± 42.38 g m^{-2} for *S. trilobata*, 243.54 ± 52.39 g m^{-2} for *H. rotundifolia*, and 149.35 ± 59.09 g m^{-2} for *G. repens*.

Curves for growth analysis with natural logarithm of the AGB (Figure 2.6), showed an initial phase of growth in the first period, followed by a semi-plateau between 70-150 DAP, and then a linear increase. The curve did not approach a final plateau to indicate the plant's maturity. Furthermore, leaf area index (LAI) was significantly different among cover crops species (Table 2.3; F = 31.36 *p*-value < 0.0001) and in all sampling dates (F = 134.20 *p*-value < 0.0001). *G. repens* showed higher LAI than *H. rotundifolia* and *S. trilobata* until 175 DAP. In the evaluation 229 DAP, however *S. trilobata* had a significantly higher LAI than *G. repens*, but *G. repens* and *H. rotundifolia* were not significantly different from each other. A regression analysis (Figure 2.7) between AGB and LAI for all plant species reflect the fit of quadratic model instead a linear model. The goodness of fit was selected according the lowest root-means-square deviation and the highest coefficient of determination (R^2).

The relative growth rates (RGR) among cover crop species (Figure 2.8) were evaluated in periods. Each period consists of an interval of sampling dates. For example, the first period is an interval between the sampling evaluation at 0 DAP and 16 DAP, and the second period is an interval between the sampling evaluation at 16 DAP and 48 DAP. Ideally, periods were planned to be in equals intervals. However, the logistic after hurricane María impeded taking measures of periods with the same time intervals. Results were only significantly different between periods (F = 4.87 *p*-value = 0.0065). Among cover crops, RGR were not significant (F = 0.22 *p*-value = 0.8069) and neither the interaction of cover crops and periods was significant (F = 1.22 *p*-value = 0.3224). Although, differences were not significant among treatments, a trend was observed in the second period, were the RGR for *G. repens* decreased (-0.047 g/g^2/day), while the RGR for *S. trilobata* and *H. rotundifolia* increased (+ 0.401 g/g^2/day and + 0.308 g/g^2/day respectively). In the third period, RGR of all species decreased and then all species increased the RGR for the fourth period.

For the analysis of crop growth rate (CGR) presented in Figure 2.9, cover crops were significantly different (F = 9.80 *p*-value = 0.0005). In addition, CRG among periods was significantly different (F = 112.02 *p*-value < 0.0001). The CGR showed a decrease in the third period after hurricane María. However, cover crops in the fourth period showed a significant increase were *S. trilobata* was the plant species with highest CGR, resulting in a mean of 61.65 ±

8.73 g m^{-2} day^{-1}, H. *rotundifolia* reflect a CGR of 39.20 ± 13.63 g m^{-2} $day^{-1}$and G. *repens* showed a CGR of 19.88 ± 12.04g m^{-2} day^{-1}. Furthermore, the net assimilation rate (NAR) showed in Figure 2.10, was significantly different for the cover crops (F = 12.25 *p*-value = 0.0001). Difference among periods did not reflect a significant difference (F = 0.90 *p*-value = 0.4503). *H. rotundifolia* had higher NAR than *G. repens* and *S. trilobata*. However, *G. repens* and *S. trilobata* were not different from each other.

Soil surface coverage and weed suppression

Common weeds were identified with Más and Lugo-Torres (2013) and were personally confirmed by Lugo-Torres. Weeds among plots were *Cyanthillium cinereum* (L.) H. Rob. (little ironweed), *Digitalia horizontalis* Willd. (Jamaican crabgrass), *Paspalum conjugatum* P. J. Bergius (sourgrass), *Amaranthus dubius* Mart. ex Thell. (spleen amaranth) and *Cyperus rotundus* L. (purple nutsedge).

Soil surface coverage and weed suppression analysis reflected a significantly difference among the three periods. In the first evaluation (35 DAP), a contrast analysis showed that soil surface cover was significantly different (*p*-value = 0.0001) among all species and the control plots. The higher coverage area was not different between *G. repens* (38.25 ± 13.13 %) and *H. rotundifolia* (38.00 ± 10.08 %), meanwhile *S. trilobata* has a mean coverage area of 21.88 ± 5.54%. Weed percentage was higher in the control plot, with a mean of 43.75 ± 11.09 %, but was not different among plant species (Figure 2.11). For the second HW evaluation (111 DAP), the coverage area was significantly different (*p*-value = 0.0001) among plant species and the control plots. The species *G. repens* (50.75 ± 13.90 %) and *H. rotundifolia* (41.25 ± 8.54 %) were not significantly different, and *S. trilobata* (35.50 ± 5.54 %) was not significantly different of *H. rotundifolia.* Also, the weed biomass recovered between treatments was not significantly different (*p*-value= 0.1377). However, the prevalence of *G. repens* and *H. rotundifolia* having greater coverage area than *S. trilobata*, changed after eight months of establishment (257 DAP) where coverage area was significantly different (*p*-value < 0.0001), and *S. trilobata* (90.75 ± 4.63 %) and *H. rotundifolia* (84.00 ± 5.07 %) had the highest coverage. In this evaluation, *G. repens* decreased

its coverage area to 47.64 ± 13.60 %. The soil surface covered by weeds was significantly higher (*p*-value < 0.0001) in control plots (62.50 ± 3.54 %) than plots with *G. repens* (10.50 ± 7.65 %). Weed percent was significantly lower in plots with *H. rotundifolia* (1.25 ± 1.44 %) and *S. trilobata* (1.75 ± 2.36 %), but not different between both species.

Although percent of coverage by cover crops and weeds were different among treatments for the first evaluation, weed biomass recovered among different plots were not significantly different (Table 2.4; *p*-value = 0.1009). Eight months after the cover crops were established and 77 days from the last HW, the weed biomass was significant different between treatments (*p*-value = 0.0029). Control plots had the highest average of weed dry biomass (65.12 ± 33.98 g m^{-2}). The plots with *H. rotundifolia*, *S. trilobata* and *G. repens* were not significantly different among each other. Plots with *S. trilobata* had an average weed biomass of 2.96 ± 1.61 g m^{-2}, *H. rotundifolia* an average of 4.04 ± 2.96 g m^{-2} and *G. repens* an average of 18.30 ± 7.30 g m^{-2}.

The labor time between treatment plots was not significantly different in the first evaluation (Table 2.12; *p*-value = 0.7811), and neither was the labor time per block (*p*-value = 0.1113). Furthermore, the labor time for the second evaluation was not significantly different (*p*-value = 0.1230). In contrast to the first and second evaluation, the last evaluation was significantly different (*p*-value <0.0001). Labor time between blocks without considering treatment plots was not significantly different (*p*-value= 0.1310). Results indicate a reduction in labor time for hand weeding among cover cropped soil in contrast to control plots.

Pest evaluation

This evaluation was limited to the aboveground parts of the cover crops. Evaluations for pest infestations at 48 and 229 DAP were not suitable for statistical analysis due to the absence of insects in cover crops species. However, an extreme light infestation (according to Modiffied Cobb scale) of whitefly pest (*Bemisia* spp) was recorded on *G. repens* at 48 days. In addition, yellow strips were not formally evaluated because results could have been altered by a nearby compost project. This project was established near the plots two days after collocating yellow strips. A massive number of flies were in the strips of plots near the compost project (Figure 2.13).

2.4 Discussion

Growth analysis

The study of the growth analysis of the plant species: *G. repens*, *H. rotundifolia*, *S. trilobata* and *T. zebrina* as cover crops, partially addressed our hypotheses due to the effects of the hurricane María. The hypothesis for the respective objective were (i) After a period of five months, at least one of the three plant species will have greater percent of soil cover than the reference plant (*G. repens*) and (ii) *T. zebrina* will adapt better than *H. rotundifolia* and *S. trilobata* due to shade tolerance variability among cover crops species. Results suggest that: (i) After the eight-month period (254 DAP), one of the plant species (*S. trilobata*) had greater LAI implying a larger coverage area than the reference plant (*G. repens*) and (ii) *T. zebrina* did not adapt after the loss of shading conditions due to hurricane María, and the study could not evaluate its performance in contrast to the other plant species. Therefore, two main points related to the results are considered: (i) *S. trilobata*'s adaptation and phenotypic plasticity in different habitat conditions, and (ii) the low tolerance of solar radiance and drought conditions of *G. repens* and *T. zebrina.*

As shown in Figure 2.5, the aboveground biomass (AGB) of the perennial cover crops did not vary widely among all cover crops after eight months of establishment. AGB was higher in *G. repens* until the evaluation at 48 DAP. This evaluation was the last before the loss of shaded conditions on the banana field. In the following evaluation, at 175 DAP, the AGB of *G. repens* was not significantly different than the other species (*S. trilobata* and *H. rotundifolia*). This could be attributed to the elimination of the shade effect after hurricane María (56 DAP) considering that *G. repens* does not tolerate high amounts of irradiance (Marin and Veloz, 1999). For the last evaluation, 229 DAP, *G. repens* was the plant species with the least aboveground biomass.

The LAI results showed a similar trend to AGB. *G. repens* had a greater LAI than other plant species until 229 DAP, where *S. trilobata* resulted with a higher LAI than *G. repens* and *H. rotundinfolia* (Table 2.3). In its native range, populations of *G. repens* are limited to humid and well-shaded environments under canopy (Concepcion and Godoy, 2003; Teo et al., 2010). There are records of using *Geophila repens* successfully as ground cover in the shade of banana canopy (Waele et al., 2006; Fongod et al., 2010; Robinson and Sauco, 2010;).

LAI indicates the photosynthetic capacity of cover crops and is related to energy capture (Hunt et al., 2002; Weiss et al., 2004). As LAI increases, shading between leaves could increase, promoting a reduction in photosynthetic activity by part of the foliage, and decreasing the net assimilation rate (NAR) (Weiss et al., 2004). The NAR, a measure of photosynthetic efficacy, is the net gain in total dry matter per unit leaf area over time (Rajput et al., 2017). Results in this study, indicate that *H. rotundifolia* had a higher NAR than *S. trilobata* and *G. repens* in the last period. Even though the LAI was not statistically different between *H. rotundifolia* and *G. repens* in the last evaluation, the observed decrease in NAR for *G. repens* could be explained considering that *G. repens* is a plant species with bigger leaves than *H. rotundifolia*. In addition, *H. rotundifolia* generated more AGB than *G. repens* at 229 DAP (Figure 2.5). However, a regression analysis between LAI and NAR did not show a relationship in this study.

The increase in AGB and LAI of *S. trilobata* after 229 DAP, could be explained by its capacity of adaptation and phenotypic plasticity in different habitat conditions. In a study in China, where *S. trilobata* is an invasive species, Si et al. (2014) evaluated *S. trilobata* in different habitat conditions: full sunlight, shady conditions, and soils with different moisture capacity. Their results showed that *S. trilobata* has two major mechanisms that work together and allow it to colonize large environmental gradients: local adaptation and phenotypic plasticity. The phenotypic plasticity index (PI) shows the ability of a genotype to produce different phenotypes as an adaptation mechanism in response to variable environmental conditions. *S. trilobata* shows a PI of 0.64, approximately 30 percent higher in comparison with other invasive species in China (Funk, 2008; Si et al., 2014).

Increase in LAI could be a result of larger leaves and not necessary more aboveground biomass or leaves (Watson, 1947). However, a correlation analysis between LAI and AGB in this study indicate a strong positive relationship. The linear correlation coefficient of Pearson (r) for *G. repens*, *H. rotundifolia* and *S. trilobata* were 0.80, 0.96 and 0.98 respectively. The positive linear relationship of LAI with AGB has been previously reported (Ramírez-García, 2012). In all cover crops species, the AGB increased with the LAI. For the regression analysis, the quadratic models gave the best fit for regression of AGB and LAI (Figure 2.7). The coefficient of

determination was high in all cover crop species, indicating a simultaneous increase in AGB and LAI.

Leaf area has been related with crop growth rate (CGR) in previous studies (Triboullois et al., 2015). The relationship between CGR and LAI for the cover crop plants was significantly positive. *H. rotunfidolia* (R^2=0.78; *p*-value<0.0001) and *S. trilobata* (R^2=0.94; *p*-value<0.0001) showed a high relationship while *G. repens* showed a weaker relationship (R^2=0.48; *p*-value=0.0030). This is consistent with the study of Triboullois et al. 2015, where the functional growth traits in cover crops was evaluated. Their study suggested that plant species with a large leaf area grew more quickly than those with smaller leaves. The results of CGR at 229 DAP showed that *S. trilobata* was the cover crop with the highest CGR and larger leaf area. However, the CGR for *H. rotundifolia* and *G. repens* did not show a significant difference.

Functional growth traits such as LAI, NAR, CGR can be used to explore differences in the performance of perennial cover crop species from their establishment until maturity. Relative growth rate (RGR) has been related to leaf functional characteristics (Tribouillois et al., 2015). Cover crop species with high growth rates demand more resources, while species with low growth rates are more conservative with the resources which can be allocated under disturbance events (Poorter and Kitajima, 2007; Tomlinson et al., 2014). Although the RGR between plant species was only significantly different in the first period, results showed an interesting trend in the second period, where *G. repens* was the only cover crop species to show a decrease in RGR. This species had a greater AGB and LAI than the rest for the evaluations that compound the second period (16 and 48 DAP). However, *G. repens* showed a significant difference between the first period and the rest. This suggest that *G. repens* could have been growing under stress even before the disturbance of the hurricane (55 DAP). Study plants were supplemented with water during the first two weeks after they were planted into the field (first period of evaluations). Commonly, *G. repens* is used without irrigation systems and growth as a rainfed crop. However, taking results into consideration, *G. repens* has low tolerance to grow as a rainfed crop with the weather conditions while the study was conducted. According to these results, an evaluation of how this species respond in plots with irrigation versus plots without supplemented water is recommended. Also, the use of *G. repens* in areas with frequently precipitation could be favorable.

T. zebrina did not show signs of growth after losing the shadow conditions and eventually died. Consequently, this species was not considered for the statistical analysis. However, it's important to recognize that *T. zebrina* could be a potential cover crop under permanent shaded conditions. Vázquez-Moreno (2005) reported that *T. zebrina* var. zebrina Bosse was used as cover crop under the canopy of coffee plantations in Cuba. A study evaluating the allelopathic effects of *T. zebrina* on *Coffea arabica*, found that using *T. zebrina* stimulated the development of *C. arabica* plants, suggesting that it could be used intercropped in coffee agroecosystems (Navarro et al., 2013). According the results with *T. zebrina*, the hypothesis proposed were *T. zebrina* will adapt better than *H. rotundifolia* and *S. trilobata* due to shade tolerance variability was not accepted. Furthermore, a study with constant shaded conditions will be required to test this hypothesis.

Soil surface coverage and weed suppression

Commonly, an explanation for the weed inhibiting effects of cover crops is their competition for nutrients, light and space (Ekeleme et al., 2003; Bickesler et al., 2009; Kumar et al., 2009). The tendency is that weed density decreases with an increase of cover crop growth (Teasdale et al., 2007; Altieri, 2012; Price and Norsworthy, 2013). When cover crops are being established, they are well suited to compete with weeds for space. Cover crops in this study followed this trend during the first two evaluations, where weed density was not reduced by cover crop species. However, after eight months (257 DAP) of establishment, *S. trilobata* and *H. rotundifolia* increased its coverage area (Figure 2.11) and weed density decreased significantly in those plots.

According to a study by Teasdale et al. (1991), if cover crops produce a biomass of more than 200 g m^{-2} and had a ground cover greater than 90 %, weed infestation could be reduced by 78 % compared to treatments without cover crops. This is consistent with the results obtained, where after 257 DAP, *S. trilobata* had a mean ground coverage of 90.75%, while *H. rotundifolia* had a mean ground coverage of 84.00 %. At 257 DAP both plant species generated a higher biomass than 200 g m^{-2}, while *G. repens* had a biomass of 149 g m^{-2} (Figure 2.5). Although weed dry biomass for plots with *S. trilobata* and *H. rotundifolia* was not significantly different between

the evaluations in 35 and 111 DAP (Table 2.4), weed percent coverage resulted in a significant difference. Both species, *S. trilobata* and *H. rotundifolia* reduced weed infestation by 98 % at 257 DAP compared with control plots without a cover crop.

Living cover crops could alter the weed seed environment by changing radiance availability, soil moisture, temperature, and nutrient dynamics (Camaal-Maldonado et al., 2001; Hartwig and Ammon, 2012). Some plant species even have allelopathic effects (Creamer et al., 1996), that influence the growth, development and reproduction of an adjacent organism (Bullock, 1992; Cheng and Cheng, 2015; Sturm, 2018). Studies on *S. trilobata* have evaluated the allelopathic effect of this plant (Cabrera-Asensio and Bastidas-Lopez, 2000; Nie et al., 2002; Zhang et al., 2004; Wu et al., 2008). Macanawai (2013) attributes to the allelopathic properties of *S. trilobata* the reduction observed in germination and growth rate of plants. The influence of the allelopathic effect of *S. trilobata* has been evaluated in seedlings of rice (*Oryza sativa* L.) (Nie et al. 2004), peanut (*Arachis hypogaeae* L.) by Nie et al. (2002), and lettuce (*Lactuca sativa* L.) by Wu et al. (2008). Allelopathy could be a mechanism that provides to *S. trilobata* an advantage over the weeds emerging from the seed bank, resulting in better coverage than the other species as cover crops. Research or evidence that mentions any allelopathic properties by *G. repens* and *H. rotundifolia* have not been found.

The evaluation of HW (Table 2.5) showed that time of labor was not different among treatments after 35 DAP and 111 DAP. In the last evaluation (257 DAP) coverage of soil by cover crops was already higher than 50 %, reflecting a significantly reduction in the average of labor time in plots with cover crops. In this evaluation, the mean of labor time was higher for control plots than cover cropped soil. The results suggest that using the plant species *S. trilobata*, *H. rotundifolia* and *G. repens* as cover crops is an effective tool to reduce labor time of hand weeding the inner roads of banana plantations. Cover crops performance for weed control is one of the multiple benefits that can be provide if the selection is appropriate. Although living cover crops implies an initial cost, reduction in labor time is a long-term remuneration while maintain desirable soil properties (Snapp et al., 2005).

The objective of this section was to determine the effectiveness of the three species interfering with other plant species naturally present in the banana field. The hypothesis proposed

was that at least one of the three plants under study will have a significant difference in the weed suppressive effects than control plots. According the results, the hypothesis proposed was accepted, suggesting the effectiveness of *S. trilobata*, *H. rotundifolia* and *G. repens* as living cover crops to suppress weeds and reduce time of labor. Grime (1977) identified three main adaptive traits in plant species: competitive, stress tolerant and ruderal. Given the above, the results in this study reflect two of those traits with *S. trilobata* and *H. rotundifolia*. Both species were competitive with weeds from the seedbank in the field of study and were stress tolerant, growing under abrupt changes in the shade of banana plants.

2.5 Conclusions

It is important to recognize that this study was conducted after the effects of a major hurricane. This atmospheric phenomenon changed the shadow composition of the banana field and could not reflect the results if shadow conditions were consistent. Taking this into consideration, and according to the functional growth traits of plant species evaluated, *S. trilobata* and *H. rotundifolia* were the two species with the highest potential as living cover crops in a perennial banana (*Musa acuminata* AAA) var. Gran Nain field. Eight months after cover plant establishment, *S. trilobata* generated higher aboveground biomass than *H. rotundifolia* and *G. repens*. However, *H. rotundifolia* had higher photosynthetic efficiency according to NAR, suggesting that this plant species demanded more nutrients than *G. repens* and *S. trilobata*. The study demonstrated that LAI and AGB were correlated, suggesting that plant species with higher leaf area index generated more aboveground biomass than those with smaller leaves. Thus, strategies to reduce weeds with *S. trilobata* and *H. rotundifolia* as cover crops, should be reflecting suppression effects after the soil has been covered 80 % or more. According this study, living cover crops reduced significatively the labor time for hand weeding. The third specific objective, to examine if the plant species could be host of certain pest was not successfully accomplished. Following the aim of this study, to evaluate the potential of three common plant species in Puerto Rico as cover crops in a field of banana, the results indicated the significant effects of *S. trilobata* and *H. rotundifolia* as cover crops to cover soil and reduce weed density.

2.6 Tables y Figures

Table 2.1. Soil fertility analysis for the banana field in July 18, 2017 before planting cover crops.[1]

Parameters	Units	Value[2]
pH		6.86
Conductivity	µS/cm	385.2
Organic Matter	(%)	1.29
P available	mg P-PO_4/Kg	6.18 M
Calcium	mg Ca/Kg	3189 H
Magnesium	mg Mg/Kg	1340 H
Potassium	mg K/Kg	80 L
Sodium	mg Na/Kg	35
CICE	meq/100g	28

[1]Inform L-17-045 [2]Soil test categories L = low, M= Medium and H = High (Sotomayor-Ramírez, unpublished)

Table 2.2. Input application in the conventional crop of *Musa acuminata* in the experimental field.

Fertilizer applications	Fungicide applications
0-46-0 Fertilizer, 113.40 g per plant at planting	CILI 30 ml per 3.79 L– monthly
12-5-10 Fertilizer, four applications per year (162g, 243g, 323g and 162g)	Banana oil 60 ml per 3.79 L – monthly

Table 2.3. Leaf Area Index of cover crop species evaluated different days after planted (DAP)[1]

	Leaf Area Index (DAP)				
DAP[1]	0	16	48	175	229
S. trilobata	0.024 a (0.004)	0.031 b (0.006)	0.061 b (0.016)	0.230 a b (0.038)	1.227 a (0.170)
G. repens	0.024 a (0.009)	0.135 a (0.040)	0.199 a (0.038)	0.345 a (0.046)	0.619 b (0.025)
H. rotundifolia	0.008 b (0.001)	0.016 b (0.003)	0.027 b (0.003)	0.140 b (0.032)	0.400 b (0.020)
[2]LSD 5%	0.008	0.082	0.068	0.148	0.346
C.V. %	25.41	78.24	41.21	35.91	28.69

1= DAP = Days After Planting [1]The standard error of the mean is in parentheses below each mean. Means consist of four repetitions. Means within a column followed by the same letter are not significantly different based on the Fisher's 2 = Least Square Difference (LSD) at P ≤0.05. C.V. % is the coefficient of variation.

Table 2.4. Effects of cover crops species on weeds biomass and weed percent coverage in different evaluation after planting cover crops.

Component	Biomass g m^{-2} (Weed percent coverage)				
	35 DAP[1]	111 DAP [1]	257 DAP[1]	Mean[2]	35 vs 257 DAP
Control	36.87 (43.75) a	144.78 (50.00)	65.12 a (62.75) a	82.26	0.1756 (0.0167)
S. trilobata	23.28 (26.75) b	119.21 (20.75)	2.96 b (1.75) c	48.48	0.0008 (0.0003)
G. repens	21.61 (22.50) b	57.86 (19.25)	18.30 b (10.75) b	32.59	0.5371 (0.0608)
H. rotundifolia	18.62 (17.25) b	85.58 (18.75)	4.04 b (1.50) c	36.08	0.0028 (0.0333)
p-value	0.1009 (0.0172)	0.1377 (0.0003)	0.0029 (<0.0001)	--	--
LSD 5%	-- (15.21)	-- 10.99	29.12 (7.62)	--	--

[1]Means consist in four repetitions. [1]Means within a column followed by the same letter are no significantly different base on the Fisher's Least Square Difference (LSD) at P ≤0.05) [2] Means consist in 12 observations.

Table 2.5. Mechanical hand weeding labor time in different periods. [123]

Component	Labor time (min)		
	35 DAP[3]	111 DAP[4]	257 DAP[5]
Treatments	0.9146	0.2698	<0.0001
Control	29.25	48.25	41.00 a
S. trilobata	27.50	56.25	2.78 b
G. repens	33.50	57.25	5.84 b
H. rotundifolia	29.50	37.75	2.70 b
Blocks	0.1310	0.9123	0.1555
Workers	0.4017	0.4478	0.9236

[1]Means of labor time consist in four repetitions. [2] Means within a column followed by different letters are significantly different base on the Fisher's Least Square Difference (LSD) at P ≤0.05) [3] Evaluation 35 DAP (First MHW) [4]Evaluation 76 days of the last Hand Weeding (HW) [5]Evaluation 77 days of the last HW.

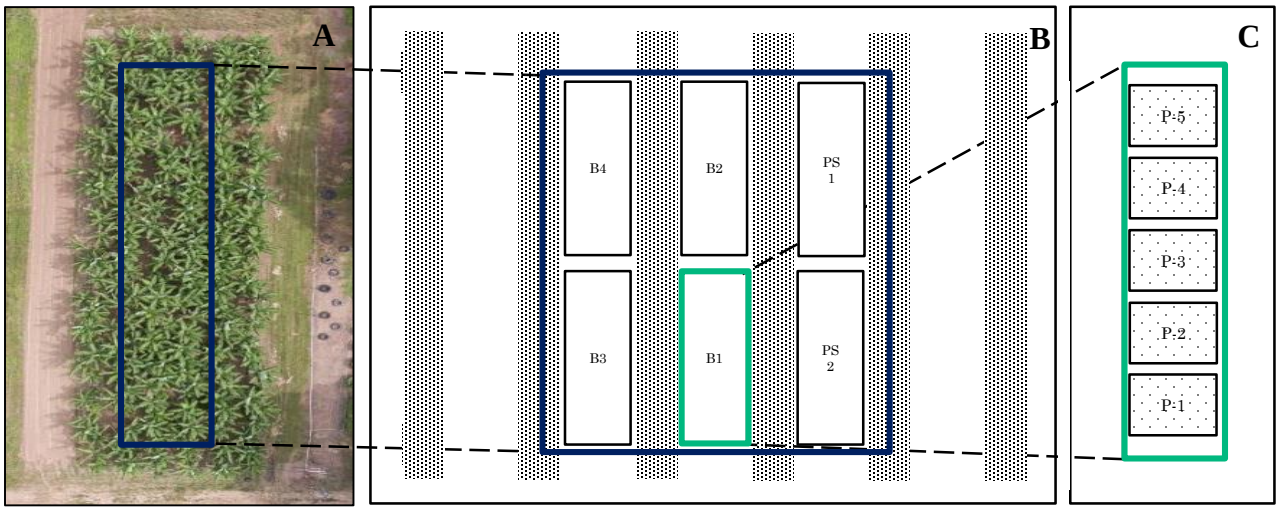

Figure 2.1. A. An aerial photograph of the study site in the Sub-experimental Station in Gurabo (Perez, 2017) **B.** Field experimental design. B is Blocks, PS is Pilot Study area. The area for the study was the four blocks. **C**. An example of the arrangement in one block, where P is plot.

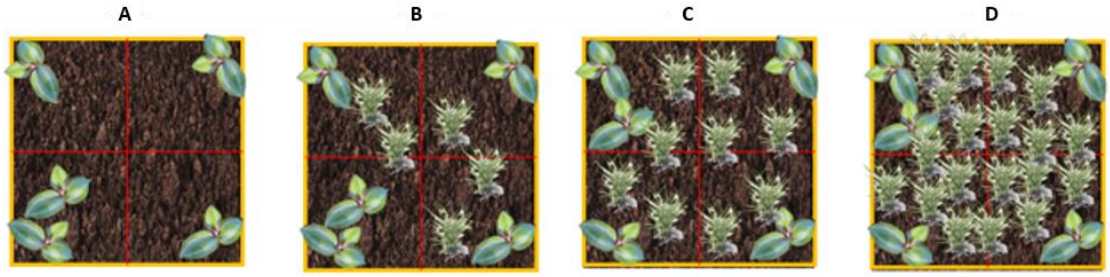

Figure 2.2. Guide for the visual assessment of weed infestation as a percentage of ground cover. The percentages were not strictly to the levels presented in the figure. The figure helps to approach a quantitative number in the visual evaluation. **A**. 0% weed coverage and 25% covered by cover crop plant. **B**. Ground has 25% of cover crop and 25% of weed coverage. **C**. 25% of coverage is by cover crops and 50% is covered by weeds. **D**. Cover crops has 25% of ground cover and 65% is weed coverage.

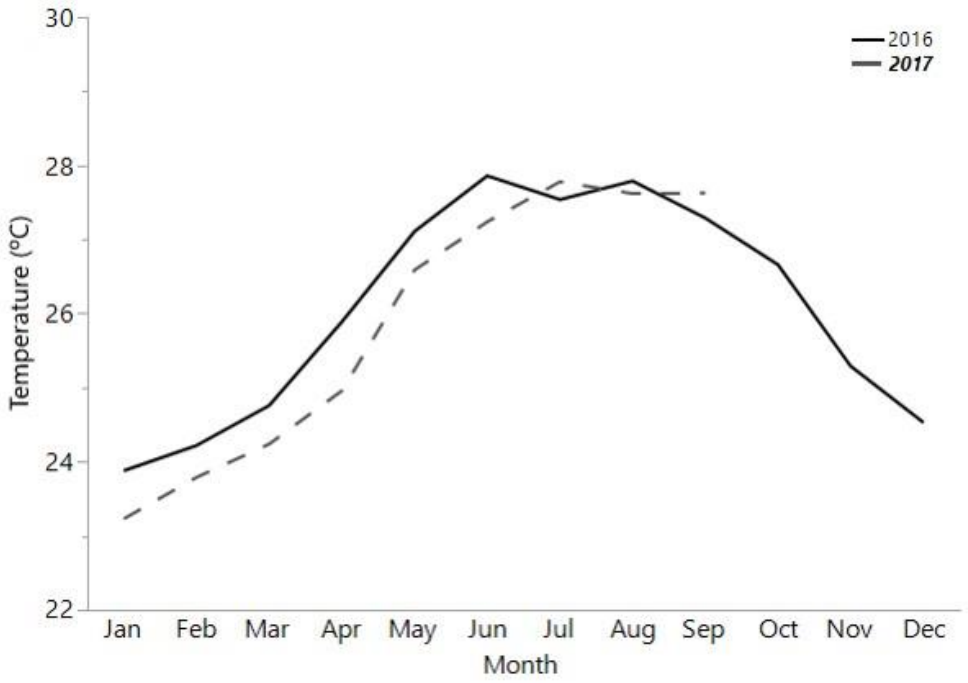

Figure 2.3. Temperature (°C) record at the Gurabo Agricultural Experiment Substation between 2016 and 2017.

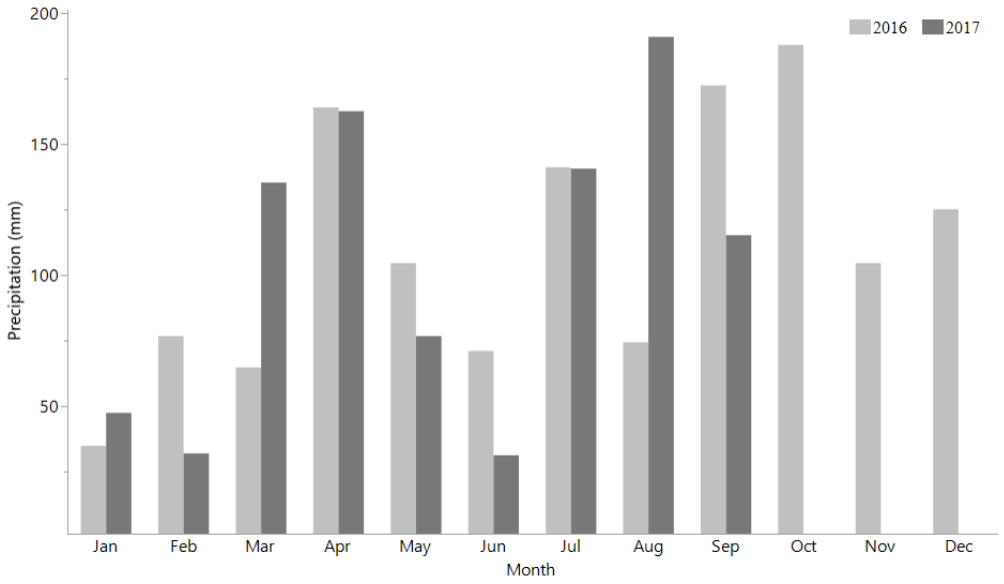

Figure 2.4. Average monthly precipitation (mm) in Gurabo Agricultural Experiment Substation between 2016 and 2017.

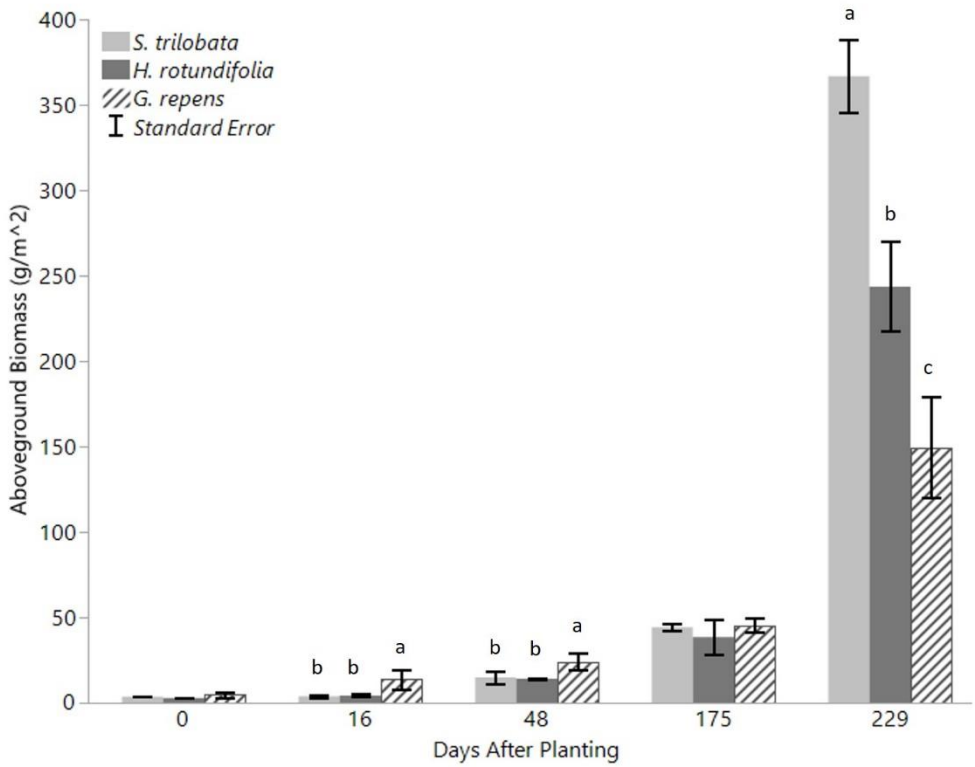

Figure 2.5. Aboveground biomass (AGB) of plant species trough days after planting (DAP). Different case letters indicate significant differences between plant species in the sampling event ($P \leq 0.05$).

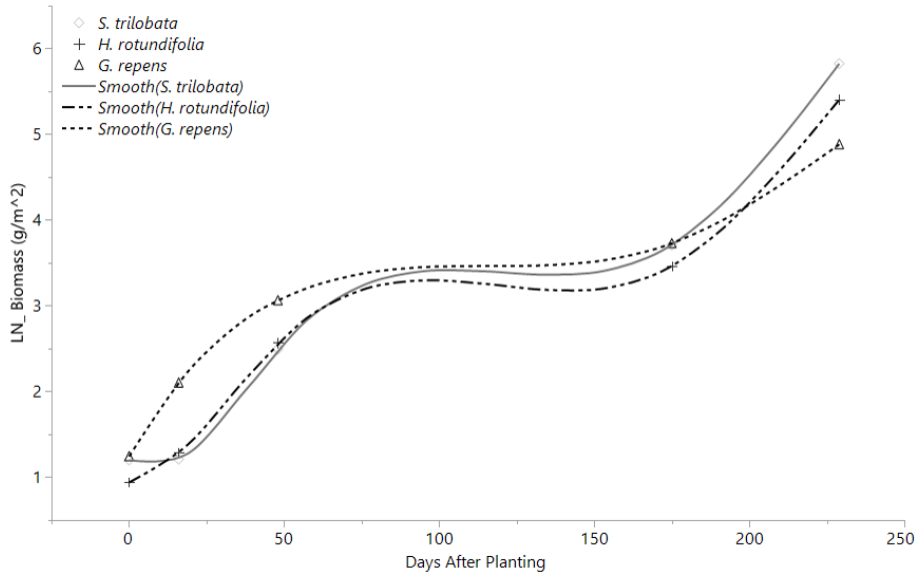

Figure 2.6. Growth curve of plant species with the natural logarithm of aboveground biomass.

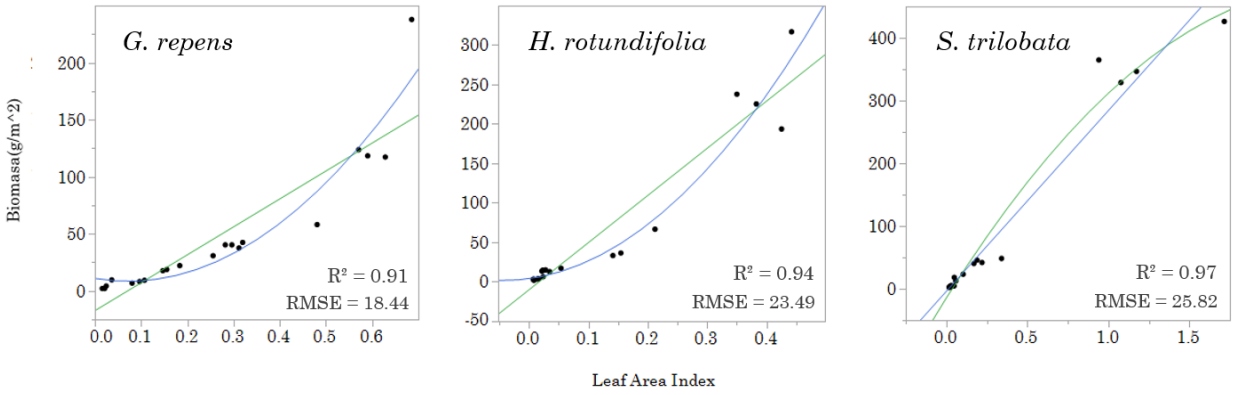

Figure 2.7. Quadratic model goodness of fit (blue lines) of regression analysis between Biomass (AGB) and LAI for all plant species. Root-means-square deviation and the coefficient of determination are presented below each curve.

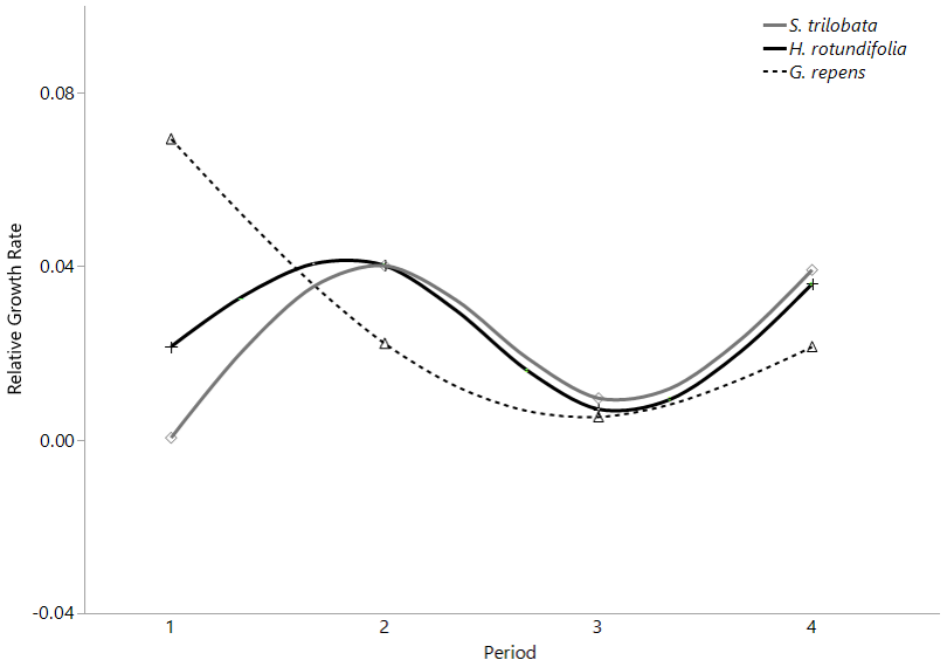

Figure 2.8. Relative Growth Rate (RGR) of cover crop species in four periods between eight months.

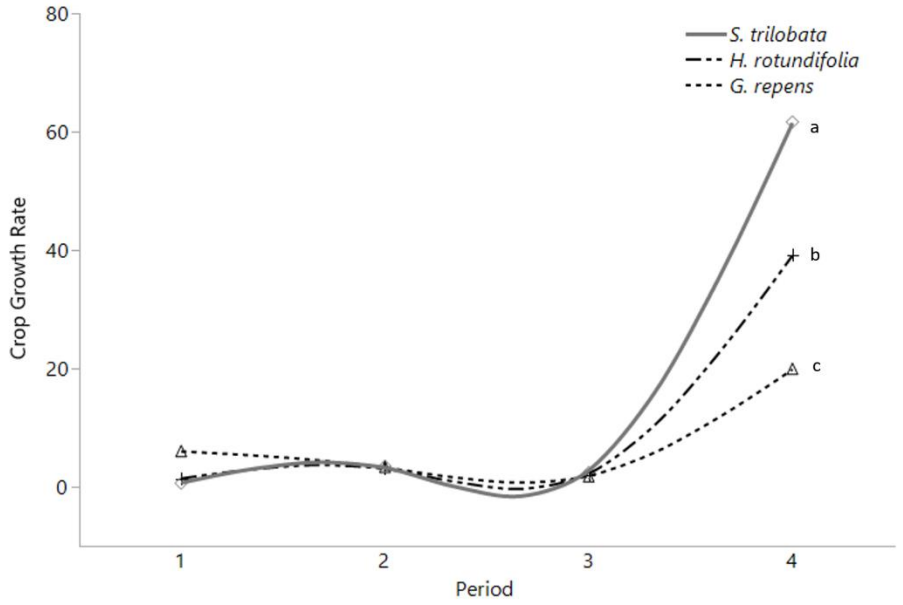

Figure 2.9. Crop Growth Rate (CGR) of plant species as cover crops in four periods between eight months.

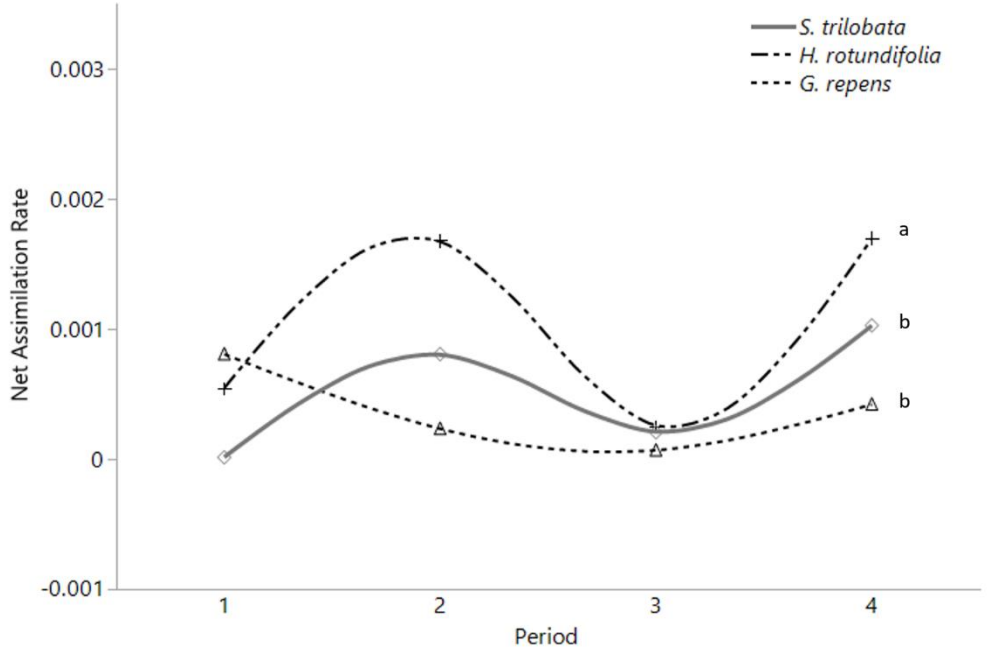

Figure 2.10. Net Assimilation Rate (NAR) of plant species in four periods between eight months.

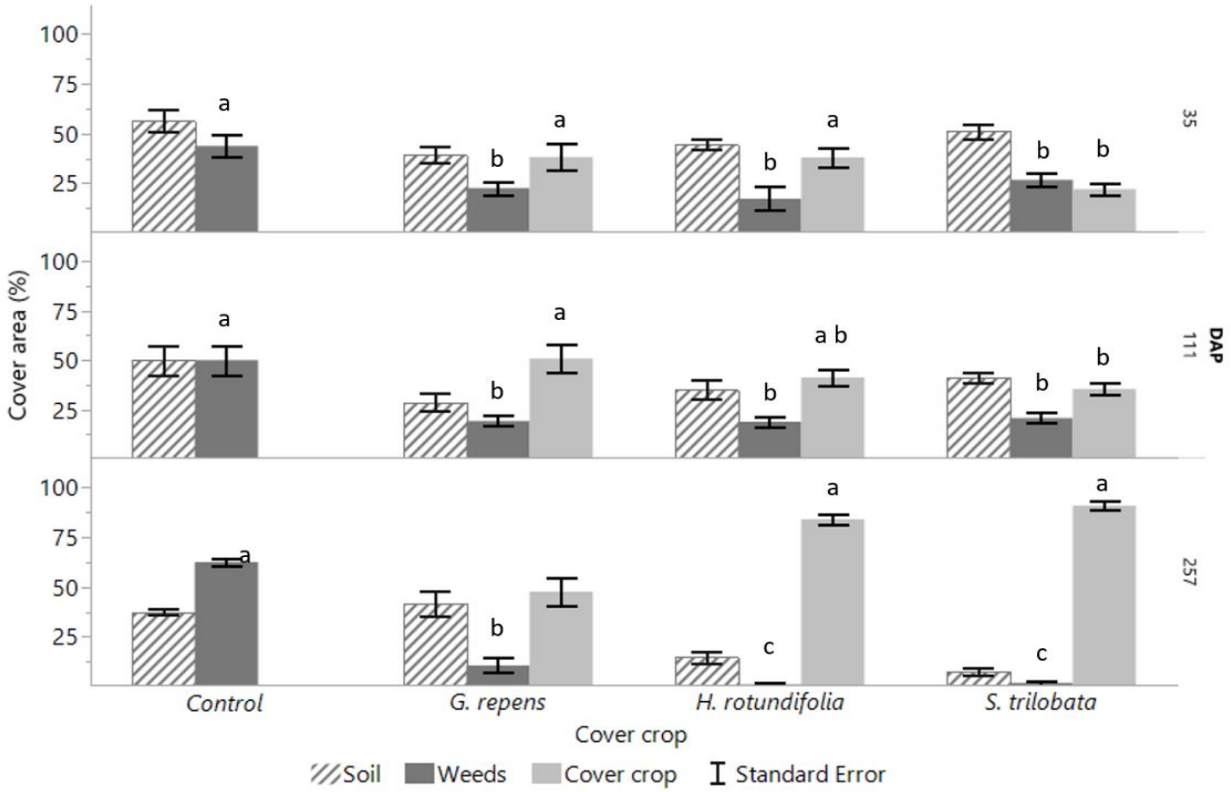

Figure 2.11. Percent of weed, soil and treatment coverage in a banana plantation in three periods. **1**. 35 DAP was conducted in August 30, 2017; **2**. 111 DAP was conducted in November 14th, 2017 and **3**. 257 DAP was conducted in April 9, 2018. Within period treatments means followed by the same letter are not significantly different based on Fisher's Least Square Difference (LSD) at *p*-value ≤ 0.05.

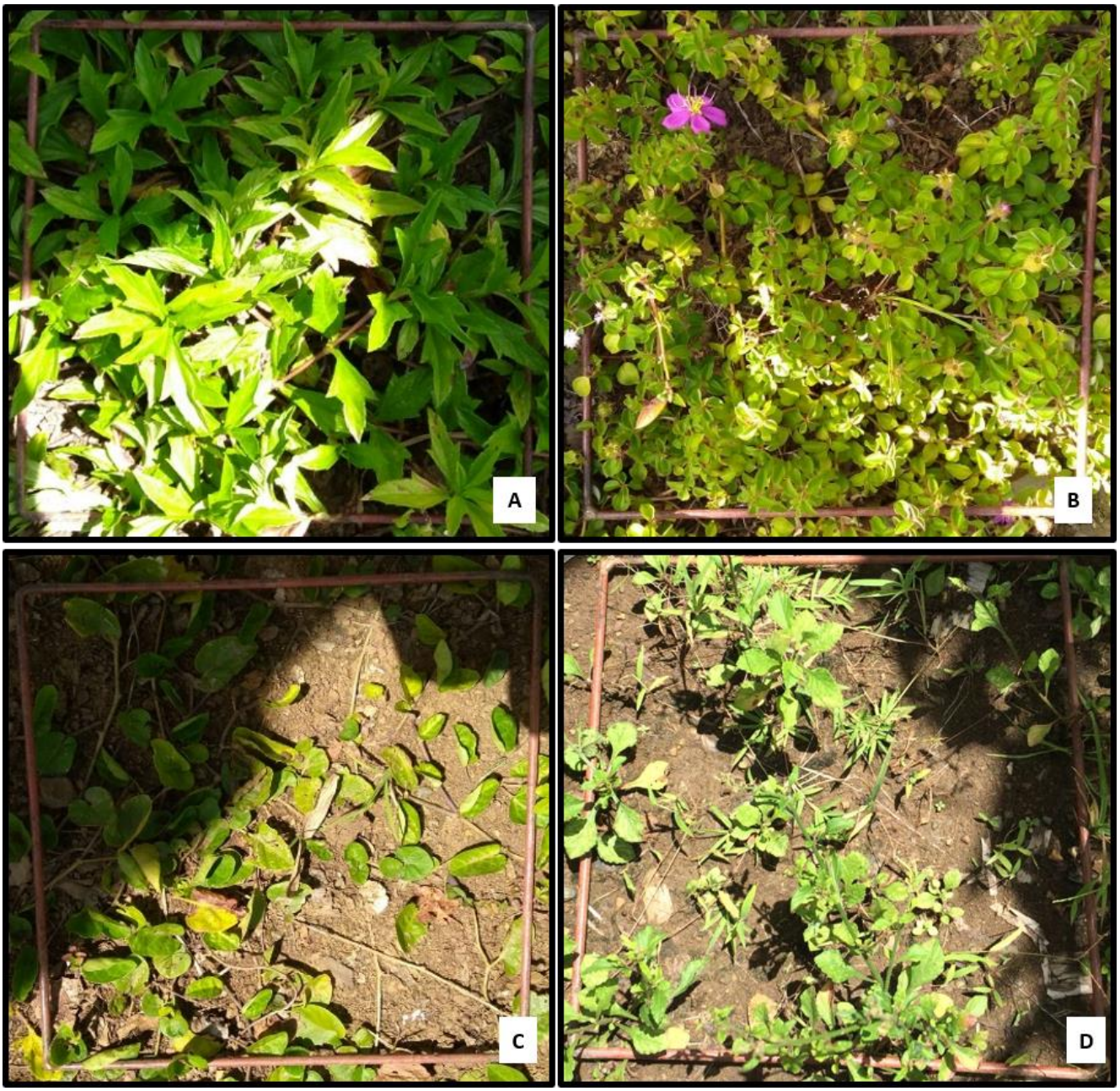

Figure 2.12. Visual assessment of soil surface coverage for treatments after eight months of planting cover crop species. **A**. *Spagneticola trilobata*. **B**. *Heterotis rotundifolia*. **C**. *Geophila repens*. **D**. Control plots without cover crops.

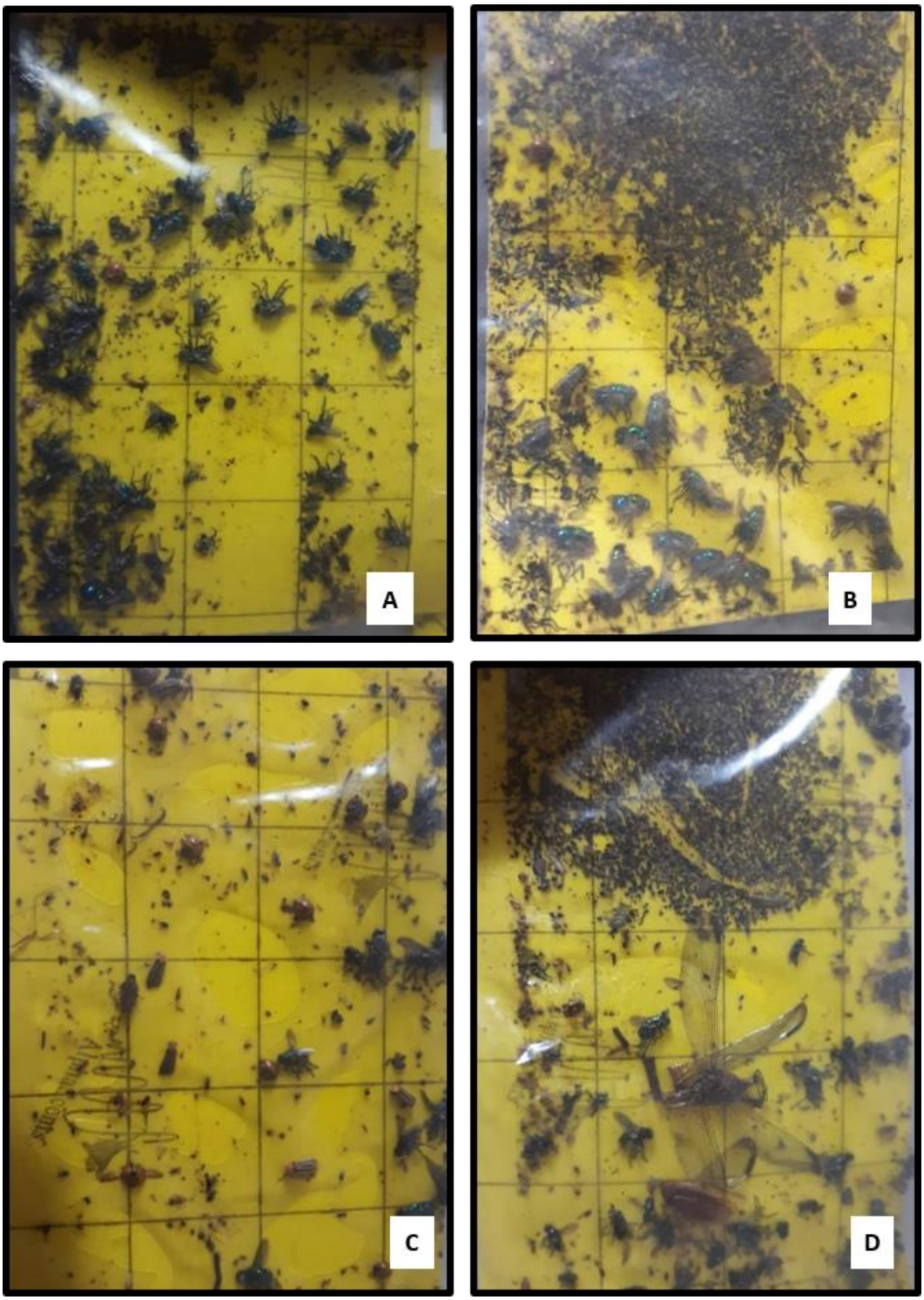

Figure 2.13. Example of yellow strips for each treatment for the block 1. **A**. Control **B**. *Geophila repens*. **C**. *Heterotis rotundifolia*. **D**. *Spagneticola trilobata*.

2.7 References

Altieri, M.A. 2012. Insect pest management in the agroecosystems of the future. *Atti Academia Nazionale Italiana di Entomologia*. 137−144

Alexander, A.G., Alison, W., Garcia, M., Velez, A., Ramirez, G., Chu, T.L., and Vel, J. 1979. Production of sugarcane and tropical grassess renewable energy source. University of Puerto Rico. Center for Energy and Environment Research

Bicksler, A., and J. Masiunas. 2009. Canada thistle (*Cirsium arvense*) suppression with buckwheat or sudangrass cover crops and mowing. *Weed Technology, 23*(4):556-563. doi:10.1614/WT-09-050.1

Blanco-Canqui, H., T.M. Shaver, J.L. Lindquist, C.A. Shapiro, R.W. Elmore, C.A. Francis, and G.W. Hergert. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agron. J.* 107: 2449-2474

Bullock, D.G. 1992. Crop rotation. Critical Reviews in Plant Sciences. 11(4):309-326

Cabrera-Asensio, I. and H. Bastidas-López. 2000. Effect of Wedelia trilobata L. (Asterales:Asteraceae) on the whitefly *Bemisa tabaci* G. (Homoptera:Aleyrodidae) *J. Agr. U. Puerto Rico.* 84(3-4)181−184

Camaal-Maldonado, J.A., J.J., Nez-Osornio, A. Torres-Barragá, A.L. Anaya. 2001. The use of allelopathic legume cover and mulch species for weed control in cropping systems. Agronomy Journal. 93. (1): 27−36

Carlo-Acosta, S.T. 2009. Promoting the use of tropical legumes as cover crops in Puerto Rico. Thesis M.S. University of Puerto Rico, Mayagüez Campus

Cheng, F. and Z. Cheng. 2015. Research Progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanism of allelopathy. Front Plant Sci. 6:1020. doi:10.3389/fpls.2015.01020

Creamer, N.G. and Baldwin, K.R. 2000. An evaluation of summer cover crops for use in vegetable production systems in North Carolina. *Hort Science*. 35(4):600−603

Di Rienzo, J.A., Casanoves, F., Balzarini M.G., González L., Cuadroda, M. y Robledo C.W. InfoStat versión 2018. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar.

Ekeleme, F., I.O. Akobundu, O. Isichei, D. Chikoye. 2003. Cover crops reduce weed seedbank in maize-cassava systems in southwestern Nigeria. *Weed Science*. 51:774−780

Fongod, A.G.N., D.A. Focho, A.M. Mih, B.A. Fonge, P. Sama-Lang. 2010. Weed management in banana production: The use of *Nelsonia cenescens* (Lam.) Spreng as a non-leguminous cover crop. *Afr. J. Environ. Sci. Technol*. 4(3):167−173

Funk J.L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. J. Ecol. 96(6):1162−1173

Grime, J.P. 1977. Evidence for the existence of three primaty strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*. 111(982):1169-1194

Hunt, R. 1990. Basic growth analysis. Unwin Hyman, London.

Hunt, R., D.R. Causton, B. Shipley, A.P Askew. 2002. A modern tool for classical plant growth analysis. *Annals of Botany.* 90(4):485−488

Kumar, N.S. 2009. Effects of plant density and weed management practices on production potential of groundnut (*Arachis hypogaea* L.) *Indian J. Agr Sci.* 43(1) 57−60

Lambers, H., F.S. Chapin III, T.L. Pons. 2008. Chapter 5: Growth and Allocation. *Plant Physiological Ecology.* 321−371

Ozalkan, C., T.S. Hasan, D. Ihsanullah and O.F. Sen. 2010. Relationship between some plant growth parameters and grain yield of chick pea (Cicer arietinum L.) during different growth stages. Turkish Journal of Field Crops. 15(1):79−83

Macanawai A.R. 2013. Impact of *Sphagneticola trilobata* on plant diversity in soils in south-east Viti Levu, Fiji. *Journal of Life Sciences.* 7(6):635−642

Marin, E. and Veloz, D. 1999. Establecimiento de la especie silvestre Geophyla repens como cobertura viva en el cultivo de banano. Universidad Earth. Guácimo, Costa Rica

Más, E.G. and M.L. Lugo-Torres. 2013. Malezas comunes en Puerto Rico e Islas Virgenes Americanas. Universidad de Puerto Rico, Recinto Universitario de Mayaguez y USDA Servicio de Conservacion de Recursos Naturales, Área del Caribe

Muñoz, M.A., W.I. Lugo, C. Santiago, M. Matos, S. Ríos, J. Lugo. 2018. Taxonomic classification of the soils of Puerto Rico, 2017. University of Puerto Rico, Mayaguez Campus

Navarro, G.B., Pelie, L.C. and Favier, M.M. 2013. Efectos alelopáticos de dos coberturas vivas *Commelina difusa* Burm. F. Y *Tradescantia zebrina* Shunltz sobre *Coffea arabica* L

Nie C.R., H.S. Li, J.H. Huang, L.Q. Cheng and M.Q. Hong. 2002. Studies of the allelopathic effects of *Wedelia chinensis merr*. on peanut (*Arachis hypogaea* L) and other c rops. *Journal of Peanut Science* 19, 30−32

Nie C.R., R.S. Zeng, S.M. Luo, H.S. Li, M.Q. Hong, L.Q. Cheng. 2004. Allelopathic potentials of *Wedelia trilobata* L. on rice. *Acta Agronomica Sinica.* 30:942−946

Poorter, L. and K. Kitajima. 2007. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology*. 88(4) 1000-1011

Price, A., J. Norsworthy. 2013. Cover crop for weed management in southern reduced-tillage vegetable cropping systems. *Weed Technology.* 27:212−217

Rajput, A., SS., Rajput and G., Jha. 2017. Physiological parameters leaf area index, crop growth rate, relative growth rate and net assimilation rate of different varieties of rice grown under different planting geometries and depths in SRI. *Int. J. Pure. App. Biosci*. 5(1):362−367

Ramírez-García, J., P. Almendros, M. Quemada. 2012. Ground cover and leaf area index relationship in a grass, legume and crucifer crop. *Plant Soil and Environment*. 58(8):385−390

Robinson, J.C. and V. Galán Saúco. 2010. Chapter 3: Morphological characteristic and plant development. *Bananas and Plantains.* 2nd Edition. 51−66

SAS, Statystical Analysis System. 2004. SAS Institute Inc. SAS/STAT® 9.1. User's Guide Cary, NC: SAS Institute Inc. Cary, N.C., USA.

Si, C.C., Z.C. Dai, Y. Lin, S.S. Qi, P. Huang, S.L. Miao, D.L. Du. 2014. Local adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical island. *Annals of Botany* 114:425–433

Snapp, S., S. Swinton, R. Labarta, D. Mutch, J.R. Black, R. Leep, J. Nyraneza, and K. O'Neil. 2005. Review and Interpretation: Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agron. J*. 97:322−332

Soil Survey Staff, Natural Resources Conservation Services, United States Department of Agriculture. Web Soil Survey.

Sturm, D.J., G. Peteinatos, R. Gerhards. Contributions of allelopathic effects to the overall weed suppression by different cover crops. Weed Research. 58(5) doi: 10.1111/wre.12316

Teasdale, J.R., C.E. Beste, W.E. Potts. 1991. Response of weeds to tillage and cover crops residue. *Weed Sci*. 39:195−199

Teasdale, J.R., L.O. Bransaeter, A. Calegari and F. Skora-Neto. 2007. Chapter 4: Cover crops and weed management. Non-chemical weed management. CAB International, Cambridge USA 49−52

Teo, S., B.R. Kurukulasuriya, H.T.W. Tan. 2010. The distribution and status in Singapore of the snake pennyworth, Geophila repens (L.) I.M. Johnst. (Rubiaceae) Nature in Singapore. 3:183−186

Tessmer, O.L., Y. Jiao, J.A. Cruz, D.M. Kramer, and J. Chen. 2013. Functional approach to high-throughput plant growth analysis. BMC Systems Biology. 7(6) S17 https://doi.org/10.1186/1752-0509-7-S6-S17

Tomlinson, K.W., L. Poorter, F. Bongers, F. Borghetti, L. Jacobs and F. van Langevelde. 2014. Relative growth rate variation of evergreen and deciduous savanna tree species is driven by different traits. Annals of Botany. 1−10

Tribouillois H. F. Fort, P. Cruz, R. Charles, O. Flores, E. Garnier et al. 2015. A functional characterization of a wide range of cover crops species: growth and nitrogen acquisition rates, leaf traits and ecological strategies. Plos One. 10(3): e0122156.

Vázquez-Moreno, L.L. 2005. Experiencia cubana en el manejo agroecológico de plagas en cafeto y avances en la broca del café. Simposio sobre Situación Actual y Perspectivas de la Investigación y Manejo de la Broca del Café en Costa Rica, Cuba, Guatemala y México. J.F. Barrera (ed.). Sociedad Mexicana de Entomología y El Colegio de la Frontera Sur Tapachula Chiapas, México, 46−57

Waele, D., Stoffelen, R. and Kestemont, J. 2006. Effects of associated plant species on banana nematodes. InfoMusa 15:1−2

Watson, D.J. 1947. The dependence of net assimilation rate on leaf area index. Annals of Botany. 22(85):37−64

Weiss, S. 2006. Developing Sustainable Tropical Leguminous Cover Crop and Green Manure Mulch Systems for Low-External-Input crop Production in the U.S. Virgin Island, Puerto Rico, and Florida. Final Report for LS12-252. SARE https://projects.sare.org/project-reports/ls12-252/

Wittwer, R.A., B. Dorn, W. Jossi, and M. Van Der-Heiden. 2017. Cover crops support ecological intensification of arable cropping systems. Sci. Rep. 7: 41911 doi: 10.1038/srep41911

Wu, J.R., S.L Peng, H.B. Zhao, and H.L. Xiao. 2008. Allelopathic effects of *Wedelia trilobata* residues on lettuce germination and seedling growth. *Allelopathy J.* 22:197–204

Zhang, Y.H., M.F. Liu, T.J. Ling, X.Y. Wei. 2004. Allelopathic sesquiterpene lactones from *Wedelia trilobata*, Journal of Tropical and Subtropical Botany 12 :533−537

Chapter 3: Short-term effects of the plant species on biological soil properties

3.1. Introduction

The use of cover crops has been demonstrated as a management tool to enhance soil quality and health (Six et al., 2006; Liu et al., 2014; Lehman et al., 2015; Finney et al. 2017). Soil organisms have been commonly used to evaluate the quality and health of soils, due to their ability to respond to management techniques (Finney et al., 2017). Kong et al. (2012) and Six et al. (2006) reported how cover crops influence microbial communities by providing resources such as carbon and habitat. For the evaluation of new cover crops, the use of soil biological parameters such as enzyme activity, microbial communities and soil organic carbon inputs, are key tools to assess short-term effects on soil health due to their relationship with soil functions and nutrient cycling (Spedding et al., 2004; Shi et al., 2018).

Studies have shown how microbial biomass, the living component of soil organic matter (Rice et al. 1996), responds to vegetation type (Finney et al., 2017; Li et al., 2018). Although cover crops are generally chosen based on their growth traits, adaptation and weed control capacity, several studies have been focused on how cover crops shape microbial communities and could promote ecological functions (Tejada et al., 2008; Acosta-Martínez et al., 2011; Finney et al., 2017; Li et al., 2018). In addition, short-term effects of cover crops and organic amendments could increase labile carbon (Gattinger et al., 2012). According to Margenot et al. (2015), Robertson and Paul (2000) and Drinkwater et al. (1998), labile soil organic matter has a greater short-term response to soil management than total soil organic carbon.

Enzyme activity could be evaluated through various enzymes such as phosphatase, urease, dehydrogenase, arymidase and β-Glucosidase. The enzymes present in the soil have multiple sources, including plants, animals and microorganism (Ferraz de Almeida et al., 2015). The use of dehydrogenase (DHA) to measure the enzyme activity has been promoted because DHA has been characterized as a tool for assessing soil quality and microbiological functions. Dehydrogenase

does not accumulate in the soil and is highly sensitive to changes in the soil environment (Wolińska and Stępniewska, 2012; Adetunji et al., 2017). *β*-Glucosidase has also been used as soil quality indicator. *β*-Glucosidase enzymes are cosmopolitan, widely distributed in ecosystems. This enzyme has been associated with carbon dynamics and is a member of glucosidases enzyme group that catalyze the hydrolysis of glycosidic bonds, which provides a source of energy for soil microorganisms. (Ferraz de Almeida et al., 2015). Enzymatic activity may respond and reflect short-term effects of soil management techniques more than other soil variables (Hai-Ming et al., 2014).

The main objective of the present study was to evaluate and characterize the short-term effects of three plant species (*Hetetoris rotundifolia*, *Spagneticola trilobata* and *Geophila repens* as reference plant) used as cover crops to improve soil biological properties in a banana (*Musa acuminata* AAA) field. *Geophila repens* is a perennial creeper, widely used as cover crops in banana fields in Costa Rica, Ecuador y Panama because doesn't climb up the banana plants, reduces soil erosion and it does not host any banana pest (Fongod et al., 2010). However, the slow growth rate has been considered as a disadvantage (Wielmaker et al., 1997; Fongod et al., 2010). Contrary, *Heterotis rotundifolia* is a fast-growing and climbing perennial plants, evaluated before as ground cover for coffee farms in Puerto Rico (Ramos et al., 2014). The other plant species, *S. trilobata* is a creeping perennial clonal herb, mat-forming with fast-growing stems (Si et al., 2014). Studies with *S. trilobata* have found that long-term effects of this species could increase soil organic matter (Wang et al., 2015) and accelerate the succession of soil microbial communities in their rhizosphere (Si et al., 2013).

The specific objectives were: (1) to assess the short-term (eight months) effects of the cover crop in soil organic carbon and soil organic matter, (2) measure and compare microbial biomass carbon among cover crops and control plots without cover crops (3) evaluate soil enzyme activity through *β*-glucosidase and dehydrogenase measurement (4) evaluate the effects of plant species on the size and composition of the soil microbial community through phospholipid fatty acid (PLFA) analysis, and (5) characterize in a qualitative and quantitative analysis the soil organic matter functional groups among cover crops by using mid-infrared spectroscopy. The hypothesis suggested for the respective objectives were that after the establishment of the plant species as

cover crops: (1) at least one species will promote a higher soil organic carbon (SOC) than the reference plant, *Geophila repens*; (2) microbial biomass carbon will be higher in cover cropped soil than control plots (3) soil enzyme activity will be higher in cover cropped soil than control plots; (4) size and composition of microbial community will be different among treatments with cover crops and control plots; size of microbial community will be greater in *H. rotundifolia* and *S. trilobata* than plots under reference plant *G. repens*, and (5) soil organic matter functional groups will be different by the influence of compounds brought into the soil by the plant species.

3.2. Materials and Methods

Study site

The study was performed at the Gurabo Agricultural Experiment Substation of the University of Puerto Rico (18.2534 N, -65.9896 W). The area is located at the agricultural coastal plains zone in eastern Puerto Rico, where the mean annual temperature is 25.2º C and annual precipitation is 1869 nm (National Weather Service, 2014). The experimental field had been planted with sugarcane (*Sacchararum officinarum* L.) beginning in the 1970's (Alexander et al., 1979). Within the last 10 years, the field was planted with banana (*Musa acuminata*) and other farinaceous crops such as plantain (*Musa* spp AAB), taro (*Colocasia escualenta*), tannier (*Xanthosoma* spp.), cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batata*) (Ortiz, C. Ortiz, personal communication).

The soil in the experimental field is a Vertisol classified as Mabi series (Very-fine, mixed, active, isohyperthermic Aquic Hapluderts) (Muñoz et al., 2018; Soil Survey Staff, 2017). Soils of this series are mainly found on alluvial fans or terraces of the Humid Coastal Plains and are characterized as very deep clay soils with slow permeability and poor drainage capacity (Soil Survey Staff, 2006). In the Table 3.1, the chemical properties of soil from 0-20 cm depth are presented. This analysis was conducted by the Central Analytical Laboratory in the Río Piedras Agricultural Experimental Station of the University of Puerto Rico before the experiment started.

Agricultural system and cover crops

The study required an established semi-perennial crop to evaluate the growth response of new cover crops to a shaded field. For this purpose, a crop of banana (*Musa acuminata* AAA*)* var. Gran Nain was planted on February 11, 2016. The experimental field was managed under conventional practices, with fertilization and fungicides applications. Tillage practices were applied before planting with a rotary tiller Bush Hog ® RTS 40-04.

As the banana crop grew, a pilot test (Appendix 2) was conducted to evaluate the most efficient techniques to propagate different plants species. For this study, three new plants species (treatments) were evaluated for intercropping in banana plantations. The plants under study as cover crops were: pink lady (*Heterotis rotundifolia*), wedelia (*Spagneticola trilobata*) and zebra plant (*Tradescantia zebrina*). In addition, *Geophila repens* was used as a reference plant, due its widely known effectiveness as a cover crop in banana and shaded fields (Waele et al., 2006). Plant specimens were obtained from the nursery in the Experimental Substation of Gurabo.

Experimental design

The field had five roads between the six rows of *M. acuminata* AAA. In every row, 34 banana ramets ("suckers") were planted every 1.83 m. However, for this study the three innermost roads were used to prevent border effects caused by solar radiation (Figure 3.1). The experimental design was arranged in a randomized complete block design with four replicates. Each block was divided into five experimental plots (4 m x 2.15 m each), four of them with the plants under study as cover crops, and one without a cover crop as a control. Between each plot, border rows of 1 m were designed to avoid border effects from the adjacent cover crops (treatments). Each experimental plot had plants of a single species, which were cut at a standardized size (12 cm) and planted every 20 cm. Study plants were supplemented with water during the first two weeks after they were planted into the field. Cover crops were planted in July 26th of 2017, 17 months' after the banana plants were planted.

Soil Sampling and Processing

To accomplish the soil analysis related to the objectives of this study, soil samples were collected in three sampling dates: July 25th, 2017 (before planting plant species), November 6th, 2017 (103 days after planting (DAP)) and April 9th, 2018 (254 DAP). From each plot, six soil sub-samples were collected from random locations and combined into a single soil sample. Before sampling, the surface debris and plants were carefully removed. Soil samples were placed in plastic

bags and stored in a compartment with ice packs to transport to the Environmental Chemistry Laboratory at the Agricultural Experimental Station (ECL-AES) in Río Piedras. For each composite sample, a subsample of about 50 g of fresh soil was used to measure the soil water content at 105 °C for 48 hours. Afterward, each sample was divided in two parts: one sub-sample was used for soil organic carbon (SOC) and enzyme activity analysis, and the second sub-sample was used for microbial biomass carbon. For the phospholipid fatty acid (PLFA) analysis, soil sampling had other specifications discussed later. The sub-samples for SOC and enzyme activity were air-dried for 24 hours and sieved through a 2-mm diameter mesh sieve to remove coarse material and roots. The sub-samples for microbial biomass carbon were sieved through a 2-mm sieve without air drying and stored at 4°C until analysis.

Soil Chemical Properties

Previous to starting the study, one composite soil sample of the study field was taken to conduct a basic soil fertility analysis (Table 3.1). The soil sample was sent to the Central Analytical Laboratory at the Agricultural Experimental Station in Río Piedras. In addition, a soil fertility analysis per plot (Table 3.2) was performed with soil samples of May 14th, 2018 (289 DAP, near 10 months). The latter analysis was performed by the Agricultural Analytical Services Laboratory in Penn State University. Nutrient levels and cation exchange capacity (CEC) were measured by Mehlich 3 extraction and soil pH was measured in a 1:1 soil: water slurry. The soil organic matter was measured by loss on ignition. Soil test categories were used according to (Sotomayor-Ramírez and Martínez (unpublished manuscript).

Soil Organic Carbon

Analysis of soil organic carbon content was conducted for each plot in three sampling dates July 25th, 2017 (before planting the cover crop plant species), November 6th, 2017 (103 DAP) and April 9th, 2018 (254 DAP). Soil samples were analyzed by the method of dichromate-oxidation Walkley and Black modified by Heanes (1984) as described in Nelson and Sommers (1996).

Detailed methodology was conducted following the protocol for experiment and analysis methods for the Project H-447 by the ECL-AES at Río Piedras (Dumas et al., Unpublished manuscript).

The soil samples were previously air dried and sieved as described above. This method requires, as additional pre-treatment, sieving samples with a 0.5 mm-diameter mesh. Soil organic carbon was measured by spectrophotometry (Shimadzu UV-1800) with a wavelength of 590 nm. The measures correspond to carbon g in the aliquot of soil. Results of soil organic carbon were calculated with the equation 3.1.

$$\text{(Eq. 3.1)} \qquad \% \, C = \frac{\text{C (g)}}{\text{weight soil (g)}} * 100$$

In addition, using the conversion factor of 1.72, SOC was calculated from the SOM measures at 289 DAP. Data from this sampling date was evaluated by loss on ignition by the Agricultural Analytical Services Laboratory at Penn State University.

Microbial Biomass Carbon

The microbial biomass carbon (C_{mic}) was estimated using the chloroform fumigation extraction method (CFEM) with 0.5 M potassium sulfate (K_2SO_4) developed by Vance et al. (1987) as described in Coleman et al. (2004). Briefly, fumigation was performed for a period of 48 hours in vacuum desiccators with ethanol-free chloroform. Afterward, the extraction with K_2SO_4, an estimation of total organic carbon (TOC) was conducted to calculate the difference between soil samples fumigated and soil samples non-fumigated. The fumigation provides a means to calculate the microbial biomass because the carbon in microbial biomass is released by microbial mineralization.

Soil samples were carefully inspected to remove residual roots after the pre-treatment sieving. Due to the sensitivity of the soil microbial community (Horwath and Paul, 1994), soil samples were stored at 4 °C until the analysis was performed a week after the soil sampling date.

The extractions were performed with soil samples of July 25th, 2017 on August 3, 2017, and soil samples of April 9th, 2010 on April 17th, 2018. The soil samples of November 6th, 2017 were not analyzed for C_{mic} because the Environmental Chemistry Laboratory was operating in part time due to power outages after Hurricane María. After the extraction procedure, TOC was measured with a Torch Combustion TOC/TN Analyzer (Teledyne Tekmar ®, Mason, OH).

The microbial biomass was calculated according to the equation 3.2 where $ugC\ /g\ soil$ is the amount of carbon trapped in microbial biomass, TOC (F) is the measure of C from fumigated samples, TOC (NF) is the measure of C from the non-fumigated samples and $K_c = 0.38$ is the proportion of microbial C evolved as CO_2 from the microbial mineralization after fumigation (Coleman et al., 2004).

$$(Eq\ 3.2) \qquad Microbial\ Biomass\ (ugC\ /g\ soil) = \frac{\mathrm{TOC\ (F)} - TOC\ (NF)}{\mathrm{Kc}}$$

β-glucosidase

The activity of β-glucosidase was determined in July 25th, 2017 (before planting plant species), November 6th, 2017 (103 DAP) and April 9th, 2018 (254 DAP). β-Glucosidase activity was determined by the method described by Tabatabai (1994). The extractions were performed before a month of storage lapsed to avoid the degradation of enzyme activity due storage (Abellan et al., 2011). Briefly, 1 g of soil (< 2 mm) was weighted in a centrifuge tube (50-mL), then 0.25 mL of toluene, 4mL of modified universal buffer (MUB) pH 6.0 and 1 mL of *p*-Nitrophenyl-β-D-glucoside (PNG) were added and mixed for a few seconds. Samples were incubated for 1 hour at 37 °C. After incubation, 1 mL of 0.5 M calcium chloride ($CaCl_2$) and 4 mL of 0.1 M Tris(hydroxymethyl)aminomethane (THAM) buffer pH 12 were added and mixed. Instead of filtering samples trough a Whatman no. 2v filter paper, samples were centrifuged 10 minutes at 10,000 rpm. An aliquot of the supernatant was moved to another vial for colorimeter readings. β-glucosidase was measured by spectrophotometry (Shimadzu UV-1800) with a wavelength of 400 nm.

A standard curve was prepared for each sampling date with a stock solution of p-nitrophenol (pNG), following the protocol for experiment and analysis methods for the Project H-447 by the ECL-AES at Río Piedras (Dumas et al., Unpublished manuscript). The activity of β-glucosidase was calculated with the equation 3.3, where V is the volume of pNP extracted from soil (10.25 mL), H is the humidity correction factor, P is the weight of soil (1.00 g) and t is the incubation time (1 hour). The β-glucosidase activity was expressed as µg pNP released g^{-1} dry soil per h^{-1}.

$$\text{(Eq. 3.3)} \qquad BG\left(ug\ \frac{pNP}{g*h}\right) = \frac{[pNP]*V*H}{P*t}$$

Dehydrogenase

Analysis of dehydrogenase (DHA) activity was conducted for each plot in three sampling dates: July 25th, 2017 (before planting plant species), November 6th, 2017 (103 DAP) and April 9th, 2018 (254 DAP). DHA was analyzed by the method of Casida et al. (1964) described in Tabatabai (1994). Briefly, the methodology consisted in using 6 g of soil, and mixing it with 0.067 g of $CaCO_3$, 1mL of TTC and 2.5 mL of dH_2O. Samples were incubated for 24 hours at 37 ° C. Then, 10 mL of methanol were added, and samples were hand mixed for 1 min. A reddish-pink color was observed in the solution, as an indicator of the redox reaction. Afterward, the solution was filtered through a glass funnel with absorbent cotton into 50 mL Pyrex™ graduated test tubes. Soil was washed with methanol until the reddish-pink color disappeared from the cotton plug. The filtered solution was evaluated by spectrophotometry (Shimadzu UV-1800) with a wavelength of 485 nm. A calibration curve was prepared from TPF standards. The activity of DHA was calculated with the equation 3.4, where [TPF] is the concentration of TPF in µg/ mL, V is the volume of soil (50 mL), H is the humidity correction factor, P is the $CaCO_3$ used and 0.99 a correction factor.

$$(Eq.\ 3.4) \qquad DHA = \frac{[TPF] * V * H}{P * 0.99 * t}$$

Phospholipid fatty acid

The phospholipid fatty acid (PLFA) analysis was used to evaluate the size, composition and structure of the soil microbial community. For the PLFA analysis, a new soil sampling was done in May 14th, 2018 (289 DAP). Soil sampling was conducted following an aseptic protocol described in Finney et al. (2017). The latex gloves were changed in every plot and tools were rinsed with isopropyl alcohol (70%) and let dry before making another sampling. For this sampling event, ten soil sub-samples (cores of 0-20 cm depth) were collected from random locations (covered by the treatment) and combined into a single soil sample. Soil samples were placed in plastic bags and stored in a compartment with ice packs to transport to the Environmental Chemistry Laboratory at Agricultural Experimental Station (ECL-AES) in Río Piedras. Because the soil was too wet to sieve, samples were freeze dried (Labconco © Freezone 6 Lyophilizer) before grinding. The aseptic protocol was performed while sieving and samples were managed. Then, soil samples were sent to be analyzed by Ward Laboratories, Inc. in Nebraska, USA. The results are expressed in concentration (ng g^{-1}) of different PLFA biomarkers that correspond to microbial functional groups (Table 3.2)

The soil microbial community size was determined using the concentrations of total biomass (ng g^{-1}). The microbial community composition was evaluated according specific PLFAs for different microbial groups. A functional group diversity index by Ward Laboratories was used to evaluate the effect of treatments. As PLFA cannot represent a specific specie, the diversity index was calculated with the Shannon equation, were quantity of fatty acids for each group was treated as the count of abundance and each functional group was treated as a specie in the Shannon equation. Three community composition ratios were evaluated: (1) fungi: bacteria, (2) predator: prey, and (3) gram (+): gram (-). In addition, a stress and community activity ratio was evaluated with the saturated and unsaturated fatty acids, monounsaturated to polyunsaturated fatty acids and Cyclo (Cy) fatty acids. Communities under stressed conditions may reflect higher unsaturated fatty acids and lower ratio of Cy (16:1/18:1) fatty acids (Bossio et al., 1998; Kaur et al., 2005). The biomarkers used for this analysis by Ward Laboratories are presented in Appendix 3.

Diffuse Reflectance Fourier Transform Mid- Infrared Spectroscopy

Diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) was performed using a Thermo Scientific™ Nicolet ™ iS™ 5 FTIR with OMNIC Specta™ software at Penn State University, PA. The soil samples were obtained from the sampling on May 14th, 2018 (289 DAP). As a protocol for soils that are shipped from outside of United States, soil samples were pre-treated with heat at 110 ºC for 16 hrs. by the Agricultural Analytical Laboratory of Penn State University. Spectra's were performed on neat soil without dilute soil with KBr (Reeves et al., 2001; Calderón et al., 2017). Soil was ground with a mortar and pestle, and the mortar and pestle were dry cleaned with Kimwipes ® between samples. Soil was pre-treated by drying at 30 °C overnight. The spectrums were performed with soil particle size < 2mm and <53 µm to evaluate if results changed according to the fraction of particle sizes. A gold target was used as the reference background and a reference spectrum was scanned once an hour. For each plot, three spectra were obtained changing the analyte (soil) with new sub-samples into the sample holder. For each spectrum, 64 co-added scans were compiled to give one spectrum with a wavenumber range between 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. Each spectrum generates a file with absorbance for each wavenumber. An average of the three spectrums was used for the analysis and a manual baseline correction was performed. To minimize fluctuations, samples were scanned by blocks on the same day.

To obtain a clear spectrum of the organic bands in the soil, mineral peaks were subtracted from the original spectrum (Chefetz et al., 1998; Margenot et al., 2015) using the spectrum from a subsample where organic carbon had been oxidized with sodium hypochlorite according to the method described by Anderson (1961) and with the modifications of Siregar et al. (2004) and Margenot et al. (2015). Briefly, 3 g of air-dried soil was mixed with 30 mL of a solution NaOCl (8% pH 8.0) and incubated for 6 hours ± 25 °C to allow oxidation. In this methodology, the long incubation time (6 hours) substitutes for heating the samples to 80 °C, which could promote a thermal alteration of the mineral fraction. The oxidation of SOC with NaOCl has been evaluated as a reliable method to remove organic carbon without affecting pedogenic oxides (Mikkuta et al., 2005; Margenot et al., 2015). At the end of the incubation, solutions were centrifuged at 20,000 rpm for 10 minutes and the supernatant was decanted. The oxidation process with NaOCl was

repeated twice for a total of three oxidations (Margenot et al., 2015). Then, soil samples were washed with 30 mL of NaCl overnight and centrifuged. Afterward, soil samples were shaken with deionized water (twice) per 6 hours, centrifuged, air-dried and re-ground. Just prior to spectral analysis, soil samples were dried at 30° C overnight. Spectra were collected on oxidized soil as described above and a manual baseline correction was conducted before subtraction. The spectral subtraction to obtain peak heights associated with organic bonds was performed by subtracting the spectra of the oxidized soil (baseline corrected) from the spectra of the original soil (baseline corrected) using OMNIC™ Specta Software version 9.8 (2008-2017 Thermo Fisher Scientific Inc.). The quantitative subtraction was performed manually. Mineral absorbances of the oxidized spectra's were subtracted from absorbances of bulk soil.

Statistical Analysis

Data sets were analyzed with SAS statistical software version 9.3 (SAS Inc. Cary, NC). Generalized linear mixed models (PROC GLIMMIX) was used to analyze data in a randomized block split-plot in time design with fixed effects of cover crops species, days and their interactions (cover crops x days), and a random effect of block and the interaction block x cover crops. Separation of least square means was performed using Least significant difference (LSD) by Fisher at $\alpha = 0.05$. Regression analysis was performed between variables to evaluate the relationship. Data for β-glucosidase enzyme activity analysis was log-transformed for statistical analysis to meet the assumptions of homoscedastic and normal distribution. For the presentation in tables and text, data were back-transformed. Phospholipid fatty acids (PLFA) analysis was conducted with mixed models (PROC MIXED).

In addition, a Pearson correlation coefficient among functional groups was conducted. Principal component analysis and Canonical discriminant analysis were performed using an extension of SAS, JMP ® version 14 (SAS Institute, Cary, NC). Soil organic matter functional groups for the Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was analyzed with an analysis of variance using InfoStat program (DiRienzo et al., 2018). In addition, a non-metric multidimensional scaling (nMDSD) analysis with the first derivate of peaks from DRIFTS

and PLFA analysis was performed in RStudio version 3.5.2. (2018) with the Vegan: Community Ecology Package (Oksanen, 2019). A Pearson correlation analysis was conducted with InfoStat program (DiRienzo et al., 2018) to evaluate relationship between aboveground biomass (AGB), relative growth rate (RGR), leaf area index (LAI), microbial community groups and DHA and β-Glucosidase enzyme activity.

3.3. Results

Soil Chemical Properties

Few differences were found after 289 DAP cover crops (Table 3.2). The parameters: cation exchange capacity (CEC), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and pH were not statistically different among treatments. Soil organic matter (SOM) among treatments was significant (F = 4.06, *p*-value = 0.0443) where *G. repens* was greater than *H. rotundifolia* and *S. trilobata* but not significantly greater than the control.

Soil Organic Carbon

Soil organic carbon (SOC) results by dichromate-oxidation Walkley-Black method were not significant among treatments (Table 3.3; F = 1.39, *p*-value = 0.30), but averaged across cover crop treatments, SOC showed a significant increase over time (Table 3.4; F = 10.85, *p*-value = 0.0004). Interaction of cover crop treatments by time was not significant (F = 0.42, *p*-value = 0.8553).

Microbial Biomass Carbon

Measures from microbial biomass carbon were not formally evaluated for this thesis. The Torch Combustion TOC/TN Analyzer (Teledyne Tekmar ®, Mason, OH) did not pass two checkpoints from measures of the C_{mic} extractions performed with soil samples of April 17th, 2018. Samples were stored to further analysis.

Enzyme activity

The presence of living ground cover for 289 days did not cause a significant increase in *β*-Glucosidase activity in the cover crop treatments (Table 3.3; F = 0.55, *p*-value = 0.66). However,

there was a significant increase in the overall β-Glucosidase activity as the experiment progressed in time (Table 3.4; F = 175.47; *p*-value < 0.0001). Similarly, dehydrogenase activity was not significantly different among treatments (Table 3; F = 1.39; *p*-value = 0.30) but showed a significant increase (Table 4; F = 10.85; *p*-value = 0.0004) over the course of the study. The Pearson correlation analysis between growth analysis traits (0, 175 and 229 DAP) and enzyme activity (0, 103 and 254 DAP) showed a significative relationship between leaf area index (LAI) and DHA with r = 0.63, *p*-value < 0.0001 and a week relationship between LAI and β-Glucosidase with r = 0.42, *p*-value = 0.0098. The correlation analysis between aboveground biomass (AGB) and β-Glucosidase activity had a r = 0.77, *p*-value <0.0001, and AGB with DHA had and r = 0.87, *p*-value < 0.0001. In a specific correlation analysis by plant species, the strongest correlation was between AGB and β-Glucosidase activity for *G. repens* with a correlation coefficient of r = 0.95 (p-value < 0.0001), *H. rotundifolia* with an r = 0.74 (*p*-value = 0.0057) and *S. trilobata* with r = 0.73 (*p*-value = 0.0073). The correlation coefficient between AGB and DHA reflected an r = 0.91 (*p*-value < 0.0001) for *G. repens*, r = 0.83 (*p*-value = 0.0006) for *H. rotundifolia* and r = 0.97 (*p*-value < 0.0001) for *S. trilobata*.

Microbial community (size and composition)

After eight months of planting cover crops species, concentrations of phospholipid fatty acids (PFLA) indicated that the total microbial biomass was not significantly different among treatments (Table 3.3; F = 3.15; *p*-value = 0.0789). This is a marginally non-significant *p*-value for the ANOVA, indicating the possibility of a trend, and in an orthogonal contrast, microbial biomass concentration was higher in soil covered with *G. repens* and *S. trilobata* compared to the no-cover control plots (estimated difference = 591 ng g^{-1}, *p*-value = 0.03). The Shannon diversity index was not significantly different among treatments (F = 1.90; *p*-value = 0.2009). Nevertheless, according the rating by the mean the diversity index (Table 3.6 and Table 3.7), *G. repens* and *S. trilobata* were in the 'Good' category, while control plots resulted 'Slighly Above Average' and *H. rotundifolia* in 'Average' category.

The effect of plant species on specific microbial groups are shown in Table 3.8 and Table 3.9. In general, total bacteria (F = 3.31; *p*-value = 0.07), total fungi (F = 2.45; *p*-value = 0.13) and protozoa biomass (F = 1.97; *p*-value = 0.19) were not significantly different among treatments. According the results presented in Table 3.8, *S. trilobata* and *G. repens* were slightly higher than *G. repens* and control plots in total bacteria concentration. After 289 DAP intercropped plots with *G. repens* and *S. trilobata* reflected and increase in Gram-negative bacteria group, in an orthogonal contrast with no cover cropped soil (estimated difference = 76.57 ng g^{-1}; *p*-value = 0.04). Group of protozoans were only present in plots with *G. repens* and *S. trilobata (*F = 1.97 *p*-value = 0.1884*).* In addition, *G. repens* was the only treatment with the presence of *Rhizobium* bacteria (F = 1.0 *p*-value = 0.4363).

The community composition ratios reflected in fungi: bacteria ratio that control plots and *H. rotundifolia* were in the 'Slightly Below Average' category, while *G. repens* and *S. trilobata* were in the 'Slightly Above Average' category (Table 3.7). In the predatory: prey ratio (expressed as protozoan to bacteria), *H. rotundifolia* and control plots did not reflect presence of predators. Differing, *G. repens* and *S. trilobata* were in the 'Excellent' category for predator: prey ratio. For the gram positive: gram negative bacteria ratio, *H. rotundifolia* has the highest value (Table 3.10).

The microbial community composition metric for stress and activity ratio of saturated: unsaturated fatty acids (sat: unsat) was not -significantly different among treatments (Table 3.11). For the Mono: Poly ratio, results showed that three of four control plots were dominated by monounsaturated fatty acids instead polyunsaturated fatty acids. All plants species reflected a plot with the presence of only monounsaturated fatty acids, reducing the number of repetitions to calculate the mean to three. Plots with *G. repens* has a mean ± S.D. (sat: unsat) in three plots of 14.61 ± 7.14. Similarly, *S. trilobata* had a mean of 16.91 ± 7.42 (n=3). Therefore, *H. rotundifolia* reflect a mean (n=3) of three plots with 64.28 ± 25.15. In the evaluation of Cyclo fatty acids, *G. repens* was the only treatment that reflect a ratio for *Pre 18:1ω7c:cy19:0* in two plots, with a mean of 15.35, while other treatments reflect only *Pre 18:1*.

A principal component analysis (PCA) for microbial groups reflected an influence in the variation among three principal components (PC-1, PC-2 and PC-3). The eigenvalue for PC1 was explained 72% of the variation, while PC-2 explained 17% of the variation and PC-3 explained7%

of the variation (Figure 1). Variation in PC-1 was positively related to gram-negative bacteria, saprophytic fungi and protozoa, while PC-2 was positively related to actinomycetes and gram-positive bacteria and negatively related to *Rhizobium* bacteria. On the other hand, PC-3 reflected a positive relation with *Rhizobium* group and a negative relation with arbuscular mycorrhiza (Table 3.12). A Pearson correlation coefficient among functional groups is presented in Table 3.13. The strongest correlation was between actinomycetes and gram-positive bacteria, *Rhizobium* bacteria and protozoa, gram-negative with saprophytic fungi, and saprophytic fungi with protozoan.

A linear canonical discriminant analysis with common covariance for microbial groups (gram positive bacteria, gram negative bacteria, arbuscular mycorrhiza fungi, saprophytic fungi, rhizobium and protozoan), DHA, soil chemical properties (pH, CEC, Mehlich 3-P, SOM) and plant species is presented in Figure 3.2. The canonical discriminant analysis with an Entropy R^2 of 94.3 (df =45 *p*-value < 0.0001), suggest that cover crop treatments were differentiated primarily by microbial groups and to a lesser extent by chemical characteristics. The first canonical variate was strongest correlated to pH (Table 3.12). The highest mean value in the first canonical variate was control plots (Table 3.13). This is congruent with the results presented in Table 3.2, where although were non-significant, control plots showed higher mean values of pH. The second canonical variate (CanVar-2) has a positive correlation with all the microbial groups and DHA, and negative correlated to CEC, and Mehlich-3 P. Results from CanVar-2 with the strong relationship among microbial groups are congruent with the results showed by the Pearson correlation analysis (Table 3.11) and results of microbial biomass (Table 3.3) where treatments with higher microbial biomass, tend have higher biomass across all the different microbial groups (Table 3.9). The strongest positive relationship in CanVar-2 was with AM fungi, in addition reflected a negative relationship with Mehlich-3 P and CEC.

Soil Organic Functional Groups

The differentiation and characterization of soil organic functional groups using DRIFTS with the subtracted spectrums did not reflect a significant difference among treatments (Figure 3.2, Table 3.14). Bands showing a strong peak were stretching vibrations found in the region 1 (Figure

3.2) between 3300 and 3400 cm^{-1} corresponding to hydroxyl groups (O-H) and amines (N-H). The region 2 with wavenumber near 2924 and 2859 cm^{-1}, represent aliphatic asymmetric and symmetric stretching vibration respectively. Further, the region 3 with a wavenumber near 1650 cm^{-1}, 1575 cm^{-1}, and 1405 $cm^{-1,}$ represent aromatic vibrational asymmetric bonds of aromatic (C=C) groups, amide (N-H) and aliphatic (C-H) bonds respectively. The region 4 had peaks near 1110 to 1080 cm^{-1} corresponding to polysaccharides symmetric stretching vibrations (C-O). Peaks in region 5 near 920 and 840 cm^{-1} correspond to aromatic functional groups less substituted. The assignment of soil organic functional groups in the regions was according Margenot et al. (2015). In a non-metric multidimensional scaling (nMDS) analysis with the first derivate of peaks from DRIFTS and PLFA analysis among treatments, results were positively associated with aliphatic type C-bond (Figure 3.3).

3.4. Discussion

Soil Organic Carbon

The first hypothesis was that one of the plant species would have a greater mean soil organic carbon than the reference plant. This hypothesis was not supported by the results obtained. Differences in the effects of treatment after 254 DAP resulted in non-significant *p*-values, meanwhile differences in the effect of sampling dates were statistically significant. The non-significant results among treatments could have been caused by the short time of cover crops growing in the soil or a low statistical power. There are two main points that could explain the significant results obtained among sampling dates: (1) a change in soil and crop management practices from before the study started to how it was managed during the study and (2) accumulation of vegetative tissues by the effects of hurricane María.

Changes in the SOC pool tend to accrue slowly over years (Schipanski et al., 2014). According to Blanco-Canqui et al. (2015), cover crops increase the SOC reservoir from 0.1 to 1 Mg ha^{-1} yr^{-1} depending on the amount of biomass produced, years of cover cropping, management of cover crops and the initial SOC level. SOC pools tend to increase if the carbon inputs of plants residues are greater than carbon losses (Kaspar and Singer, 2011). Cover crops that are grown to incorporate biomass into the soil have been an effective technique to increase SOC (Steenwerth and Belina, 2008). However, permanent cover crops, as in this study, are not incorporated into the soil. Therefore, carbon inputs from living cover crops are derived from other small sources such as fine root turnover, root exudations and litter decomposition (Maul and Drinkwater, 2010; Kong and Six 2012). From the results of this study, inputs of SOC were in small sources, enough to reflect a difference over a short time period, but not enough to reflect a difference among plant species. This suggests the need for long-term studies with permanent cover crops to evaluate their possible effects on enhancing SOC content. The latter is especially important in soils with conventional management and with low SOC content, such as the soil used for this experiment.

Although differences among treatments were not significant, sampling dates reflect an increase in SOC after 254 DAP. Before planting cover crops, the inner roads of the banana fields were in conventional management with routine herbicide (paraquat) applications. Previous studies

show how management could influence the quantity and quality of soil organic carbon and decrease SOC in conventional management systems due to SOM being exposed to degradation factors (Shrestha et al., 2008; Razafimbelo et al., 2008). In a study conducted by Razafimbelo et al. (2008), ploughed tillage practices with residue removed was compared with no-tillage and mulching practice to evaluate the influence in soil organic carbon. Razafimbelo et al. (2008) results, showed higher content of macroaggregates and SOC in no-tillage treatments. Results from this study showed similar results as SOC increased 10 months after tillage was conducted for the preparation of the field.

On the other hand, the significant increase in SOC observed after 254 DAP could be an effect produced by hurricane Maria, where winds damaged and uprooted the whole banana plants of the field experiment. Even though banana plant aboveground biomass was removed, root systems remained in the soil, acting as inputs of carbon. According to Turner (2003), banana plants contribute to carbon stocks in the soil though nodal roots. However, in a study conducted by Kamusingize (2017), evaluating the potential of 14 banana plantations to sequester carbon in Uganda, the contribution of soil organic carbon by plants was small and not significant in relation to the carbon stocks in the soil.

In a separate evaluation of soil parameters conducted after 289 DAP, soil organic carbon measured through soil organic matter revealed a significant difference among treatments. In this evaluation, *G. repens* was higher in SOC than *H. rotundifolia* and *S. trilobata*. This result could be attributed to *G. repens*, which showed a loss of aboveground biomass after hurricane María, as adding greater inputs of carbon from aboveground biomass into the soil. Also, in control plots, weed plants were growing in wide intervals without hand weeding (HW). Despite weeds being removed from control plots, the lapse of time while weeds grew could have contributed inputs of soil organic carbon. According to Filho et al. (2012), residues have a significant impact on storing C in the soil. In a study by Sainju et al. (2003), soils with perennial weeds had higher organic C concentrations than soils cover cropped with *Arachis glabatra* Benth, due to continuous C inputs from aboveground and belowground plant biomass. Our study suggests that all cover crops increase SOC after 229 DAP, however a significant difference showed that *G. repens* has positive effects as a permanent cover crop, increasing SOC after 289 DAP.

Enzyme activity

The third hypothesis was that at least one of the plant species would promote a higher enzyme activity (Dehydrogenase and *β*-Glucosidase) than control plots. The results obtained indicate that effects among treatments in the soil enzyme activity was not significantly different. However, a significant difference among sampling dates reflect an increase in DHA and *β*-Glucosidase after 254 DAP. The overall increase in enzyme activity could be a result of the change in management and the proliferation of vegetation, cover crops and weeds in control plots.

DHA plays an important role in the biological oxidation of SOM (Wolińska and Stępniewska, 2012). According to a study by Blazier et al. (2005) where the DHA activity was evaluated among different management practices, fertilization and the use of herbicides reduced activity of the enzyme, while the untreated control plots did not have a decrease in DHA activity. The results of Blazier et al. (2005) suggest that soil microbes are dependent on the availability of labile C sources. In our study, DHA activity was twelve times greater in the last evaluation (254 DAP) as compared to the first evaluation before cover crops had been planted. The lowest results, before planting cover crops, could be an exacerbated effect of C decrease influenced by the field preparation management before conducting our study (Carter et al., 2002; Błońska et al., 2017).

Measures in *β*-Glucosidase enzyme activity showed the same trend as DHA, a significant increase among sampling dates, with the last evaluation three times higher than the first evaluation. *β*-Glucosidase is an important enzyme in the C cycle by catalyzing glucose formation, an energy source for the microbial community (Tabatabai, 1994; Hai-Ming et al. 2014). Living vegetation such as permanent cover crops in this study, provide highly labile C sources through root exudates (Blazier et al., 2005). According to Vance and Chaping (2001), the abundance of root exudates could compensate for the low organic matter quality in soils. Also, the no-tillage activity in the inner roads while the study was conducted could have influenced the results after 254 DAP. Pandley et al. (2014) evaluated the effects of conventional tillage and no tillage system on soil enzyme activities under rice crops. Most of the enzymes (including *β*-Glucosidase) increased following a reduction in tillage (Pandley et al., 2014). The correlation analysis showed a strongest relationship (r=0.83) between DHA and *β*-Glucosidase enzyme activity in plots with *G. repens*. In addition, *G. repens* showed a correlation coefficient of r=0.68 between soil organic matter and

DHA enzyme activity. Blońska et al., 2017, reported analogous results in a study evaluating enzyme activity in differently managed soils where there was a strong positive relationship between enzyme activity and soil organic matter.

Soil microbial community

The fourth hypothesis was that plots with *S. trilobata* and *H. rotundifolia* plant species would be higher in total soil microbial biomass than the reference plant, *G. repens* and control plots without cover crop. Soil microbial biomass is commonly used to evaluate short-term effects of soil management and changes in soil functions (Li et al., 2018). Studies have shown that plants can accelerate the succession of soil microbial communities to promote microbial functions and facilitate the growing conditions for the plants (Kulmastiski et al. 2008). One of the main factors that influences the size of microbial biomass is the quality of SOM (Chen et al., 2015). In this study, the results of total soil microbial biomass (indicated by phospholipids fatty acids), don't support the postulated hypothesis.

Although the total soil microbial biomass results were not significant among treatments, results suggest a trend where the highest concentrations of total microbial biomass were in *S. trilobata* and *G. repens*. However, these results indicate little evidence about the latter suggestion and require other studies with more statistical power due to the high variation among measures. The results could be affected by measures in one of the blocks in the experimental design that reflected the lowest values for all treatments. This block reflected low drainage capacity compared with the other blocks after rain events (personal observation). If the statistical analysis was conducted without these block measures, results would have been significant (p-value=0.0369) with a strong effect on the total soil microbial biomass by *S. trilobata* and *G. repens*. Similarly, Finney et al. (2017) also found a higher total soil microbial biomass under cover cropped soils than control plots without coverage.

The influence in soil microbial communities has not been previously reported for *G. repens* as a cover crop. The presence of beneficial soil bacteria termed 'plant growth promoting bacteria' such as *Rhizobium* in *G. repens* could be a further theme to investigate. Besides, *S. trilobata* has

been reported as a plant species that promotes changes in soil microbial community, specifically in fungal groups (Si et al., 2014). This is consistent with our results, where S. *trilobata* and *G. repens* showed the highest concentration in saprophytic fungi and arbuscular mycorrhizal fungi. On the other hand, the influence of *H. rotundifolia* in soil microbial communities has not been reported before, although pharmaceutical research has described it as a plant with antibacterial activity (Dougnon et al., 2017).

For the community composition ratios, protozoan groups were only present in *G. repens* and *S. trilobata*, suggesting that a suitable environment was available in those plots compared to control and *H. rotundifolia* plots at 289 DAP. The protozoan group is one of the most important bacterial predators in soils (Acosta-Martínez et al., 2008). The protozoan population tends to be scarce if the soil is low in moisture, high in temperatures or managed with herbicides (Acosta-Martínez et al., 2008; Mayzlish and Steinberger, 2004). Results for gram+: gram – ratios showed a higher abundance of gram-positive bacteria in plots with *H. rotundifolia*. Buyer et al. (2010) attributed a decrease in gram-positive bacteria, observed in his study with cover crops in tomato systems, to the available carbon in cover-cropped treatments, which fomented other groups over gram-positive bacteria. Buyer et al. (2010) study, showed that gram-positive bacteria responded positively to black polyethylene cover instead a cover cropped soils. Although, the complexity of biological communities due to interactions and environmental variables, Buyer et al. (2010) study, indicates that cover cropping was the most important variable in the effects observed in soil microbial community structure. Our results for aboveground biomass (AGB) and coverage in Chapter II, indicate that *H. rotundifolia* and *S. trilobata* were the species with the highest above ground biomass and soil cover. However, according results for microbial community, *S. trilobata* and *G. repens* were the two plant species with the most notable effects with higher soil microbial biomass. The importance of cover crops species aboveground biomass is not only for soil coverage, but also to promote microbial community complexity.

Soil Organic Functional Groups

The DRIFTS analysis identified that soil samples were more abundant in O-H, N-H, C=C and C-O functional groups. Although difference among treatments were non-significant, the nMDS analysis reveals that microbial groups are related to functional groups in the soil organic matter. This analysis reflects that microbial communities are positively related to aliphatic C-H bonds in soil organic matter (Figure 3.5). In a study conducted by Margenot et al. (2015), fields that received higher inputs of composted green manures resulted in higher band intensities for aliphatic groups. Margenot et al. (2015) explained that aliphatic C-H bonds were the most sensitive to change in the evaluated organic tomato fields. Calderon et al. (2011) suggested that aliphatic C-H bonds reflect the presence of labile C. The aliphatic C-H bonds tend to be related with other functional groups such as amides and aromatic C=C bonds. Higher amounts of aliphatic C-H bonds have been reported in decomposition processes, thus increasing labile C (Glacometti et al., 2013; Veum et al., 2014). Evaluating the DRIFTS for labile carbon among treatments could reflect clearer differences in the effect of cover crops in soil organic matter functional groups. Aranda et al. (2011) reported higher inputs of aliphatic C-H bonds and greater microbial biomass in cover cropped soils.

The nMDS reflected a lower relationship between phospholipid fatty acids and polysaccharide type bonds (Figure 3.5). Polysaccharide type bonds, such as those found in cellulose, have been related to plant residues in soil (Ouellete et al., 2016). The lower relationship between microbial biomass and polysaccharide bonds could be related to the relative abundance of polysaccharides vs. aliphatic organic compounds. There is a preference for aliphatic compounds as a growth substrate for microbial communities. Another mechanism may be that active microbial populations deplete the polysaccharide residue pool more quickly than the aliphatic pool. The abundance of polysaccharide C bonds tend to decrease through the decomposition process. Tseng et al. (1996) found that 50 percent of the polysaccharide bonds decreased when substrates when were composted. However, the measure of DRIFTS in this study did not separate polysaccharides from the SOM pool or plant-derived polysaccharides. In addition to aliphatic C-bonds and polysaccharides types bonds, other peaks could reflect changes in microbial communities and the short-term effects of cover crops in SOM functional groups. Thus, the DRIFTS technique can be

an effective, sensitive, economical and quick way to assess different cover crops and their short-term effects on soil biological parameters

3.5 Conclusions

The use of living cover crops to enhance soil quality could increase total soil organic and enzyme activity after 8 months. This study suggests that inputs of SOC from living cover crops are in small quantities, enough to reflect a difference over a short time period, but not enough to reflect a difference among plant species. Permanent and living cover crops such as the plant species used in this study provided a source of highly labile C. In our study, DRIFTS results reflected higher amounts of aliphatic C-H bonds, which are related to labile C sources and an increase of microbial biomass. The plant species with the greatest concentrations of microbial biomass were *S. trilobata* and *G. repens*, reflecting the highest trophic levels with the presence of predators (protozoan groups). Among plant species, *H. rotundifolia* showed the highest value and variance for the stress and community ratio. Taking this into consideration, the more suitable living cover crops in this study were *G. repens* and *S. trilobata*. Further studies should be conducted, taking into consideration the evaluations of more sensitive parameters, such as labile soil organic carbon, microbial biomass carbon and nitrogen, soil respiration in the field, and macro an mesofauna community. In addition, less depth of sampling or split soil sampling depth into the first 5 cm and > 5-20 cm could be more effective to differentiate the legacy effect of specific plant species on soil quality.

3.6 Tables and Graphics.

Table 3.1. Soil chemical analysis for the banana field in July 18, 2017 before planting cover crops.[1]

Parameters	Units	Value[2]
pH		6.86
Conductivity	S/cm	385.2
Organic Matter	(%)	1.29
P available	mg P-PO_4/Kg	6.18 M
Calcium	mg Ca/Kg	3189 H
Magnesium	mg Mg/Kg	1340 H
Potassium	mg K/Kg	80 L
Sodium	mg Na/Kg	35
CICE	meq/100g	28

[1]Inform L-17-045 [2]Soil test categories L = low, M= Medium and H = High (Sotomayor-Ramírez, unpublished)

Table 3.2. Mean of soil chemical properties (±SE) among treatments at 289 days after planting cover crops.

Treatment	pH	OM	CEC	P	K	Mg	Ca
		%	$cmol_c\ kg^{-1}$	$mg\ kg^{-1}$	---------- mg/Kg ----------		
Control	6.20 (0.34)	2.47 a b (0.16)	30.63 (1.31)	18.25 (1.50)	174.50 (16.54)	1305.50 (173.94)	3272.48 (291.66)
S. trilobata	5.95 (0.17)	2.42 b (0.23)	30.78 (2.66)	17.25 (2.36)	174.75 (47.08)	1258.00 (195.92)	3179.90 (273.72)
G. repens	5.96 (0.12)	2.56 a (0.20)	30.78 (2.00)	16.75 (2.75)	160.25 (12.82)	1233.75 (174.45)	3069.15 (172.99)
H. rotundifolia	5.97 (0.23)	2.46 b (0.16)	31.40 (1.89)	18.00 (3.56)	164.50 (6.95)	1298.50 (239.39)	3177.63 (189.18)
p-value	0.0811	0.0443*	0.6416	0.4570	0.5457	0.4870	0.2360
C.V. %	3.61	2.26	4.17	11.63	13.10	7.89	5.60

Means consist in four repetitions. * *p*-values results significant (α=0.05) SE = Standard Error, C.V. % = variation coefficient, OM = Organic Matter, CEC = Cation Exchange Capacity, P= Phosphorous available, K is potassium available, Mg = Magnesium and Ca = Calcium

Table 3.3. Effect of treatments on soil organic carbon, dehydrogenase, β-Glucosidase enzyme activity, and phospholipid fatty acid.

Component	**SOC** (%)	**DHA** (µg TPF*g $^{-1}$)	**ß-Glu** (µgPNP * g^{-1})	**PLFA** (ng g^{-1})
Control	1.40	0.82	50.20	1544.30
S. trilobata	1.37	0.92	72.04	2347.34
G. repens	1.50	0.79	46.60	2243.56
H. rotundifolia	1.29	0.75	53.90	1569.29
p-value	0.3090	0.8477	0.6594	0.0789
C.V. %	15.21	40.65	60.65	25.06

C.V.% = Coefficient of variation, SOC = Soil Organic Carbon, DHA = Dehydrogenase enzyme, ß-Glu= ß-Glucosidase enzyme activity PLFA = phospholipid fatty acids.

Table 3.4. Effect of sampling dates on soil organic carbon, dehydrogenase and β-Glucosidase enzyme activity.

DAP	**SOC** [1] (%)		**DHA** [2] (µg TPF*g $^{-1}$)		**ß-Glu** [3] (µgPNP * g^{-1})	
0	1.45	a	0.16	c	28.43	c
103	1.31	b	0.43	b	49.05	b
254	1.52	a	1.87	a	89.58	a
p-value	0.0004		<0.0001		<0.0001	

C.V. % = Coefficient of variation, SOC = Soil Organic Carbon, DHA = Dehydrogenase enzyme, ß-Glu= ß-Glucosidase enzyme activity PLFA = phospholipid fatty acids.

Table 3.5. Mean of soil organic carbon (± SE) among treatments in different sampling dates.

Component	Soil Organic Carbon (%) in different days after planting (DAP)			
	0	**103**	**254**	**0 vs 254** (*p*-value)
Control	1.27 (0.14)	1.37 (0.15)	1.44 (0.17)	0.0556
S. trilobata	1.27 (0.10)	1.28 (0.16)	1.45 (0.19)	0.0335
G. repens	1.34 (0.40)	1.42 (0.32)	1.59 (0.25)	0.0118
H. rotundifolia	1.26 (0.19)	1.28 (0.16)	1.39 (0.19)	0.1233
C.V. %	18.56	16.79	13.53	--

The p-value for 289 DAP was 0.0443. SE = Standard Error. Means consist in four repetitions (n=4). C.V. = coefficient of variation.

Table 3.6. Shannon Functional Group Diversity Index

Treatment	Shannon Diversity Index
Control	1.33
S. trilobata	1.40
G. repens	1.47
H. rotundifolia	1.28
p-value	0.2009

Table 3.7. Functional Group Diversity Index by Ward Laboratory Inc.

Total Biomass (ng g^{-1})	Diversity Index	Fungi: Bacteria	Rating
< 500	< 1.0	< 0.05	Very poor
500^{+} – 1000	1.0^{+} – 1.1	0.05^{+} – 0.1	Poor
1000^{+} – 1500	1.1^{+} –1.2	0.1^{+} – 0.15	Slightly Below Average
1500^{+} – 2500	1.2^{+} – 1.3	0.15^{+} – 0.2	Average
2500^{+} – 3000	1.3^{+} – 1.4	0.2^{+} – 0.25	Slightly Above Average
3000^{+} – 3500	1.4^{+} – 1.5	0.25^{+} – 0.3	Good
3500^{+} – 4000	1.5^{+} – 1.6	0.3^{+} – 0.35	Very Good
> 4000	> 1.6	> 0.35	Excellent

Ranking tables were created by Ward Laboratory Inc. with n=1000, taking the mean as average and the degrees of standard deviation to create the ranking limits between rates categories: very poor, poor, slightly below average, average, slightly above average. good, very good and excellent.

Table 3.8. Mean of phospholipid fatty acids (± SE) among treatments for three main microbial groups.

	Microbial groups PLFA concentration (ng g^{-1})		
Treatment	**Total Bacteria**	**Total Fungi**	**Protozoa**
Control	641.45 (128.80)	97.59 (66.18)	0.00
S. trilobata	892.61 (209.16)	206.60 (134.14)	4.40 (5.10)
G. repens	902.23 (290.27)	236.06 (159.75)	7.54 (9.52)
H. rotundifolia	632.08 (100.68)	73.12 (37.43)	0.00
p-value	0.0713	0.1303	0.1884

SE = Standard Error. Means consist in four repetitions (n=4). C.V. = coefficient of variation.

Table 3.9. The concentration of phospholipid fatty acids (PFLA) (ng g^{-1}) in functional groups to evaluate the community composition of the soil in a banana field with living crops.

Treatment	Gram (+)	Gram (–)	Actinomycetes	Rhizobia	AM fungi	S fungi
Control	476.02	165.42 b	148.82	0.00	51.45	46.13
S. trilobata	652.35	240.26 ab	196.78	0.00	81.29	125.31
G. repens	633.06	269.18 a	205.69	6.51	81.98	154.09
H. rotundifolia	485.80	146.28 b	147.14	0.00	28.23	44.89
p-value	0.1338	0.0612	0.1840	0.4364	0.1957	0.1227

Gram (+) = Gram positive bacteria. Gram (-) = Gram negative bacteria. AM = Arbuscular Mycorrhiza fungi. S fungi = Saprophytic fungi.

Table 3.10. Soil microbial community composition ratios in response to the short-term effect of the intercrop treatment in a banana field in Gurabo, Puerto Rico.

Treatment	Fungi:bacteria	Predator: Prey	Gram (+): Gram (-)
Control	0.155	All Prey	2.94
S. trilobata	0.233	0.50	2.73
G. repens	0.238	0.51	2.57
H. rotundifolia	0.115	All Prey	3.39

Gram (+) = Gram positive bacteria. Gram (-) = Gram negative bacteria.

Table 3.11. Stress and community activity ratios with saturated and unsaturated fatty acids

Treatment	Sat: Unsat
Control	3.75
	(1.18)
S. trilobata	3.53
	(1.82)
G. repens	2.98
	(0.54)
H. rotundifolia	4.31
	(1.57)
p-value	0.5151

Sat = saturated fatty acids. Unsat = Unsaturated fatty acids.

Table 3.12. Loading of each biomarker group on the eigenvectors of the Principal Component Analysis.

Biomarker group	PC1	PC2	PC3
A	0.3462	**0.5319**	0.2906
R	0.3021	**-0.5769**	**0.4822**
GN	**0.4177**	-0.0750	0.0288
AM	0.3722	0.0774	**-0.7521**
S	**0.4264**	-0.2053	-0.1901
P	**0.4108**	-0.2505	0.0060
GP	0.3540	**0.5174**	0.2832

The values with the largest influence for each principal component are in bold. A = Actinomycetes, R = *Rhizobium* bacteria, GN = Gram-negative bacteria, AM = Arbuscular Mycorrhizae fungi, S = Saprophytic fungi, P = Protozoan and GP = Gram-positive bacteria

Table 3.13. Pearson correlation coefficient among functional groups to evaluate microbial community of soil in a banana field intercropped with perennial cover crops.

	A	R	GN	AM	S	P	GP
A	1.0000						
R	0.2344	1.0000					
GN	0.6460**	0.6624	1.0000				
AM	0.5967*	0.3727	0.7354***	1.0000			
S	0.5878*	0.7275**	0.9129***	0.8293***	1.0000		
P	0.5763*	0.7677***	0.8335***	0.7245***	0.9434***	1.0000	
GP	0.9630***	0.2592	0.7041***	0.6158*	0.6008*	0.5633*	1.0000

Significance results * $P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.005$ A = Actinomycetes, R = *Rhizobium* bacteria, GN = Gram-negative bacteria, AM = Arbuscular Mycorrhizae fungi, S = Saprophytic fungi, P = Protozoan and GP = Gram-positive bacteria N=16

Table 3.14. Statistic of diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) bands among treatments.

Wavenumber cm^{-1}	Region	Functional group assignment	*p*-value
3400	1	$_{\nu}$(N-H), $_{\nu}$(O-H)	NS
2924	2	Aliphatic $_{\nu\text{-as}}$ (C-H)	NS
2850	2	Aliphatic $_{\nu\text{-s}}$ (C-H)	NS
1650	3	Aromatic $_{\nu}$ (C=C)	NS
1575	3	Amide $_{\delta}$(N-H) and $_{\nu}$ (C=N)	NS
1470	3	Aliphatic $_{\delta}$(C-H)	NS
1405	4	Aliphatic $_{\delta}$(C-H)	NS
1390	4	Aliphatic δ(C-H), potential contributions from carboxylate s(C-O)	NS
1270	4	Phenol $_{\nu\text{-as}}$ (C-O), carboxylic acid $_{\nu}$(C-O)	NS
1110	4	Polysaccharide $_{\nu}$(C-O)	NS
1080	4	Polysaccharide $_{\nu}$(C-O)	NS
920	2	Aromatic δ(C-H)	NS
840	2	Aromatic δ(C-H) less substituted	NS

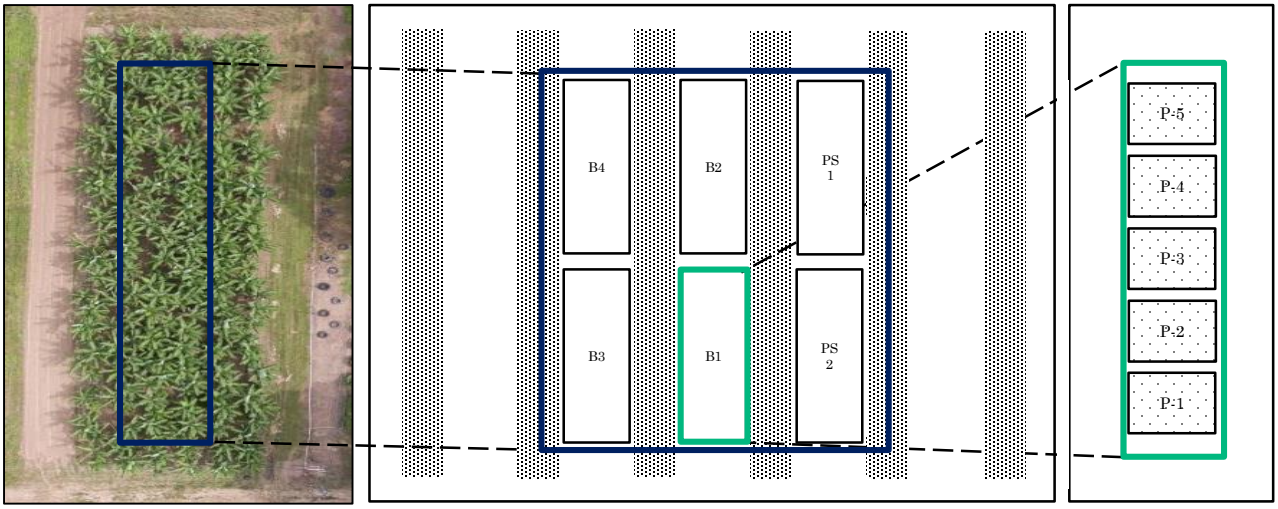

Figure 3. 1. A. An aerial photograph of the study site in the Sub-experimental Station in Gurabo (Perez, 2017) **B.** Field experimental design. B is Blocks, PS is Pilot Study area. The area for the study was the four blocks. **C**. An example of the arrangement in one block, where P is plot.

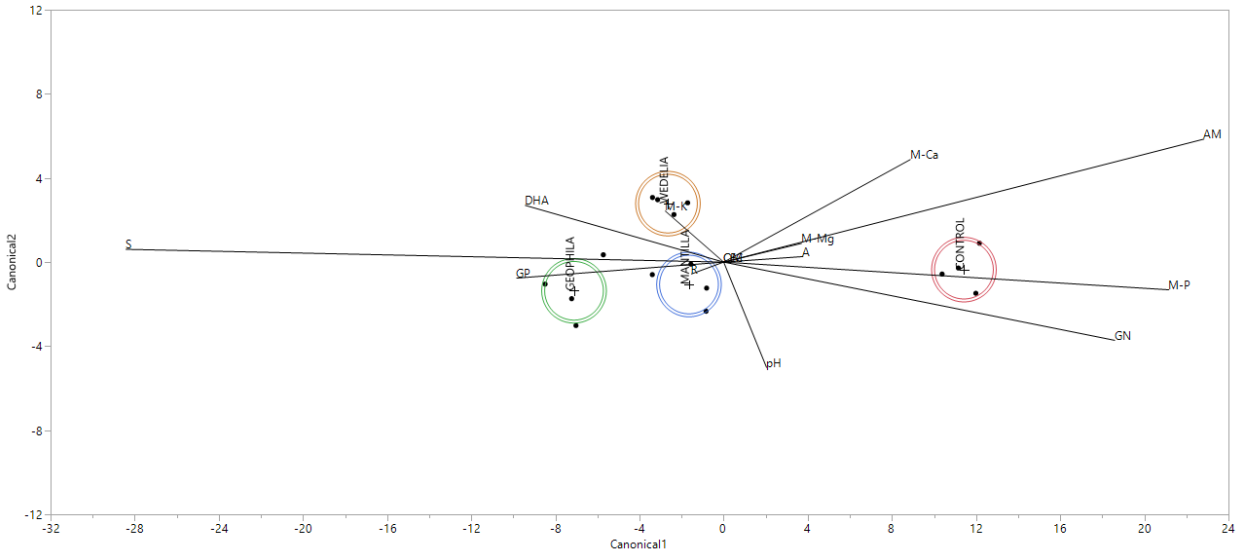

Figure 3. 2. Canonical discriminant analysis for microbial groups and plant species evaluated as living cover crops intercropped with a banana field.

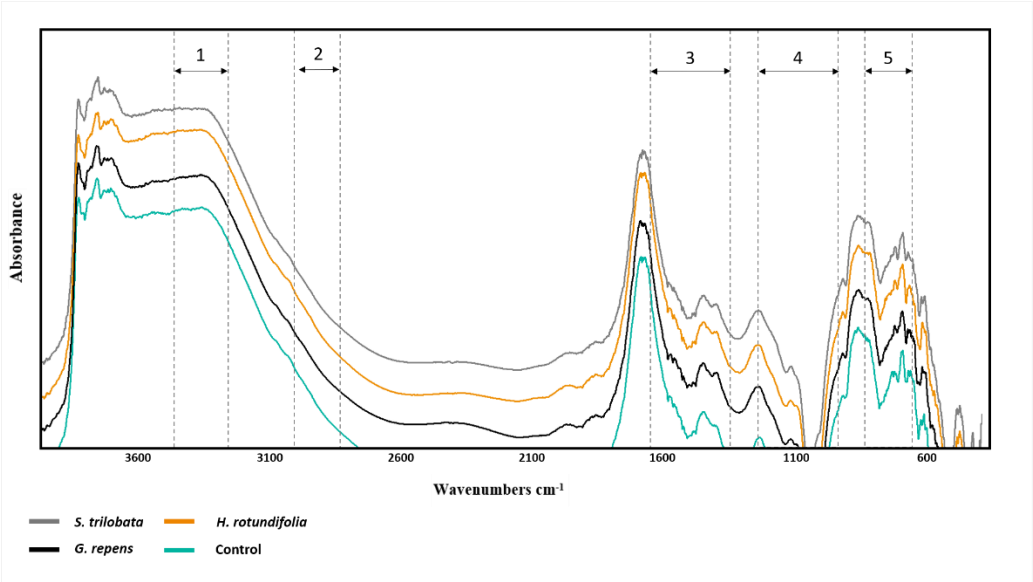

Figure 3. 3. Mean diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectrum of treatments. Regions of interest are presented with numbers. Spectra are offset vertically to improve visualization. Region 1, between 3300 and 3400 cm^{-1} correspond to hydroxyl groups (O-H) and amines (N-H). Region 2 near 2924 and 2859 cm^{-1}, represent aliphatic asymmetric and symmetric stretching vibration respectively. Region 3 near 1650 cm^{-1}, 1575 cm^{-1}, and 1405 $cm^{-1,}$ represent aromatic vibrational asymmetric bonds of aromatic (C=C) groups, amide (N-H) and aliphatic (C-H) bonds respectively. The region 4 near 1110 to 1080 cm^{-1} correspond to polysaccharides symmetric stretching vibrations (C-O). Peaks in region 5 near 920 and 840 cm^{-1} correspond to aromatic functional groups less substituted. The assignment of soil organic functional groups in the regions was according Margenot et al. (2015).

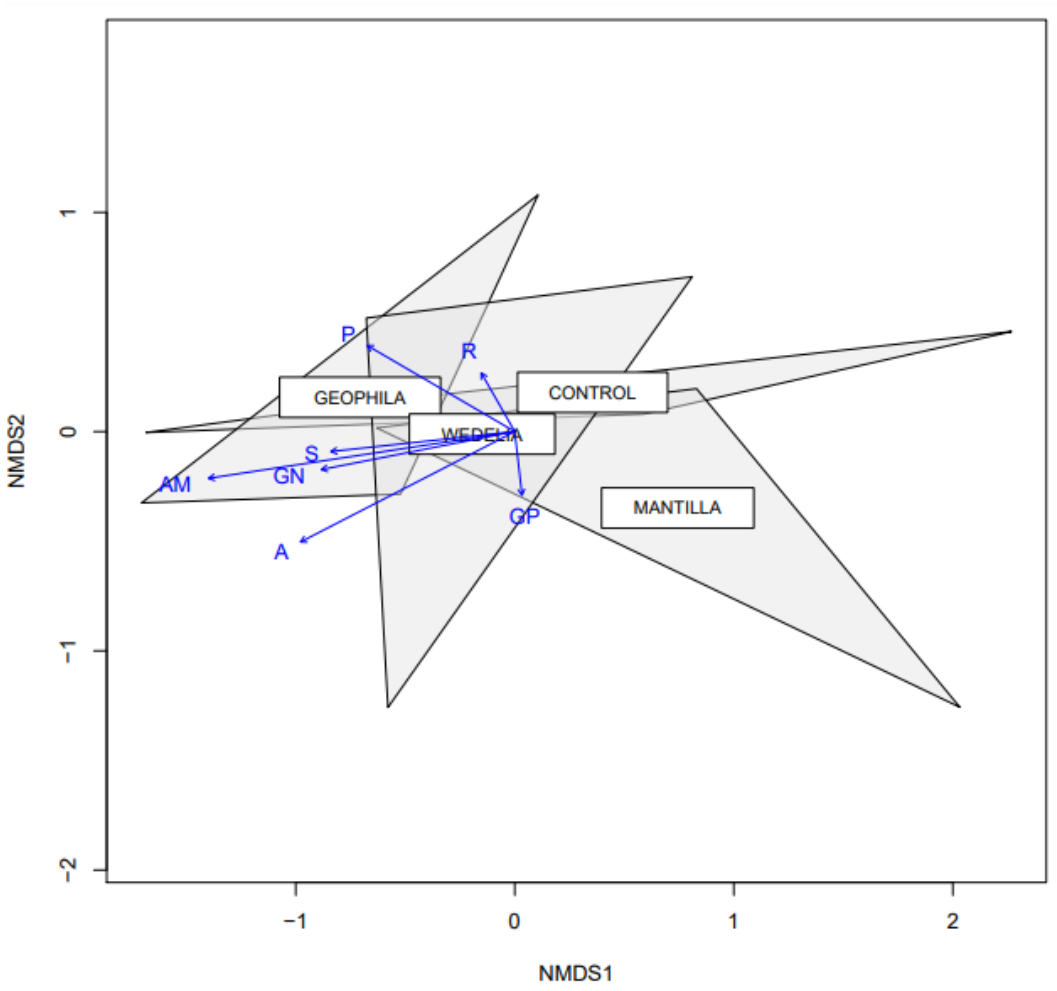

Figure 3. 4. Biplot of the first two axes for the non-metric multidimensional scaling (nMDS) analysis of PLFA and first derivate of peaks from DFRITS in the cover crops species. Geophila = *Geophila repens*, Wedelia = *Spagneticola trilobata* and Mantilla = *Heterotis rotundifolia.*

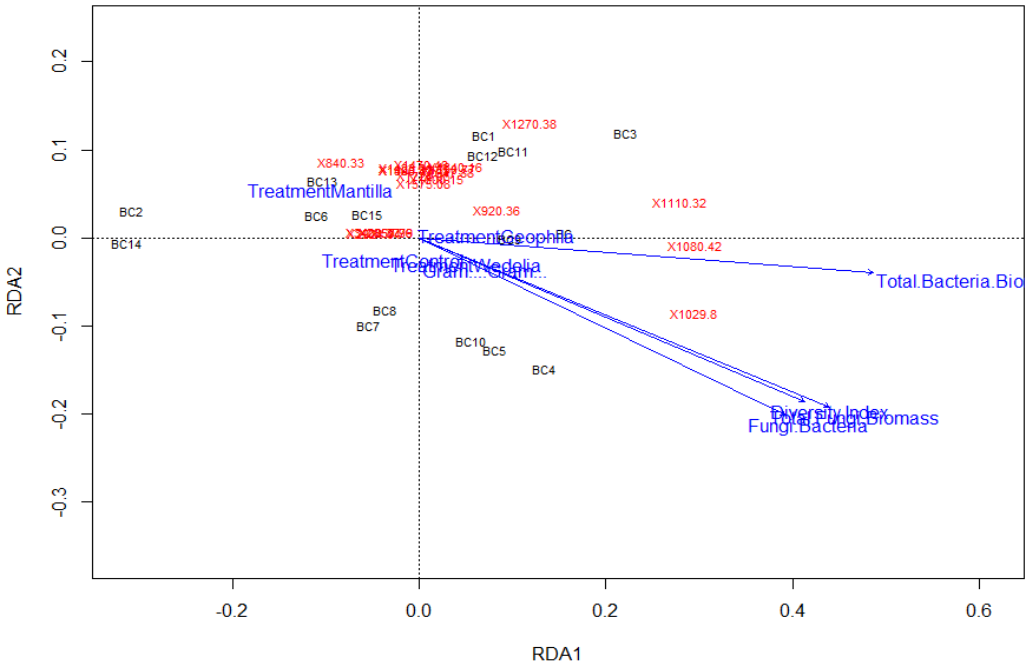

Figure 3.5. Redundancy Analysis plot showing relationship among microbial community groups and aliphatic type-C bonds near a wavenumber of 1120 cm^{-1}. Blue vectors are microbial groups.

3.7 References

Acosta-Martínez, V., R. Lascano, F. Calderon, J.D. Booker, T.M. Zobeck, D.R. Upchurch. 2011. Dryland cropping systems influence the microbial biomass and enzyme activities in a semiarid sandy soil. *Biol Fertil Soils*. 47:655−667

Acosta-Martínez, V., Acosta-Mercado, D., Sotomayor-Ramírez, and L. Cruz-Rodríguez. 2008. Microbial communities and enzymatic activities under different management in semiarid soils. *Applied Soil Ecology.* 38: 249−260

Adetunji, A.T., F.B. Lewu, R. Mulidzi, and B. Ncube. 2017. The biological activities of B-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition. 17*(3): 794−807

Aranda, V., M.J. Ayora-Cañada, A. Dominguez-Vidal, J.M. Martín-García, J. Calero, R. Delgado et al. 2011. Effect of soil type and management (organic cs. Conventional) on soil organic matter quality in olive groves in a semi-arid environment in Sierra Mágina Natural Park (S Spain). *Geoderma.* 164: 54−63. doi:10.1016/j.geoderma.2011.05.010

Blanco-Canqui, H., T.M. Shaver, J.L. Lindquist, C.A. Shapiro, R. Wesley-Elmore, C.A. Francis, and G.W. Hergert. 2015. Cover crops and ecosystem services: insights from studies in temperate soils. *Agronomy Journal.* 107 (6): 2449−2474

Blazier, M.A., T.C. Hennessey, and S. Deng. 2005. Effects of fertilization and vegetation control on microbial biomass carbon and dehydrogenase activity in a juvenile loblolly pine plantation. *Forest Science.* 51(5) 449−459

Bossio, D.A., Scow, K.M. Gunapala, N., and Graham, K.J. 1998. Determinats of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acids profiles. *Microbial Ecology*. 36:1−12

Calderon, F.J., J.B. Reeves, H.P. Collins, and E.A. Paul. 2011. Chemical differences in soil organic matter fractions determined by diffuse-reflectance mid-infrared spectroscopy. *Soil Sci. Soc. Am. J.* 75, 568−579

Carter, M.C., T.J. Dean, M. Zhou, M.G. Messina, and Z. Wang. 2002. Short-term changes in soil C, N, and biota following harvesting and regeneration of loblolly pine (*Pinus taeda* L.). *For. Ecol. Manage.* 164:67−88

Chen, X., D. Wang, X. Chen, J. Wang, J. Diao, J. Zhang. 2015. Soil microbial functional diversity and biomass as affected by different thinning intensities in a Chines fir plantation. *Appl. Soil Ecol.* 92:35−44

Coleman, D., Crossley, D.A., and Hendrix, P. 2004. Fundamental of Soil Ecology. Second Edition.

Dougnon, V.T., J.R. Klotoé, M. Senou, G.O. Oladélé, G. Dougnon, K. Fabiyi, A. Amadou, A. Aniambossou, P. Assogba, H. Bankolé, J. Dougnon, and L. Baba-Moussa. 2017. Chemical composition, cyatotoxicity, and antibacterial activity of selected extracts of Euphorbia hirta, Citrus aurantifolia, and Heterotis rotundiflia on Enteropathogenic bacteria. *EC Microbiology* 12.4: 180−195

Drinkwater, L.E., P. Wagoner, and M. Sarrantonio. 1998. Legume-based cropping systems have reduced carbon and nitrogen lossess. *Nature.* 396:262−265

Ferraz de Almeida, R., E. Rezende, and E. Pinheiro da Mota. 2015. Soil quaility: Enzymatic activity of soil B-glucosidase. *Glob. J. Agric. Res. Rev.* 3 (2): 146−150

Filho, C.C., A. Lourenco, M. DeF. Guimaraes y I.C.B. Fonseca. 2002. Aggregate Stability under Different Soil Management Systems in a Red Latosol in the State of Parana, Brazil. *Soil. Till. Res.* 65:45−61.

Finney, D.M., and J.P. Kaye. 2017. Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. J. Appl. Ecol. 54(2):509−517

Gattinger, A., A. Muller, M. Haeni, C. Skinner, A. Fliessbach, N. Buchmann, P. Mader, M. Stolze, P. Smith, N. El Hage, and U. Niggli. 2012. Enhanced top soil carbon stocks under organic farming. Proceeding of the National Academy of Sciences of the United States of America. 109 (44) 18226-18231

Giacometti, C., M.S. Demyan, L. Cavani, C. Marzadori., C. Ciavatta and E. Kandeler. 2013. Chemical and microbial soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. *Applied Soil Ecology*. 64: 32−48

Hai-Ming, T., X. Xiao-Ping, T. Weng-Guang, L. Ye-Chun, W. Ke and Y. Guang-Li. 2014. Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. *Plos One.* 9(6): e100443.

Kamusingize, D., J. Mwanjalolo-Majaliwa, E. Komutunga, S. Tumwebaze, K. Nowakunda, P. Namanya and J. Kubiriba. 2017. Carbon sequestration potential of East African Highland Banana Cultivars (Musa spp. AAA-EAHB) cv. Kibuzi, Nakitembe, Enyeru and Nakinyika in Uganda.

Kaspar, T.C. and J.W. Singer. 2011. The use of cover crops to manage soil. American Society of Agronomy and Soil Science Society of America, 5585 Guilford Road, Madison, WI 53711, USA. Soil Management: Building a Stable Base for Agriculture. doi:10.2136/2011.soilmanagement.c21

Kaur, A., Chaudhary, A., Kaur, A. Choudhary, R. and Kaushik. R. 2005. Phospholipid fatty acid A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science. Vol. 89, No. 7. Pag 1103−1112

Kong, A.Y. and J. Six. 2012. Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems. *Plant and Soil.* 356(1-2):315−330

Kulmatiski, A., K.H. Beard, J.R. Stevens, S.M. Cobbold. 2008. Plant-soil feedbacks: a meta-analytical review. *Ecol Lett.* 11 (9):980−92

Lehman, R.M., V. Acosta-Martinez, J.S. Buyer, C.A. Cambardella, H.P. Collins, T.F. Ducey, J.J. Halvorson, V.L. Jin, J.M.F. Johnson, R.J. Kremer, D.K. Lundgren, J.E. Manter, J.L. Maul, and D.E. Smith. 2015. Soil biology for resilient, healthy soil. *J. Soil Water Conserv.* 70:12−18

Li, L., M. Xu, M.E. Ali, W. Zhang, Y. Duan, and D. Li. 2018. Factors affecting soil microbial biomass and functional diversity with the applications of organic amendments in three contrasting croplands soils during a field experiment. Plos One. 13(9):e0203812

Liu, Y., J. Wang, D. Liu, Z. Li, G. Zhang, Y. Tao, J. Xie, J. Pan, F. Chen. 2014. Straw mulching reduces the harmful effects of extreme hydrological and temperature conditions in citrus orchards. *Plos One* 9(1) e87094

Margenot, A.J., F.J. Calderon, T.M. Bowles, S.J. Parikh, and L.E. Jackson. 2015. Soil organic matter functional groups composition in relation to organic carbon, nitrogen, and phosphorus fractions in organically managed tomato fields. *Soil Sci. Soc. Of Am.* 79 (3) 772−782

Maul, J.E. and L.E. Drinkwater. 2010. Short-term plant species impact on microbial community structure in soils with long-term agricultural history. *Plant and Soil*. 330: 369−382.

Mayzlish, E. and Y. Steinberger. 2004. Effects of chemical inhibitors on soil protozoan dynamics in a desert ecosystem. *Biol. Fertil. Soils*. 39: 415–421.

Moorman, and M. Beare. 1996. Role of microbial biomass carbon and nitrogen in soil quality. In: J.W. Doran and A.J. (eds). Methods for assessing soil quality. S*oil Sci. Soc. Of Am.* Madison, Wisconsin.

Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In Methods of soil analysis, part 3-Chemicals methods. SSSA Book Series: 5, Bighman, *J.M. Ed. American Society of Agronomy,* Madison, Wisconsin.

Ouellette, L., R.P. Voroney and L.L. Van Eerd. 2016. DRIFT Spectroscopy to Assess Cover Crop and Corn Stover Decomposition in Lab-incubated soil. *Soil Sci. Soc. Am. J.* 80:284−293 doi:10.2136/sssaj2015.10.0371

Pandey, D., M. Agrawal, and J.S. Bohra. 2014. Effects of conventional tillage and no tillage permutations on extracellular soil enzyme activities and microbial biomass under rice cultivation. *Soil and Tillage Research.* 136: 51−60.

Razafimbelo, T.M., A. Albrecht, R. Oliver, T. Chevallier, L. Chapuis-Lardy, L. Feller. 2008. Aggregate associated C and physical protection in a tropical clayey soil under Malagasy conventional and no-tillage systems. *Soil Tillage Res*. 98, 140−149.Rice, C.W., T.B.

Robertson, G.P., and E. Paul. 2000000. Decomposition and soil organic matter dynamics. Methods in Ecosystem Science. Springer Verlag, New York. 104−116

Sainju, U.M., T. Terril, S. Gelaye, and B.P Singh. 2003. Soil Aggregation and Carbon and Nitrogen pools under rhizome peanut and perennial weeds. *Soil Science Society of America Journal.* 67 (1) 146−155

Schipanski, M.E., M.E. Barbercheck, M.R. Douglas, D.M. Finney, K. Haider, J.P Kaye, A.R. Kemanian, D. Mortesen, M.R. Ryan, J.F. Tooker, C.M. White. 2014. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems.* 125:12−22

Shrestha, B.M., G. Certini, C. Forte, B.R. Singh. 2008. Soil organic uses in a mountain water shed of Nepal. *Soil Sci. Soc. Am. J.* 72: 1563−1569 https://doi.org/10.2136/ sssaj2007.0375.

Shi, Y., N. Ziadi, C. Hamel, S. Bittman, S. Hunt, R. Lalande, J. Shang. 2018. Soil microbial biomass, activity, and community composition as affected by dairy manure slurry applications in grassland production. *Applied Soil Ecology.* 125: 97−107 https://doi.org/10.1016/j.apsoil.2017.12.022

Six, J., D. Frey, R.K. Thiet, and K.M. Battern. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70(2):555−569

Sotomayor-Ramírez, D. and G. Martínez. 2018. Soil testing as a nutrient management tool in the Caribbean. Unpublished Manuscript, Research Project 68-F352-16-501 USDA-NRCS

Spedding, T. A., C. Hamel, G.R. Mehuys, and C.A. Madramootoo. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biol Biochem.* 36, 499–512.

Tabatabai, M. 1994. Soil enzymes. Methods of Soil Analysis: Part 2-Microbiological and Biochemical Properties. 775−833

Tseng, D.Y., R. Vir, S.J. Traina, and J.J. Chalmers. 1996. A Fourier-transform infrared spectroscopic analysis of organic matter degradation in a bench-scale soild substrate fermentation (composting) system. *Biotech. Bioeng*. 52: 661−671

Tejada, M., J.L. Gonzalez, A.M. García-Martínez, and J. Parrado. 2008. Effects of different green manures on soil biological properties and maize yield. *Bioresource Technology,* 99: 1758-1767

Turner, D.W. 2013. Factors affecting the physiology of the banana root system. In: Turner D.W., Rosales FE (Eds) Banana Root System: Towards a better understanding for its productive management. Proceedings of an international symposium held in San Jose, Costa Rica. Pp. 107-113

Vance E.D., and F.S. Chapin III. 2001. Substrate limitations to microbial activity in taiga forest floors. *Soil Biol. Biochem*. 33:173−188

Veum, K.S., K.W. Goyne, R.J. Kremer, R.J. Miles, K.A. Sudduth. 2014. Biological indicators of soil quality and soil organic matter characteristics in an agricultural management continuum. *Biochemistry*. 117, 81−99.

Waele, D., Stoffelen, R. and Kestemont, J. 2006. Effects of associated plant species on banana nematodes. *InfoMus*a 15:1−2

Wolińska, A. and Z. Stępniewska. 2012. Dehydrogenase Activity in the Soil Environment, Dehydrogenases, Rosa Angela Canuto, IntechOpen, DOI: 10.5772/48294.

General Conclusion

Overall, the selection of cover crops should be addressed to meet a specific objective. An important characteristic required for plants to be used as living cover crops under a banana canopy is a tolerance to shaded conditions. Additionally, the aim of this study was to evaluate potential plant species that are commonly found in Puerto Rico that could suppress weeds, cover soil in a short lapse of time and enhance soil microbial activity. The results of this work indicated that *Spagneticola trilobata* was the most suitable plant species according to functional growth traits (Chapter II) and soil microbial community (Chapter III). This plant species generated more above-ground biomass and had a higher leaf area index than *Heterotis rotundifolia* and *Geophila repens*. Furthermore, the soil microbial size and composition reflected that *Spagneticola trilobata* and *Geophila repens* were the plant species with the greatest concentrations of microbial biomass and highest trophic complexity. Although *G. repens* did not show the greatest results in the aboveground evaluation, results from below-ground parameters indicated that this species had positive effects in enhancing soil quality over a short-term period. Results from this study demonstrate the positive effects of living cover crops intercropped with banana crop, by reducing both weed density and the labor time for hand weeding. In addition, this study offers evidence of how specific plant species promote different microbial community groups and how they are related to aliphatic C-H bonds, labile C sources in soil. Moreover, this study reflects the potential living cover crops that could adapt to a change in the agroecosystem, after the disturbance of a major hurricane. In contrast to *Spagneticola trilobata*, *Heterotis rotundifolia* and *Geophila repens*, the plant species *Tradescantia zebrina* did not show signs of growth after the hurricane. The results of this study are important to develop effective management practices to enhance soil health under banana canopy. In addition, these plant species should be evaluated under the canopy of others perennial orchards, such as coffee and citrus plantations.

Appendix 1. Fertilization recommendation for banana fields

Table 1.1. Fertilization recommendation for banana fields in Puerto Rico according 'Conjunto Teconologico' by the Agricultural Experimental Station (Lopez y Espinosa, 1995).

Nutrient	Soil level		
	Low	**Medium**	**High**
Phosphorous (ppm)	**<10**	**10-20**	**>20**
Kg P2O5/ha/year	100	50	0
Potassium ($cmol_c$/ kg)	**<0.20**	**0.20-0.50**	**>0.50**
Kg K2O/ha/year	700	600	500
Calcium ($cmol_c$/ kg)	**< 3**	**3-6**	**> 6**
Kg CaO/ha/year	1160	560	0
Magnesium ($cmol_c$/ kg)	**< 1**	**1-3**	**> 3**
Kg MgO/ha/year	200	100	0
Nitrogen		**Indifferent**	
Kg N/ha/year		350-400	

Appendix 2. Pilot studies to select plants species

Table 2.1. Percent of stem cuttings of potential plants species after a month of planting in a pilot study conducted in 2016.

Plant species	Family	Number of cuttings		Percent of survival
		Planted	Sprouted	
Heterotis rotundifolia	Melastomataceae	200	123	62
Euphorbia prostate	Euphorbia	70	17	24
Arachis pintoi	Fabaceae	275	205	75
Geophila repens	Rubiaceae	200	170	85
Indigofera spicata	Fabaceae	40	0	0
Alysicarpus vaginalis	Fabaceae	72	14	19

Table 2.1. Leaf area index and aboveground dry weight of potential plant species for cover cropping under a canopy of banana crop. [1]

Plant species	Leaf area index		Dry weight (g/plant)	
	Direct	Transplant	Direct	Transplant
Heterotis rotundifolia	152	159	17.5	20.0
Geophila repens	306	619	16.4	36.9
Indigofera spicata	0	195	0.0	13.7
Alysicarpus vaginalis	59	190	4.2	7.5
Spagneticola trilobata	221	476	170	45.6
Tradescantia zebrina	1298	1282	195.6	191.1

[1]Pilot Study conducted in 2017. Evaluations were after four mounts of planting cover crops to compare the development by direct planting and by transplanting.

Appendix 3. Biomarkers for phospholipid fatty acids

Table 3.1. Biomarkers used by Wards Laboratories to identify microbial groups according phospholipid fatty acids

Biomarkers	Specific Group	Family	Class
10:0 2OH		Gram -	Bacteria
10:0 3OH		Gram -	Bacteria
11:0 iso 3OH		Gram -	Bacteria
12:0 2OH		Gram -	Bacteria
14:0 iso		Gram +	Bacteria
14:0 2OH		Gram -	Bacteria
14:0 iso 3OH		Gram -	Bacteria
15:0		Gram +	Bacteria
15:0 iso		Gram +	Bacteria
15:0 anteiso		Gram +	Bacteria
16:0 iso		Gram +	Bacteria
16:1 w5c	*Arbuscular Mycorrhizal*		Fungi
16:1 w7c		Gram -	Bacteria
16:1 w9c		Gram -	Bacteria
16:0 2OH		Gram -	Bacteria
16:0 10-methyl	*Actinomycetes*	Gram +	Bacteria
17:0		Gram +	Bacteria
17:0 iso		Gram +	Bacteria
17:0 anteiso		Gram +	Bacteria
17:0 10-methyl	*Actinomycetes*	Gram +	Bacteria
17:0 cyclo		Gram -	Bacteria
18:0 10-methyl	*Actinomycetes*	Gram +	Bacteria
18:1 w5c		Gram -	Bacteria
18:1 w7c		Gram -	Bacteria
18:1 w9c	*Saprophytes*		Fungi
18:2 w6c	*Saprophytes*		Fungi
18:3 w3c	*Saprophytes*		Fungi
19:0 iso		Gram -/Gram +	Bacteria
19:0 anteiso		Gram -/Gram +	Bacteria
19:0 cyclo w8c	*Rhizobia*	Gram -	Bacteria
19:0 cyclo w9		Gram -	Bacteria

19:0 cyclo w6		Gram -	Bacteria
20:1 w9c	*Arbuscular Mycorrhizal*		Fungi
20:2 w3c			Protozoa
20:2 w6c			Protozoa
20:3 w3c			Protozoa
20:4 w6c			Protozoa
22:1 w9c	*Arbuscular Mycorrhizal*		Fungi
20:5 w3c	*Saprophytes*		Fungi