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The effects of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp (*Crotalaria juncea* L.) as legume cover crops on soil physical, biological and chemical properties in an agricultural field in Puerto Rico.

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I dedicate my thesis to my amazing wife Bleyette Maceno Dieudonné and my two sons Lensey Evaldy and Nick Dorsey Maceno.

Dedication is gone to my parents Dieufene Maceno and Alicia Geduis, my sisters Julanie, Monique and Jina Maceno, and to my brother Jean Marie Maceno.

It is also dedicated to the Scientific Committee and to all people who believe in efficiency.

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Abbreviations, Acronyms and Units

DHA	Dehydrogenase Activity (µg $TPFg^{-1}min^{-1}$)
LOI	Loss-On-Ignition (Percentage)
EV	Volumetric water content (m^3water/m^3 soil)
EC	Bulk Electrical Conductivity (dS/m)
Ka	Dielectric Permittivity (F/m)
Ts	Soil temperature (ºC)
Ti	Internal temperature of the wireless sensor (ºC)
MANOVA	Multivariate analysis of variance
ANOVA	Analysis of variance

Summary

The intensification of agricultural land and the massive use of synthetic fertilizers to increase crop productivity represents a global concern. The concern is whether an acceptable standard level of environmental and/or ecological quality can be met and maintained while simultaneously intensifying production through chemical inputs. Though useful, studies have shown the intensification of land use through mechanization, agrochemical inputs, and unsustainable crop management practices provoke harmful impacts on the environment. These effects are mainly contamination of surface and groundwater by adding pesticides, excesses of nutrients through leaching and erosion, reduced soil fertility and quality, loss of biodiversity and ecosystem services. Since the past decades, studies have been emphasized on developing sustainable agricultural management or mitigation practices to integrate into the farming systems. Studies conducted on cover crops reported that they provide several benefits to ecosystems. For instance, cover crops are economically feasible and ecologically sustainable, leading to greater crop productivity, soil and water conservation, and maintenance and recovery of soil fertility. Besides, cover crops as a sustainable management practice can mitigate the harmful effects on the environment. The use of cover crops in tropical regions has been a common practice. Therefore, the integration of some tropical legume cover crops in the farming system could be profitable for the Caribbean region, particularly the island of Puerto Rico.

This study seeks to evaluate the effects of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp (*Crotalaria juncea* L.) as legume cover crops on soil properties and earthworms' dynamics in an agricultural field in Puerto Rico. Based on knowledge gaps, this investigation was focused on assessing electrical conductivity, dehydrogenase activity, dielectric permittivity, aggregate stability, bulk density, pH, strength, temperature, texture, total Carbon and Nitrogen, volumetric water content and water-holding capacity as soil attributes. A wireless sensor network was installed in the field to collect data on volumetric water content, soil

temperature, electrical conductivity and dielectric permittivity. Measurements were conducted on the percentage of germinated seeds, plant density and height, seedlings vigor, canopy cover estimation, flowering dates and resistance of attack of pests to evaluate the performance of jack bean and sunn hemp in the field. In addition, the effect of jack bean and sunn hemp on earthworms' density and biomass was studied.

A multivariate analysis of variance was performed to determine the effects of jack bean and sunn hemp on the percentage of germinated seeds, plant density, seedling, canopy cover estimation, plant height, and flowering date. The findings show that plant species have significant effect on plant density and height, and canopy cover estimation. Sunn hemp grew higher (0.95 ± 0.15) and denser (713.5 ± 203.85) than jack bean. Noted that jack bean had greater coverage during the whole study. Percentage of germinated seeds, plant density, canopy cover estimation, and seedling vigor assessed on jack bean decreased at the second growing period except plant height. Period of planting may be one cause to explain the decrease in the mean of the studied variables. Jack bean had a better performance in the field based on the findings and field observations. In addition, jack bean was more resistant to natural disturbances or disasters and can rapidly recuperate compared with sunn hump.

Multivariate analysis of variance was also performed for the effect of jack bean and sunn hemp on electrical conductivity, dehydrogenase activity, dielectric permittivity, soil aggregate stability, soil bulk density, soil pH, soil strength, soil temperature, soil texture, total Carbon and Nitrogen, volumetric water content and water-holding capacity during two growing periods. Based on the findings, plant species had no significant difference on these evaluated soil properties except on soil temperature during the second planting. Total carbon was slightly modified after the first planting. This result could be resulted due to the rapid decomposition of the biomass of jack bean added in the soil during the first planting round. Therefore, plant species can have a positive effect on total carbon in the soil.

In addition, two earthworm samplings were conducted during this study. The findings showed that jack bean and sunn hemp had no significant effect on the density and biomass of the population of earthworms during both planting rounds. I sampled *Pontoscolex* sp., *Pontoscolex corethrurus*, *Pontoscolex spiralis, and Amynthas or Polypheritima* sp. during this study. I found *Pontoscolex* sp. was the most abundant species collected during the study. I also found more than 80 % of sampled earthworms were immature. Additionally, immature worms were mostly found in plots of jack bean and sunn hemp. In addition, the density of earthworms was (18 m^2) in jack bean, (44 m^2) in sunn hemp and (40 m^2) in control plot. The mean of fresh biomass was (3.58 ± 0.26) in jack bean, (8.14 ± 0.31) and (7.23 ± 0.18) in control plot. During the second planting, the density was (94 m^2) in jack bean II, (74 m^2) in jack bean I, and (32 m^2) in control plot. Finally, the mean of fresh biomass was (9.74± 0.52) in jack bean II, (5.61 ± 0.22) in jack bean I, and (5.22 ± 0.40) in control plot.

CHAPTER 1. General Introduction

Agricultural intensification and large chemical inputs

In every region of the world, the intensification of crop-based agriculture has been associated with a significant augmentation in the use of synthetic fertilizers (Morris et al., 2007). The objective of using large chemical inputs is to raise food production. The millennium project in 2008 stated food production will have to double in 30 years to help solve the food crisis (Roberts, 2009). Total production can be increased through more intense land use by reducing the amount of fallow, increasing the numbers of crops grown per year, and by cultivating new agricultural land (Wingeyer et al., 2015). Roberts (2009) reported the world will not be able to meet its food production goals without using fertilizers. The purpose of using large chemical inputs is to balance the loss of soil nutrients from previous crops. Though useful, there are major concerns of harmful impacts on the environment from large chemical inputs to increase crop productivity.

A previous study highlighted that the doubling of global food production during the past two decades has been accompanied by a massive increase of the use of synthetic fertilizers and extensive use of irrigation and energy (Wingeyer et al., 2015). For instance, heavy machinery or repeated tillage operations (Stewart and Texas, 2015), and the use of synthetic nitrogen, phosphorus, and pesticide applications, and intensive use of irrigation are common practices to increase food production (Gomiero, 2016). Commercial fertilizers are responsible for 40 to 60 % of the world's food production (Roberts, 2009). For example, in India, the contribution of fertilizers towards an increase in food grain production is estimated to be 50 percent (Tiwari, 2007). In Puerto Rico, the majority of farmland is dominated by industrial agriculture: a system of chemically-intensive food production implemented single-crop farms (Santiago et al., 2016). Despite these benefits, the use of chemical fertilizers and pesticides have negative impacts on the environment and consumers (Santiago et al., 2016). A matter of concern is whether an acceptable standard level of environmental and or ecological quality can be met and maintained while simultaneously intensifying production through the chemical inputs.

Global environmental impacts

Previous studies have documented the environmental effects of agricultural intensification to increase global crop productivity (Santiago et al. 2016; Vos et al. 1984; Firbank et al. 2009; Zhao et al. 2015; Issaka and Ashraf, 2017). These effects are mainly contamination of surface and groundwater by adding pesticides, excess of nutrients through leaching and erosion, reduced soil fertility and quality, and loss of biodiversity and ecosystem services. Vos et al. (1984) suggested that such monocultures will be vulnerable to invasion by weeds and would have high incidence of diseases and pests. Entire regions of the world now are dominated by virtual monocultures of crops such as corn, rice, and others (Killebrew and Wolff, 2010; Tilman, 1999). These monocultures have replaced natural ecosystems that once contained hundreds to even thousands of plant species, thousands of insect species, and many species of vertebrates (Tilman, 1999). In comparison with the point source pollution caused by industrial activities, non-point source pollution by agricultural activities is a much bigger matter of concern (Lin et al. 2018). For example, nitrogen and phosphorus loss into the aquatic environment in agricultural settings are the two main causes of non-point source pollution, stimulating eutrophication in both agricultural areas and adjacent natural habitats (Vos et al., 1984).

Since the past two decades, the application of fertilizers is almost an imperative in farming systems due to the decline of soil fertility. This results from the intensive use of cropland and inability to rapidly replenish nutrients consumed by the growth of previous crop (Epa, 2001). However, the intensive use of pesticides and fertilizers in agricultural practices increases the potential for contamination of surface and groundwater supplies. The United States Geological Survey stated in the year 1999 at least 143 different pesticides and 21 transformation products have been found in groundwater, including pesticides from every major chemical class in the world (Aktar et al., 2009). For example, excess concentrations of Nitrate ($N0_3$-N) in drinking water can cause blue baby syndrome (methemoglobinemia) in infants and is also a health hazard for ruminant animals (Beauchamp et al., 1992). Besides, nutrient contamination in water bodies reduces oxygen levels and harms fish and plant populations (Vos et al., 1984). In addition, the disturbance of delicate aquatic ecosystems can occur (Issaka and Ashraf, 2017; Killebrew and Wolff, 2010). One important consequence of eutrophication is that it contributes to depauperating of plant assemblages through the increase of a small number of potential dominant species that are better able to capitalize on increased nutrients availability (Firbank et al., 2008).

Earth's ecosystems sustain biological diversity and provide myriad services of benefit to humanity including the purification of air and water. However, many of them are being disrupted by large-scale land use change and other environmental alterations, especially in tropical settings (Laurance et al., 2014). Agricultural intensification through increased fertilization within fields in cropland expansion at large-scales is considered to be a key driver of biodiversity loss and the decline of ecosystem services (Zhao et al., 2015). There is a great deal of evidence documenting how farming practices influence species richness and abundance of taxa, and other biodiversity threats posed by agricultural intensification (Firbank et al., 2008). Therefore, proper land use strategies and good crop management practices are needed to conserve soil fertility and sustain ecological integrity.

Another major concern is soil erosion induced by land use change and inappropriate soil management practices. Soil erosion is a natural and inevitable process that can become a serious environmental and economic problem when it is accelerated by human activities (Del et al., 1998). For example, erosion is a major problem in areas with expanding population, agricultural production, construction and increasing urbanization (Issaka and Ashraf, 2017). Besides, Millennium Ecosystem Assessment identified unwise land use choices and harmful crop or soil management practices as the major drivers of increasing soil erosion (Labriere et al., 2015), in combination with environmental factors such as topography, rainfall, natural vegetation, and erodibility of the soil (Gumbs, 1997).

Several Caribbean islands are characterized by steep slopes with a high percentage (58 percent) of the land area having slopes greater than 30° on which farming is routinely practiced (Persaud, 1997), leading to intensification in soil erosion (Pimentel and Burgess, 2013). Acosta (2009) reports soil erosion is one of the most chronic environmental problems on citrus farms in Puerto Rico. Farms in many Puerto Rican municipalities have steep slopes and high annual rainfall (mean annual rainfall of 1500 mm in the north and 508 mm in the central mountain area) that can cause severe soil erosion (Acosta, 2009). In addition, soil erosion in Puerto Rican coffee plantations without ground cover was found to be ten times greater than from adjacent areas of coffee with natural ground cover (Del et al., 1998). Poor management of agricultural land induces soil erosion that leads to reduced productivity, or, in extreme cases to the abandonment of the land (Gomiero, 2016).

The environmental effects of soil erosion are numerous and widespread. For instance, soil erosion has multiple consequences both on and off site, including decreased crop yields, increased atmospheric CO_2 concentration, decreased water quality (turbidity and particle-born, pollutants), sedimentation of reservoirs, and disturbed hydrological regimes such as increased flood risk due to riverbed filling and stream plugging (Labrière et al., 2015). Moreover, soil erosion is a major cause of soil degradation because it involves the removal of the most fertile topsoil where organic matter and nutrients are concentrated (García-Ruiz et al., 2015). Soil erosion adversely hinders the growth of plants, agricultural yields, quality of water and recreation (Issaka and Ashraf, 2017). For example, soil erosion has been estimated to reduce yields on about 16 % of agricultural land, especially cropland and pasture in Africa and Central America (Gomiero, 2016). In addition, soil erosion reduces the valuable diversity of plants, animals, and soil microorganisms (Pimentel and

Burgess, 2013). Appropriate practices of soil management are needed to reduce and offset these severe consequences.

Justification of the study

Historically, cover crops have been an integral part of agricultural production systems and extensively used to provide a wide array of services (Scholberg, 2010). Cover cropping was largely abandoned by the late 1950s when conventional agriculture turned to synthetic fertilizers (White, 2014). The abandonment of using cover crops was due to inexpensive chemical fertilizers available to farmers (Acosta, 2009). Prior to this change, the uses of cover crops in the farming systems in tropical regions was a common practice. A return to the use of some tropical legume cover crops could be profitable for the Caribbean regions, particularly the island of Puerto Rico, to decrease the environmental and ecological risks provoked by unsuitable agricultural practices. Previous studies reported that cover crops can mitigate land degradation by improving nutrient management and providing protection against soil erosion (Stewart and Texas, 2015). Previous investigations conducted on cover crops state cover crops provide benefits to ecosystems; however, appropriate soil management practices are the key for providing these benefits.

Cover crops have been long used to reduce soil erosion and water runoff and improve water infiltration, soil moisture retention, soil tilth, organic carbon and Nitrogen (Reddy, 2003; Mosjidis, 2011; Mennan et al., 2009). Cover crops carpet the soil, protecting them from exposure to rainfall within or between cropping seasons (Bargout and Raizada, 2013), and providing permanent soil cover as a physical barrier to raindrops (Acosta, 2009). Residues from cover crops can be incorporated as green manure to supply macro and micronutrients for increasing soil fertility for the next crop (Gill and Mcsorley, 2011).

Cover crops are economically feasible and ecologically sustainable, leading to greater crop productivity, soil and water conservation, and maintenance and recovery of soil fertility (Mubiru et al, 2009). There are few reasons to evaluate cover crops for using in modern cropping systems.

For instance, the use synthetic fertilizers in the modern agriculture has been associated with concerns such as high fertilizer prices, high energy costs, water quality concerns, soil tilth, and compaction issues, and concerns about replacing organic matter with increased residue removal practices (Hoorman, et al., 2009). Regardless of the benefits provided when using cover crops, they still need to be integrated into agricultural management practices.

Overall and specific objectives

The overall aim of this study is to investigate the effects of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp (*Crotalaria juncea* L.) as legume cover crops on soil properties in an agricultural field in Puerto Rico, with the goal of promoting the integration of those cover crops in the farming system as a sustainable management practice. Based on the knowledge gap that exists concerning the benefits of these two cover crops, this research was focused on measuring water holding capacity, volumetric water content, soil texture, soil strength, soil pH, total Carbon and Nitrogen, soil texture, soil bulk density, soil temperature, electric conductivity, dielectric permittivity and dehydrogenase activity to evaluate the effects of these cover crops. The objective of the second chapter was to evaluate the performance of the given cover crop while growing in the field. The third chapter assessed the effect of those planted cover crops on the soil attributes listed above. Lastly, the objective of the fourth chapter is to evaluate the effects of jack bean and sunn hemp on earthworm populations.

Research questions

Is there any difference in the studied variables on jack bean and sunn hemp with planting times?

What are the effects of jack bean and sunn hemp on water holding capacity, volumetric water content, soil texture, soil strength, soil pH, total Carbon and Nitrogen, soil texture, soil bulk density, soil temperature, electric conductivity, dielectric permittivity and dehydrogenase activity?

What is the effect of jack bean and sunn hemp on the density of earthworms?

Hypotheses

Hypothesis I. In chapter two, I hypothesize plant species have a significant effect on the percentage of germination, plant density, seedling vigor, canopy cover estimation and plant height. In addition, planting period has a positive effect on the studied variables on jack bean species and rotation plots.

Hypothesis II. I hypothesize in chapter three, jack bean and sunn hemp have positive effects on water holding capacity, volumetric water content, soil texture, soil strength, soil pH, total Carbon and Nitrogen, soil texture, soil bulk density, soil temperature, electric conductivity, dielectric permittivity and dehydrogenase due to their benefits they provide to the soil.

Null hypothesis (H_0): the effects of jack bean and sunn hemp is equal on the assessed soil attributes during two different periods.

Alternative hypothesis (H_1): The effect of jack bean and sunn hemp is different on the assessed soil properties during two different periods.

Hypothesis III. In chapter four, I hypothesize that jack bean and sunn hemp have significant effects on the density of earthworms during the two planting rounds.

Null hypothesis (H_0): The density of earthworms is equal in jack bean and sunn hemp plots.

Alternative hypothesis (H_1): The density of earthworms is different in jack bean and sunn hemp plots.

The relevance of this study

Several studies promote the integration of cover crops in modern agricultural sciences as a sustainable management practice capable of mitigating the harmful effects of agricultural intensification and large chemical inputs to the environment. Legume cover crops could increase crop yield through improved nutrient supply/availability and/or improved soil water holding capacity. They also offer other benefits such as increased soil organic matter, cation exchange capacity, microbial activity and reduction of soil temperature (Amede and Kirkby, 2001). Acosta (2009) reported that the use of legume cover crops in farming systems can be a viable management practice in Puerto Rico. Another study reported the integration of legume cover crops into farm systems as an option to improve physical and chemical soil properties (Bernardino and Arturo, 2014).

In Puerto Rico, the majority of the farmland is dominated by industrial agriculture which has a significant input of pesticides and chemical fertilizers (Santiago et al. 2016). A recent study identified 124,187 ha (306,873 acres), or 14 percent of Puerto Rico, as well-suited to mechanized agriculture with slopes under 10 percent (Gould et al., 2017). Noted that this agricultural practice has an impact on the environment and consumer health (Santiago et al., 2016). For instance, pesticides residues accrue in the physical body, and this exposition seriously harms the health of the population in the long term. In addition, synthetic fertilizer and pesticides lost by leaching go and contaminate the water bodies. These impacts could potentially be reduced through the use of legume cover crops such as jack bean and sunn hemp. However, the effects of these cover crops on soil property attributes are not well understood and merit further study to evaluate the benefits provided to the soil. Therefore, the study will be conducted to fill this knowledge gap and many uncertainties that come with the growth of these crops.

General literature review

Cover crops are plants that are grown to provide soil cover and improve the physical, chemical, and biological characteristics of soil (Florentín et al., 2010; Nielsen et al., 2015), and to benefit other crops (Mutch and Martin, 2010). Farmers grow cover crops in a variety of ways, including growing them year round as a living mulch, planting after harvest or intercropping (White, 2014). There are three major categories of commonly grown cover crops: grasses, legumes, and brassicas (Dabney et al., 2010). Legume cover crops fix atmospheric nitrogen into a form that plants and microorganisms can use while non-legume species recycle existing soil nitrogen and can reduce the risk of excess nitrogen leaching into groundwater (Mutch and Martin, 2010). Cover crops have long been used in the tropics for soil and water conservation, especially on steep land, and to improve soil structure (Acosta, 2009).

Key benefits provided by cover crops

Generally, cover crops are used to pursue the following objectives: provide soil cover for non-tillage, reduce pests, disease and weed infestation, improve soil structure, promote biological soil preparation, and add biomass to the soil (Florentín et al., 2010). Reducing soil water erosion is one of the main reasons for/ growing cover crops (Kaspar et al., 2011). Cover crops provide a variety of agronomic benefits which can be translated into economic benefits by decreasing input costs and stabilizing yield variability (Duzy and Kornecki, 2013). Additionally, cover crops have the potential to prevent off-farm environmental degradation while improving soil quality (Kaspar and Bakker, 2015). Cover crops can provide many different benefits in modern cropping systems (Hoorman et al., 2009: Fageria et al., 2014)**.** Besides, the Midwest Cover Crops Council (MCCC) stated that cover crops have the potential to reduce cropping systems costs by reducing the use of herbicide, nitrogen fertilizer and irrigation.

Soil physical properties

Cover crops increase porosity and soil water infiltration and storage, and improve soil quality by increasing soil organic matter, soil aggregation, rooting depth, and decreasing soil compaction (Schmidt et al., 2018; Kaspar and Bakker, 2015; Stewart and Texas, 2015). There is also a body of evidence that supports the ability of cover crops to increase soil organic matter and soil carbon, and to improve soil physical properties which enhance soil water dynamics (Basche et al., 2016; Schmidt et al., 2018). Living cover crops can also significantly alter soil temperatures; for example, in one study cover crops decreased the amplitude of day and night temperature more than the average temperature, resulting in less variability (Hoorman et al., 2009). Remediation or prevention of soil degradations requires integrated management solutions that, for agricultural soil, include cover crops or crop residues management to reduce raindrop impact, maintain higher infiltration rates, increase soil water storage, and ultimately increase crop production (Stewart and Texas, 2015).

Soil organic matter (SOM)

One of the most significant effects of the use of cover crops is to increase organic matter content in the topsoil (Veiga, 2017; Fageria et al., 2014; Isik et al., 2014; Amede and Kirkby 2001), which in turn should result in larger, more stable aggregates that are less susceptible to detachment (Kaspar et al., 2011). Soil organic matter (SOM) is important in promoting good soil structure and cation exchange capacity. Noted that cover crops can be a main source of organic matter and can help maintain it gradually in the soil (Penn State University College of Agricultural Sciences, 2010).

Biomass/cover crops

Cover crops can produce enough aboveground biomass and nitrogen during the growing season. Some reports of biomass production from the Northern Great Plains in the USA would suggest that cover crop production is sufficient to produce both profitable forage and wind erosion protection (Nielsen et al., 2015). Acosta (2009) reported jack bean generated the largest average amount of biomass (3,579 kg/ha) and accrued more nitrogen in the biomass (95kg/ha) during the growing season than velvet bean. In Florida, Sunn hemp crop also produced about 3628.74 kg/acre of dry biomass and fixed 81.65 kg/acre of N at three months (Valenzuela and Smith, 2002). Another study showed early planted cover crops with more growing degree days had higher dry biomass production with a higher organic carbon and N content than late-planted covers (Dabney et al., 2001).

Chemical properties

Nitrogen and nutrients recycling management

Cover crops can be utilized to manage N in agricultural soils by altering N cycling and availability (Kaspar et al., 2011). Cover crops play a key role in nutrient cycling in the soil and add labile organic carbon to the system, bringing economic benefits with minimal long-term impact on the soil and on water and air quality (Veiga, 2017). Cover crops can have multiple benefits for N management whether occupying the land for six weeks or six months: they can reduce N leaching losses, reduce erosional N losses, fix N, immobilize N and increase crop N uptake (Dabney et al., 2010). Legume cover crops increase cation exchange capacity in the soil (Amede and Kirkby, 2001). Moreover, legume cover crops fix nitrogen from the air adding up to 45.36 to 68.04 kg/acre of this essential nutrient whereas non-legume cover crops recycle leftover nitrogen from the soil, storing it in the roots and aboveground plant material, where a portion will be available to the following crop (Hoorman et al., 2009; Bernardini and Arturo, 2014).

Reducing fertilizers and operating costs

The use of cover crops in the farming system can also be economically profitable for farmers. Legume cover crops promote the economical use of nitrogenous fertilizers and greater biological balance of N in the soil (Amede and Kirkby, 2001), thus decreasing the effects of pests and diseases (Mubiru and Coyne, 2009). When the residues of cover crops become completely decomposed, nitrogen is available for the new cropping season. The demand for N fertilizer purchase will be less which results in monetary savings for the farmers. One study claimed that cover crops have the potential for eliminating pre-herbicides that control the early-season weeds, whereas late-season weeds can be managed with post herbicides on a needed basis (Reddy et al., 2003). In addition, cover crops, when applied using no-till farming techniques, can reduce costs by reducing the use of heavy machinery and expensive equipment (Schmidt et al., 2018).

Biological properties

Cover crops increase the potential for macro- and microfaunal activity in soils because they increase the total inputs of organic materials to soils (Kaspar et al., 2011). Based on the accumulation of organic residues left on the soil surface by the cover crops, the intensity of biological activity on the soil surface increases (Hoorman et al., 2009). Kaspar et al. (2011)

reported that after 3 years with crimson clover or cereal rye cover crops soils had greater total bacterial and fungal propagule density and fluorescein diacetate hydrolytic activity (FHA) than the soil without a cover crop. Additionally, cover crops increase inocula of mycorrhizal fungi in soils which are important in enhancing early growth and survival of some crops, particularly cotton (Dabney et al., 2001).

Reduce disease and weed infestation

Weeds are the most important limiting yield factors and weed control without using herbicides is an expensive time-consuming task (Isik et al., 2014). Weed growth is a major source of inefficiency diverting limited resources (nutrients, water, light, and labor) and results in about one-third of yield losses in major crops (Connolly et al., 2018). Isik et al., (2014) reports using cover crops for weed control is one of the broadly applied alternative methods. A study in Puerto Rico found that, on a one-year cropping sequence, velvet bean (*Mucuna deerigiana*) planted prior to planting tomatoes resulted in suppressed growth of purple nutsedge in the tomato Crop (Semidey and Flores-lópez, 2006). Purple nutsedge is a persistent weed and one of the most difficult to control in vegetables and agronomic crops due to its tolerance of many control practices, including herbicides (Webster and Grey, 2014). In a study on weed control and grape yield with coverage management Veiga (2017) observed up to a 95% reduction in weed infestation when cover crops were used compared to the control crop without them. Weed suppression by cover crops has been a key element in the successful adoption of no-till agriculture in South America (Dabney et al., 2001). Moreover, cover crops can also suppress weeds and enhance the natural enemies of insect pests (Timper et al., 2006)**.**

Moreover**,** many cover crops have the ability to release allelochemicals in the environment and create unfavorable conditions for weed germination and establishment (Mennan et al., 2009). For example, Sunn hemp is considered to be allelopathic to other plants which could its ability to suppress weeds (Mosjidis et al., 2011). Allelopathy has been defined as any direct or indirect harmful effect produced in one plant through toxic chemicals released into the environment by another (Creamer et al., 1996). In addition to allelopathic impacts, the effect of cover crops on weed infestation can also result from both cover crops being left on the surface of the soil in the form of mulch, preventing the overgrowth layer of weeds. (Błażewicz-Woźniak et al., 2015). Cover crops can also suppress pests such as insects and nematodes in the fields that could impact greatly yields (Mosjidis et al., 2011). For example, leguminous cover crops such as sunn hemp and 'Iron Clay' cowpea (*Vigna unguiculata*) provide viable methods for nematode management (Kokalis-Burelle et al., 2008; Wang et al., 2002). Additionally, cover crops can be used as natural biological agents to control weeds in crops and to reduce the need for herbicides (Semidey and Flores-lópez, 2006).

Conclusions

Since the past few decades, the intensification of agricultural land and the vast application of synthetic fertilizers is very debating in the world because of the harmful consequences. We agree that the increased of crop production is necessary due to the augmentation of the global population which raises the demand for food. Presently, researchers accentuate their studies to find agricultural management practices that can maintain crop productivity and lessen the harmful effect of conventional agriculture on the environment. Previous studies have been conducted on the integration of legume cover crops in the farming system. Based on the findings of the previous studies the integration of cover crops in the farming systems seem to be considerable. The outcomes of those studies reported many benefits of using cover crops in the farming system and it is a sustainable agricultural practice. Besides their potential benefits, they can decrease the large chemical inputs using when planting cash crops which are economically favorable for the farmers. Today, the evaluation of cover crops in the farming system is fundamental to help farmers in their decision.

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Chapter II. The assessment of the performance of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp (*Crotalaria juncea* L.) during two planting rounds.

Abstract

The use of cover crop in agricultural systems is a sustainable management practice based on the findings of previous studies on cover crops. Cover crops provide a considerable soil cover to prevent the soil from erosion and influence soil surface temperature and evaporation. Cover crops improve nutrients and water management, increase nitrogen availability in the soil as benefits to cash crop and preserve biodiversity. Jack bean and sunn hemp are among the cover crops that provide those benefits and well adapted in tropical regions. An experimental field was conducted on jack back and sunn hemp by evaluating the percentage of germinated seeds, plant density, seedling vigor, canopy cover estimation, plant height, and flowering date to measure the performance of those cover crops. A MANOVA statistical test was performed to determine the effects of jack bean and sunn hemp on the assessed variables and planting regime. Results show that plant species have significant effect on plant density and height, and canopy cover estimation. I found sunn hemp plants grew taller and denser than jack bean plants. Meanwhile, jack bean provided a greater coverage to the soil than sunn hemp plants during the study. The mean of percentage of germination, plant density, canopy cover estimation, and seedling vigor of jack bean decreased at the second growing period. Only the mean of plant height was increased during the second planting round. In addition, the mean of density of jack bean plants evaluated after fourty days after sowing was higher than the mean observed at twenty days. Lastly, jack bean had a greater performance in the field than sunn hemp based on the findings and field observations.

Key words: canopy cover estimation, cover crops, crop production, jack bean, flowering dates, the percentage of germinated seeds, plant density and height, Puerto Rico, seedling vigor, soil quality, and sunn hemp.

Introduction

Growing cover crops in cropping systems is a key strategy to improve soil quality and consequently crop yields (Fageria et al., 2014).This sustainable practice needs to capture worldwide farmers due to its benefits. Several studies have shown the benefits of using cover and cash crop in rotations since earliest times. Cover crops can be managed to achieve certain purposes such as: conserving N, adding N or C to an agricultural systems, optimizing the C:N ratio of residues, supplying residues for erosion control or improving the availability of N to a subsequent crop (Dabney et al., 2010; Amede and Kirkby, 2001). The usefulness of cover crops is a sustainable management practice used to improve soil and enhance soil fertility, water quality, weed control/suppression, pest, and pathogen populations and preserve biodiversity in agro-ecosystems (Acosta, 2009; Mulinge et al, 2017).

The choice to use legume cover crop in the farming systems could be a critical decision of the farmers. Researchers have reported the use of legume cover crops is an ecologically sustainable practice that plays an important role in the recovery of soil fertility (Mulinge et al, 2018; Muribu and Coyne, 2009). There are several species of tropical legumes that have been studied for their potential as cover crops and they have been reported to influence soil surface temperature and evaporation within the plant root zone leading to improve nutrient and water management (Mulinge et al, 2017; Florentín et al., 2010). Additionally, the use of those crops is an economical choice for the farmers which decrease the use of inorganic fertilizers and lessen the impacts on the environment.

Legume cover crops biologically fix atmospheric Nitrogen (N), which subsequently becomes available to next crop during residues decomposition (Reddy et al., 2003; Mosjidis, 2011). Legume cover crops usually have C: N ratios lower than 20 and reduce the amount of nitrogen fertilizer required for a given yield level (Dabney et al., 2001). Moreover, leguminous crops can serve as sinks for plant nutrients that might otherwise be lost by volatilization or leaching (Mwalimbwala, 2015). Besides, legumes cover crops provide other considerable benefits such as providing cover to reduce maintenance and improving soil physical properties, increasing soil organic matter, cation exchange capacity, microbial activity and reduction of soil temperature (Amede and Kirkby, 2001); and mitigate disease problems (Morel et al., 2012; Mwakimbwala, 2015). Another benefit of leguminous crop is reduction of herbicides, pesticides, and fertilization costs (Florentin et al., 2010). Among leguminous crops, jack bean and sunn hemp are two common that provide the benefits listed above. Moreover, sunn hemp and jack bean have been tested in many regions in the world for those reasons. Based on the sharp increase of chemical inputs in the agriculture the evaluation of these two cover crops in tropical regions is an important decision for the farming systems.

In tropical regions, sunn hemp is principally used as a green manure crop for soil improvement and is an excellent rapid-growing green manure to be included in rotation with vegetable, ornamental to add nitrogen and organic matter to suppress weed, to reduce root-nod nematodes (Li et al., 1982; Rotar and Joy, 1983). In addition, sunn hemp provides a viable method to nematode management in the cropping season (Kokalis-Burelle et al., 2008; Wang et al., 2002). Therefore, using sunn hemp as a cover crop may offer alternatives to nematicides (Wang et al., 2002). Besides nematodes suppression, crop residues from sunn hemp also help to improve soil organic matter, crop vigor and health (Gill and Mcsorley, 2011). It can produce 5,000 to 12,500 lb./acre (5,600 to 14,000 kg/ha) of dry biomass and fix to 182 lb./acre (204 kg/ha) (Brunner et al., 2009). Sunn hemp is used as green manure crop, was released in 1982 by the National Resources of Conservation Seirra (NRCS), formerly the Soil Conservation Service and the University of Hawaii Institute of Tropical Agriculture (Wang et al. 2002; Mosjidis, 2011: Balkcom and Reeves, 2005).

Other potential uses of sunn hemp are forage, paper fiber, and as an alternative fuel crop (Brunner et al. 2009; Acosta, 2009). Sunn hemp is grown mainly in Brazil, India and West Pakistan for its fiber. It is used in the production of twine, rug yarn, tissue paper, fishnets, sacking, canvas and cordage and biomass for fuel (Rotar and Joy, 1983; Sheahan, 2012). Sunn hemp is also helpful as a tool for breaking up soil compaction due either to naturally heavy clay subsoil layers or hard pans resulting from machinery traffic (Valenzuela et al., 2002). Its importance as a cover crop is due to its biomass production, Nitrogen accumulation, reduced pests and pathogen infestation, and weed suppression when planted (Price et al., 2006).

Jack bean attains rapid initial soil cover due to the great size of its leaves and it possesses a vigorous taproot with an outstanding capacity to decompact soil (Florentín et al., 2010). A study conducted in Nicaragua showed that farmers were attracted to the performance of jack bean due to its vigorous growth, good soil cover and outstanding level of adaptation to drought stress based on green forage yields (Mwakimbwala, 2015). Generally, jack bean produces a great amount of dry biomass and is an excellent fixation of nitrogen. Acosta (2009) reported dry biomass and N accumulation from jack bean are greater than four other cover crops tested in farms in Puerto Rico. As a cover crop jack bean produces phytochemicals that act as a pesticide, bactericide and a fungicide (Sheahan, 2012). Jack bean also has good nutritive quality for feed formulation and utilization (Akande and Ramsey, 2016). Jack bean seeds can be used as an animal ingredient since they are good sources of starch and protein (Akande and Ramsey, 2016). The mature jack bean seed has high crude protein content (20-32%), and amino-acid profile that makes it suitable for use as a substitute for fish feed while the fully ripened seeds are sometimes used as a coffee substitute (Eke et al., 2007).

Sunn hemp and jack bean are both well-adapted legume cover crops in tropical regions. For instance, sunn hemp is grown in Puerto Rico and Guam under similar condition to Hawaii (Wang et al., 2002). These leguminous can play an important role in the agro-ecosystems and provide several benefits. In Puerto Rico, few studies have been conducted on these two legume cover crops which assessed a few potential benefits provided by these cover crops. There is a need for further studies because these legumes are not well investigated in the island. There is a gap in knowledge on the effects of sunn hemp and jack bean on soil physical, and biochemical properties in Puerto Rico.

Industrial agriculture dominates Puerto Rican farmlands; a system of chemically-intensive food production implemented in enormous single-crop farms (Santiago et al., 2016). Moreover, a recent study reported 14 percent of Puerto Rico as well-suited to mechanized agriculture (Gould et al., 2017). Findings from previous studies have shown large scale of chemical inputs: fertilizers and pesticides have harmful consequences on the environment and consumers. Today, few Puerto Rican farmers use those legume cover crops in the farming systems as sustainable management practices to decrease the impact on the environment. Therefore, this study fills a void for farming systems in which I evaluate the potential benefits of jack bean and sunn hemp.

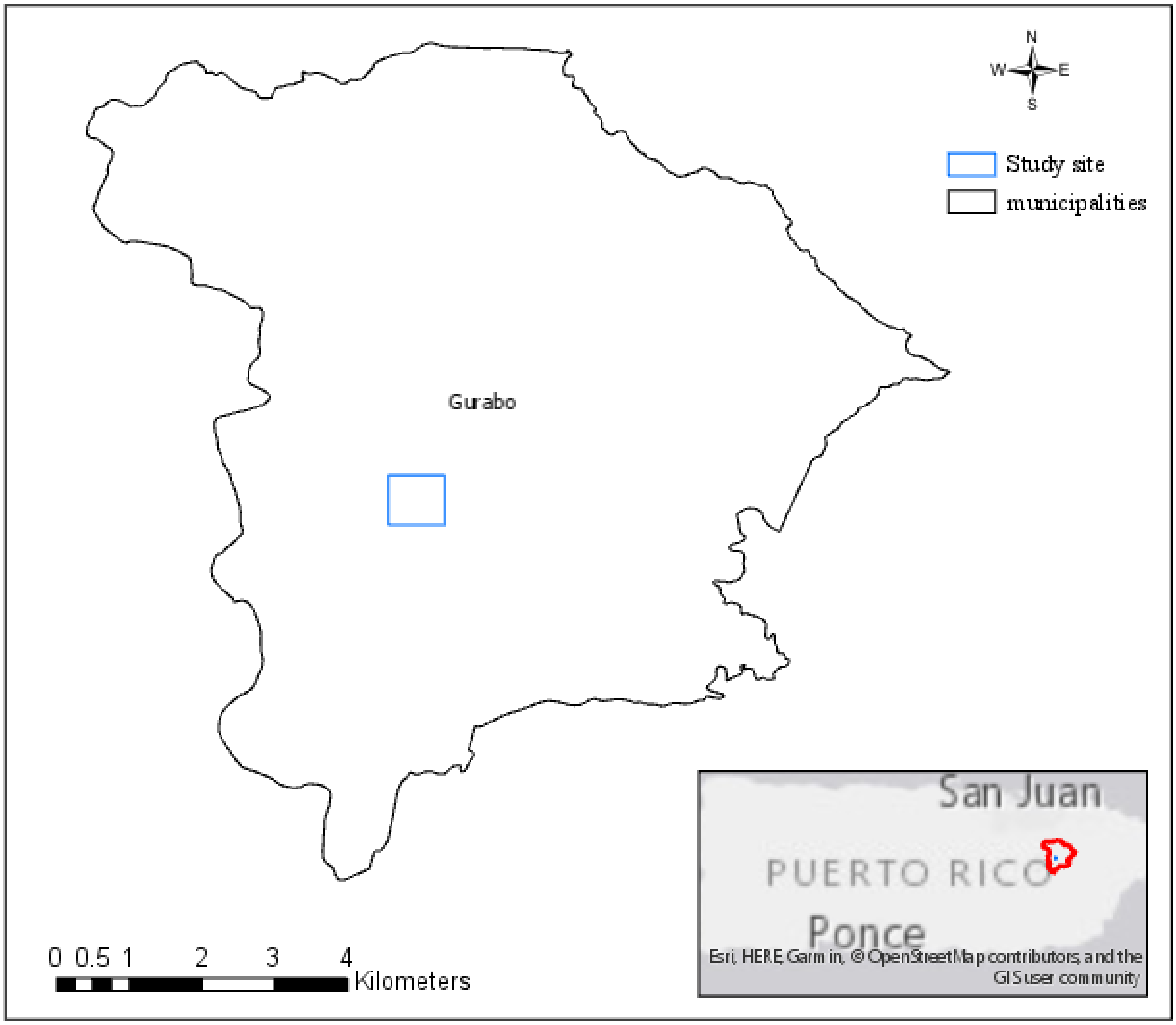
The aim of this chapter is to evaluate the performance of jack bean and sunn hemp by evaluating the percentage of germination, plant density and height, seedling vigor, canopy cover estimation, flowering date, resistance to pests' attack, and weed suppression. I also evaluated the effect of two different periods on the same variables listed above on jack bean species.

Methodology

Description of the study site

This study was conducted at the Agricultural Experimental Station at Gurabo of the University of Puerto Rico, Mayagüez campus. The geographical coordinates of the experimental station are 18˚15' 15. 84" N and 65˚58' 22.58" W. Gurabo climate is semitropical and has 1869 mm yr^{-1} as mean annual precipitation and temperature is 25.2˚C. This area is uniform and a flat region at the altitude less than 10 m and it is in the central eastern part of the island and covers 72.1 km^2 of land. The soil texture of the study area is formed on the alluvial fine texture and it is mainly silt soil. It was designated for Investigators and researchers to conduct academics studies. The highest point in Gurabo is at 367 m above sea level.

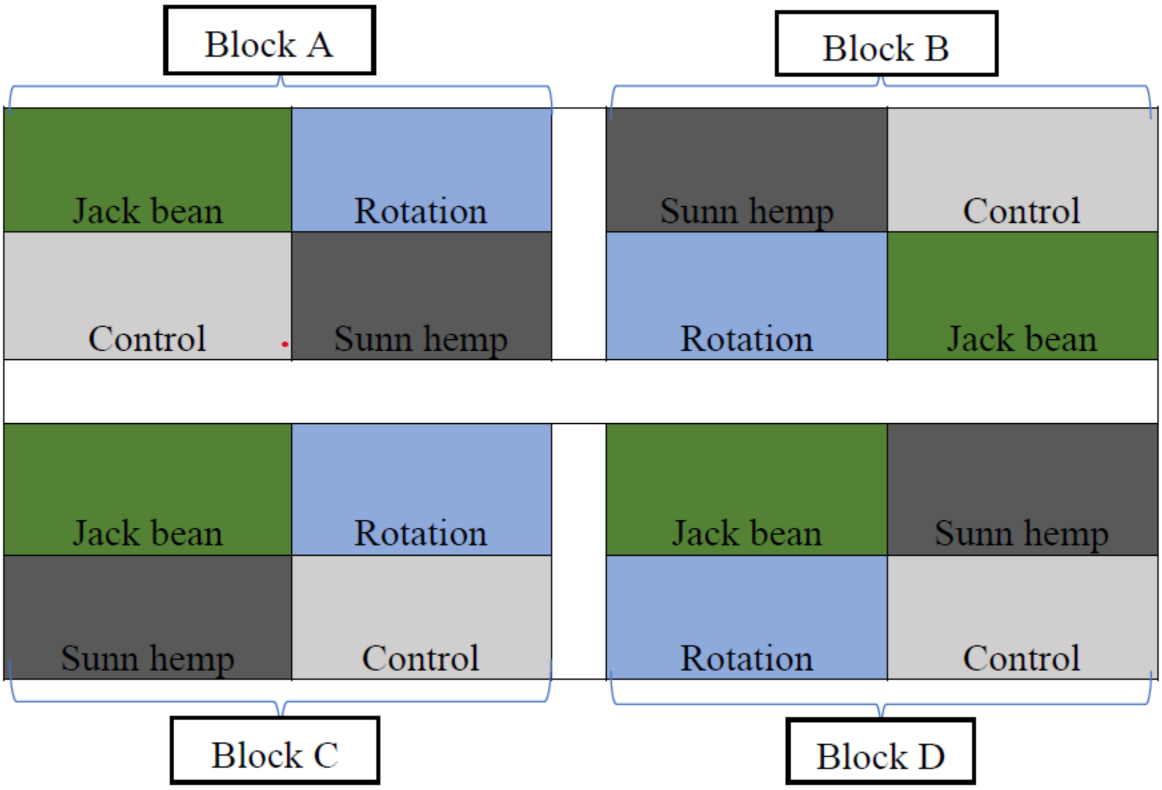
FIGURE 1: Map showing where the field experimental was established during the planting rounds in an agricultural land in Puerto Rico.



Experimental Design

The experimental design is a completely randomized block design with four replications which means treatments are repeated four times. This experimental design is divided in four blocks and contains four experimental units per block. Each experimental unit is about 7.5 m^2 (5 m x 1.5 m) and the whole experimental area is 207 m^2. Jack bean and sunn hemp were planted in one subplot per block (Figure 2). In addition, each block has a control and a rotation plots, but rotation plots were planted with jack bean at the next planting round. Block design was chosen to account for soil heterogeneity in the field.

Figure 2 : Experimental design established in the field at the first planting of jack bean and sunn hemp. It has 16 subplots divided in four blocks (A, B, C, and D) and each block contains four experimental units or plots. In addition, each block has a jack bean, sunn hemp, rotation and control plots.



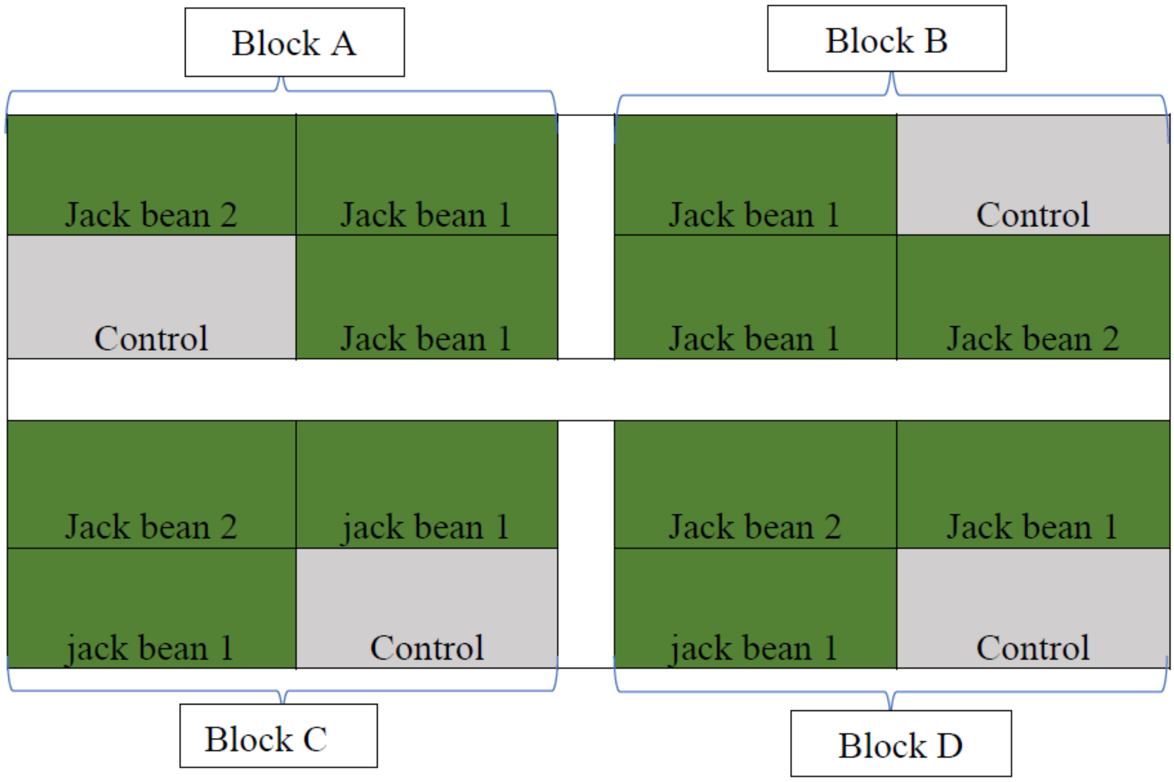
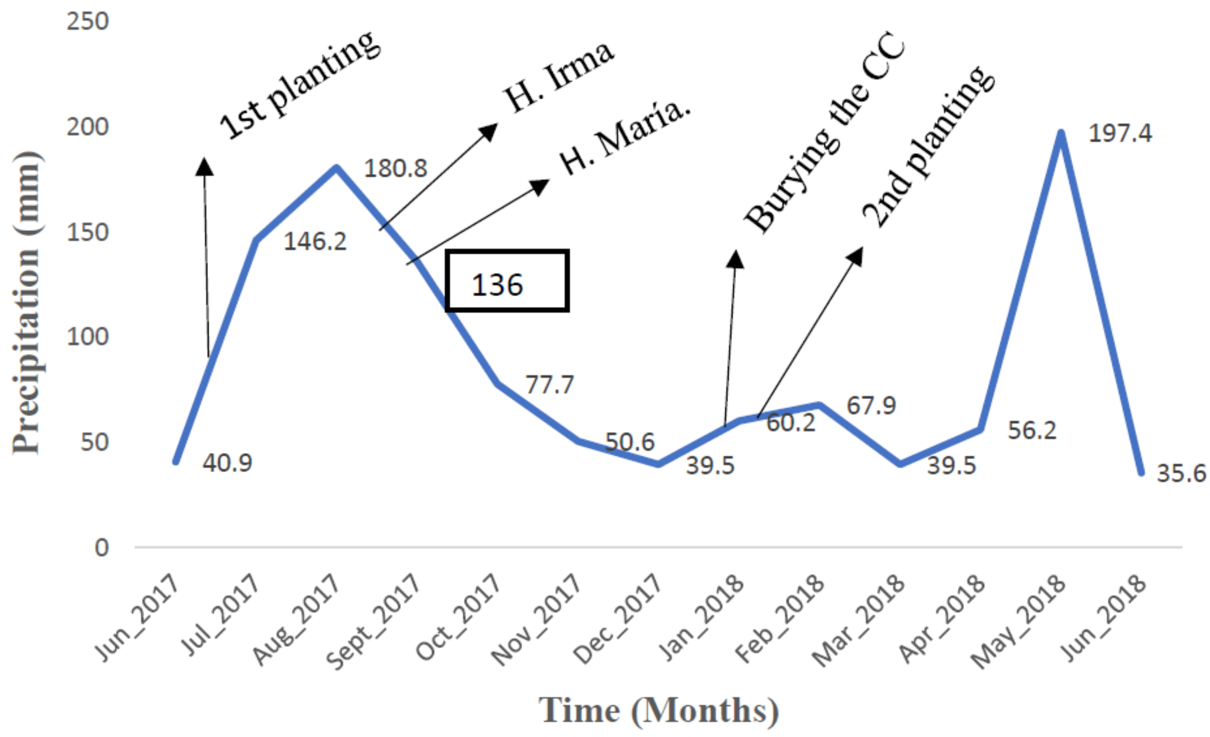


Figure 3. Experimental design established at the second growing period. It also contains sixteen plots split in four blocks (A, B, C, and D); however, jack bean was planted in three plots in each block. Jack bean 2 means that jack bean was planted for a second time in the same plot and 1 when jack bean was planted in plots for the first time.

History of the field experiment and planting strategy

Figure 4: Representing the history of field and important dates during the study.



The history of the field is crucial to understand the findings of this investigation. Some expected results may be modified related to the previous used of the field. Previous crops were planted in the area; however, I assume that adding nutrients may need reasonable time to deplete in the soil. Beyond the previous used of my field, two hurricanes hit the island while occurring the study, but the precipitation pattern is typical to Puerto Rico. Jack bean and sunn hemp were planted in a minimum tillage system which is 0-20 cm depth. In addition, a slight plowing was conducted to incorporate the biomass before the second growing round. Noted that, a rotavator was used to conduct this operation in the field experiment.

Jack bean and sunn hemp were sown directly at 2-3 cm depth manually due to the size of the plots. Jack bean was planted using a spacing of 0.5 m between rows and 0.20 m between plants and the density of sowing was two seeds per hole. Afterward, jack bean was planted at 0.40 m between plants at the second growing season which decreased the density on subplots. Previous studies reported that sunn hemp seeds can be sown by hand using 40- 60 lb./acre (45-67 kg/ha) or drill at 30-50 lb./acre (34-56 kg/ha) (Brunner et al., 2009) in rows (50 cm) apart (Valenzuela et al., 2002). Based on previous studies, sunn hemp was sown at an optimum distance which was 15 cm between plants. Planting depth was between 2-3 cm to ensure that soil moisture level is adequate. Monitoring and evaluating of these cover crops were conducted until the final assessment. An interval of time was described to evaluate each given variable in the field.

Jack bean [*Canavalia ensiformis* (L.) DC.]

Botanical description of jack bean

Jack bean is native to tropic Africa and South and Central America but is naturalized and cultivated worldwide stated by USDA (2009). Jack bean is a legume crop belonging to the family Fabaceae (Patel et al, 2016). Jack bean is an annual plant, shrub, erect or climbing 1 to 2 m height and its leaves have a length of 6 to 12 cm, oval-elliptical, white hair with regular density. Jack bean leaves and dense cover protect the soil from erosion when there is heavy rainfall. The plant produces an average of 7 pods linear, slightly curved 20 to 30 cm long by 3.5 wide capsules (Mwakimbwala, 2015).

Figure 5 : A plot of jack bean [*Canavalia ensiformis* (L.) DC.] at the first month in field experiment during the first planting period.

In addition, jack bean has proven to be useful species in the tropical soil reclamation efforts because of its deep penetrating root systems provides high drought tolerance (Acosta, 2009; Florentin et al., 2010). Jack bean can be grown in soils with high lead concentration and has the potential to be used for restoration of lead-contaminated soils (Sheahan, 2012). It grows best at altitudes up to 1800 m, temperature 15-30 ^{o}C, soil pH of 4.5- 8.0, and tolerates a wide range of rainfall (650- 2000 mm), evenly distributed throughout the year (Eke et al., 2007). In spite of early production of the first seedpods its vegetative development continues for a long period of time during which it also produces seeds (Florentín et al., 2010).

Sunn hemp (*Crotalaria juncea* L.)
Botanical description

Sunn hemp (*Crotalaria juncea* L.) is a leguminous cover crop which is commonly used in the world. Sunn hemp is a tall growing (1-3 m) herbaceous annual that is probably native to Indo-Pakistan subcontinent (Mosjidis and Wehtje, 2011). It can reach a height of over 4 ft. (1.2 m) in 60 days when grown under favorable conditions. It can attain a height of over 6 ft. (1.8 m) in approximately 90 days (Rotar and Joy, 1983). It has a long taproot with vigorous lateral roots and a thick, ribbed, pubescent (covered in short, soft hairs) stem and root nodules are lobed (Sheahan, 2012). Sunn hemp is naturally adapted to hot, semiarid, and arid areas and is drought resistant. It should receive a minimum of 1 inch (25 mm) of moisture per week to maximum growth, and it does not tolerate waterlogging (Valenzuela and Smith, 2002). Tropic Sun is a rapid growing crop that is good or uses a green manure and adding organic matter and nitrogen to the soil (Wang et al, 2002). Sunn hemp is a legume that when grown as summer annual can produce over 2268 kg biomass and can fix over 45 kg N ha^{-1} (Acosta, 2009). Sunn hemp possesses many characteristics of a cover crop, being poor or non-host to a large group of pest and pathogens, competitive with weed without becoming a weed, growing vigorously to provide good ground coverage performing symbiosis with rhizobium to fix nitrogen and being a green manure (Wang et al., 2002).

Figure 6 : A plot of sunn hemp after two months in the field experiment at the first planting round (June to November).

Collection timeline

A timetable was developed to define when every activity conducted in the field while a spreadsheet was created to collect data in the field. Data were collected on germinated seeds, seedling vigor, plant density, plant height, canopy cover estimation, flowering date and resistance to pests' attack. Those evaluations were conducted on both planting rounds but weed suppression and flowering date were missed at the first growing round because of the Hurricane María on the planted cover crops in the field experiment at the Agricultural Experimental station in Gurabo.

The assessment of variables on jack bean and sunn hemp

Percentage of germination

Sun hemp and jack bean seeds were produced in the island at the Agricultural Experimental Substation of the University of Puerto Rico. However, the percentage of germinated seeds (PG) was calculated from each subplot in the experiment to evaluate the viability of the seeds. The evaluation of the percentage of germinated seeds was started from five to fourteen days after planting in the field experiment. To assess the percentage of germination every single plant was counted from each experimental unit during the evaluation period. To calculate the percentage of germination this formula was been used PG= Germinated seeds up to fourteen days/ Total seed sown in the experimental * 100.

The first sowing of jack bean and sunn hemp was conducted on June 20th, 2017, at the field experiment at the substation of Gurabo. The second planting was sown in the field experiment on December 20th, 2017, in which jack bean was the only planted cover crop. All plants were counted from five to fourteen days after the sowing date in every subplot to calculate the amount of the percentage of germination on each experimental unit.

Seedling vigor assessment

Seedling vigor assessment is used to describe the performance of both legume cover crops. This evaluation is based on seed properties which determine the rapid uniform emergence and development of normal seedling under a wide range of field conditions. This assessment is based on rapid germination, seedling growth rates, homogeneity, and size of the first leaves. Once the germination percentage process was completed after fourteen days of planting in the field then seedling vigor was evaluated from a rank of one to four (1= Bad, 2= Slightly less good, 3= good and 4= very good). This assessment was conducted on July 5th, 2017 on the first planting round and January 08, 2018 on the second planting. Noted that this evaluation was conducted one time per growing season. Every single subplot has been evaluated on the category of 1 to 4 based on the provided criteria.

Plant density

The number of plants in a subplot may influence the yield and the soil attributes. In this case, plant density was assessed to determine the effects of jack bean and sunn hemp on the evaluated soil attributes in this study. This evaluation was conducted after the evaluation of the percentage of germinated seeds and seedling vigor. The first evaluation was conducted on July 28th, 2017 at the first planting round. In addition, to evaluate the effect of time on plant density, this measurement was conducted in two different periods during the second growing cycle. Therefore, these measurements were conducted on January 11th, and February 1st, 2018. Every single plant was counted from each experimental unit (5 m x 1.5 m) to determine the plot density.

Plant height

Plant height was measured in the field to evaluate the growing process of the legume cover crops. This measurement was conducted twice on those cover crops from each growing season. The first measurement took place one month after the cover crops were planted in the field. The second evaluation was conducted right after the flowering stage. Unfortunately, I could not conduct the second measurement because the Hurricane María has affected the experiment in the first planting round. Three plants were selected from every subplot to have an average plant height. Those three plants have been divided into three categories: highest, high and less high then we calculated the average. Noted that each category was randomly selected then I chose one per group.

Canopy cover estimation

Canopy cover estimation can reduce soil erosion effect, maintain soil moisture and provide other benefits to the soil properties. For instance, canopy cover can prevent the soil from raindrop and sun to reach the soil directly. Canopy cover estimation of both legume cover crops was measured during the growing seasons. An area of 1.5 m^2 was delineated in every single subplot to estimate the percentage of canopy cover from each legume cover crop. We evaluated the spaces where the sun reached directly the soil under the selected area to obtain an estimation of cover. This measurement was conducted two months after planting and the ultimate estimation was conducted at the flowering stage.

Flowering date

The flowering date is the crucial step during the growing season of the cover crops. The flowering dates were noted or monitored when 50 % of the total population was blooming in every experimental unit. Generally, the flowering stage of the species of Canavalia starts at three months after planting. Unfortunately, the evaluation of flowering date has missed in the first growing season which was planted on June 20th, 2017, because hurricane María hit this experiment before getting to this stage/step. Consequently, flowering dates were noted at the second planting round in the field experiment. Noted that the flowering dates were different in subplots of the same species of jack bean.

Resistance to pests attack

Some cover crops are more resistant to the attack of pests or disease damage. Pest damage was assessed in the field experiment at the end of the growing season. Every single healthy and infected plant was counted to evaluate the incidence of the attack of pest or diseases damage. Afterward, the Modified Cobb scale was selected to estimate the amount of leaf area infected by the attack of pests (Acosta, 2009).

TABLE 1: Description of leaf areas infected by pests attack based on the modified Cobb scale.

Scale	Description of the damage in the leaves
0	No visible infection.
1	1-5% leaf area infected or damaged.
2	6-10 % leaf area infected or damaged.
3	11-25 % leaf area infected or damaged.
4	26-40 % leaf area infected or damaged.
5	65-100% leaf area infected or damaged.

Source: Acosta (2009)

Weed suppression

Cover crops have been known to be important for weed suppression during their growing cycle. In this study, area of 0.75 m^2 has been delineated from subplots within the cover crops to evaluate the performance of the planted cover crops on weed suppression. Plots have been established one month and a half from the date the cover crops were planted in the field. Afterward, weed density was counted in the selected area while establishing the design in the field; however, at the end of the growing cycle weeds density were re-counted to evaluate weeds that are being suppressed by the cover crops.

Data analysis

The observed data were primarily tested for normality and homogeneity. MANOVA was performed to evaluate the effects of those two plant species on the dependent variables. The analysis of variance was used to evaluate separately each response variable when the global multivariate analysis of variance is significant. The significant level was set at $\alpha = 0.05$ and R statistical software version 3.3.2. was used to perform the tests.

Results

Table 2 : Mean (±) of the dependent variables : Percentage of germination (%), plant density (m^2), seedling vigor, canopy cover (%) and plant height (m).

Variables	First planting round		Second planting round
	Jack bean	Sunn hemp	Jack bean
Germinated seeds (%)	91.32 ± 6.17	88.5 ± 9.23	82.63 ± 16.13
Plant density (NP/m^2)	94.5 ± 6.86	713.5 ± 203.85	60.5 ± 4.42
Seedling vigor	4 ± 0	3.5 ± 0.58	3.25 ± 0.75
Canopy cover %	93.25 ± 2.36	83.25 ± 2.36	52.5 ± 17.77
Plant height (m)	0.67 ± 0.15	0.95 ± 0.15	0.79 ± 0.10

When comparing the effect of planting these two plant species at two different periods in the field experiment, I found results are lightly different on the studied variables. For instance, the percentage of germinated seeds for jack bean species was 91.32 during the first growing cycle, but decreased to 82.63 at the second planting round. In addition, the average of plant density of jack bean species decreased from 94.5 to 60.5 ($plant/m^2$) whereas seedling vigor decreased from 4 to 3.25 at the second period. The same trend occured with canopy cover estimation of jack bean which decreased in the second growing round. Unlike, variable height of jack bean species was lightly increased from 0.67 to 0.79 m (Table 2). In addition, sunn hemp species has a high performance on evaluated variables but it has higher density in the plots. The purpose of sowing sunn hemp at hight density was to have a coverage of the soil.

The outcome of the multivariate analysis of variance has shown that plant species have significant effect on the evaluated variables. For instance, plant species have significant difference on plant density, canopy cover estimation and plant height but there was no significant difference on percentage of germination and seedling vigor (Table 3). Generally, sunn hemp grows denser in field which is crucial to have an rapid initial coverage. Therefore, sunn hemp has sown at higher density to meet this purpose during this study. Besides, both plant species have high percentage germinated seeds it mostly due to the viability of their seeds. Therefore, there is no significant difference between on the percentage of germination on both plant species. Based on the criteria, both plant species have a considerable seedling vigor in the field; they were in the same category (4 : very good) of evaluation. Though sunn hemp was denser in the field ; jack bean had greater canopy cover estimation. Jack bean can produce a large initial coverage due to its large leaves. In addition, sunn hemp grew taller than jack bean it is due to the morphology of these two species. Sunn hemp is a shrub which can reach up 2 m at the end of the growing season whereas jack bean is like a creeping plant.

Table 3 : Summary of the analysis of variance for the effect of plant species on the percentage of germination, plant density, seedling vigor, canopy cover estimation, and plant height.

	Df	**Sum Sq.**	**Mean Sq.**	**F Value**	**P (>F)**
Percentage of germination	1	15.93	15.93	0.25	0.63
Seedling vigor	1	0.5	0.5	3	0.13
Plant density	1	766	766	36.84	**0.0009**
Canopy cover estimation	1	200	200	35.82	**0.00097**
Plant height	1	0.16	0.16	7.027	**0.038**

Figure 7 : Mean and standard error of the percentage of germinated seeds of jack bean and sunn hemp at the first growing round.

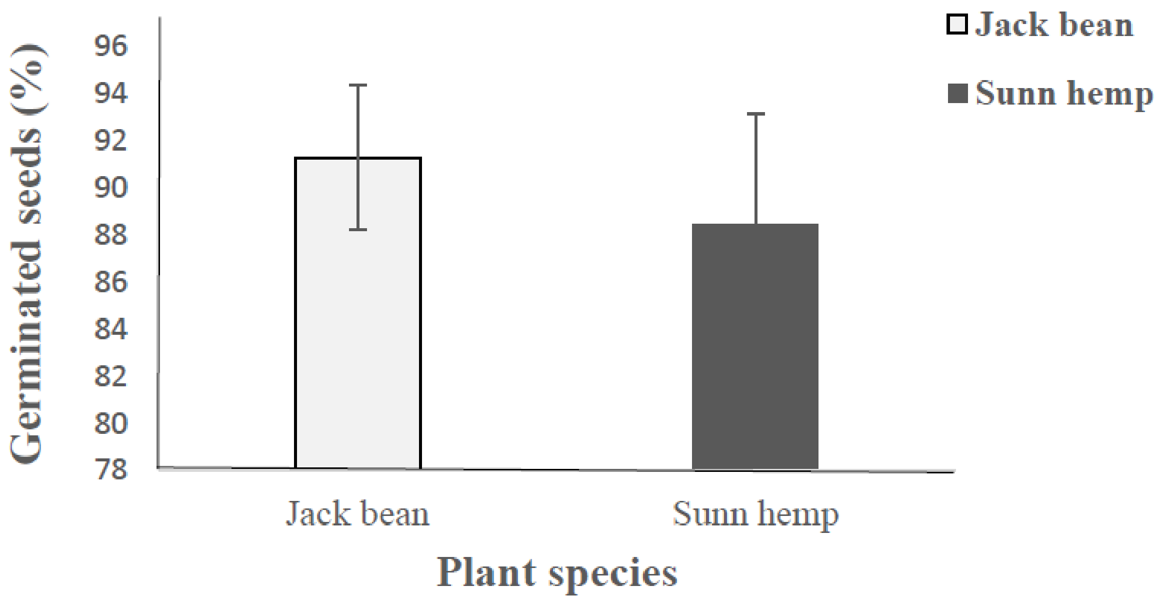


Figure 8 : Mean and standard error of plant density (A), seedling vigor (B), canopy cover estimation (C), and plant height (D) of jack bean and sunn hemp at the first planting round.

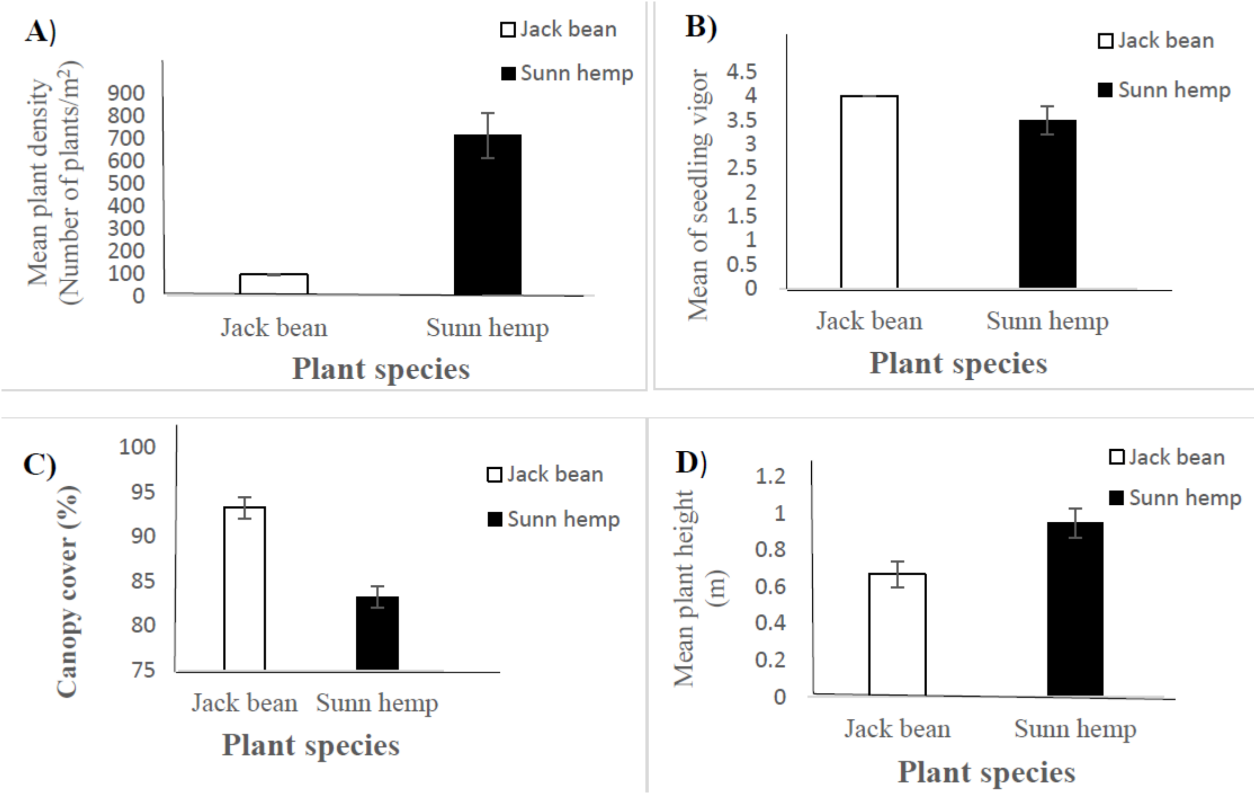
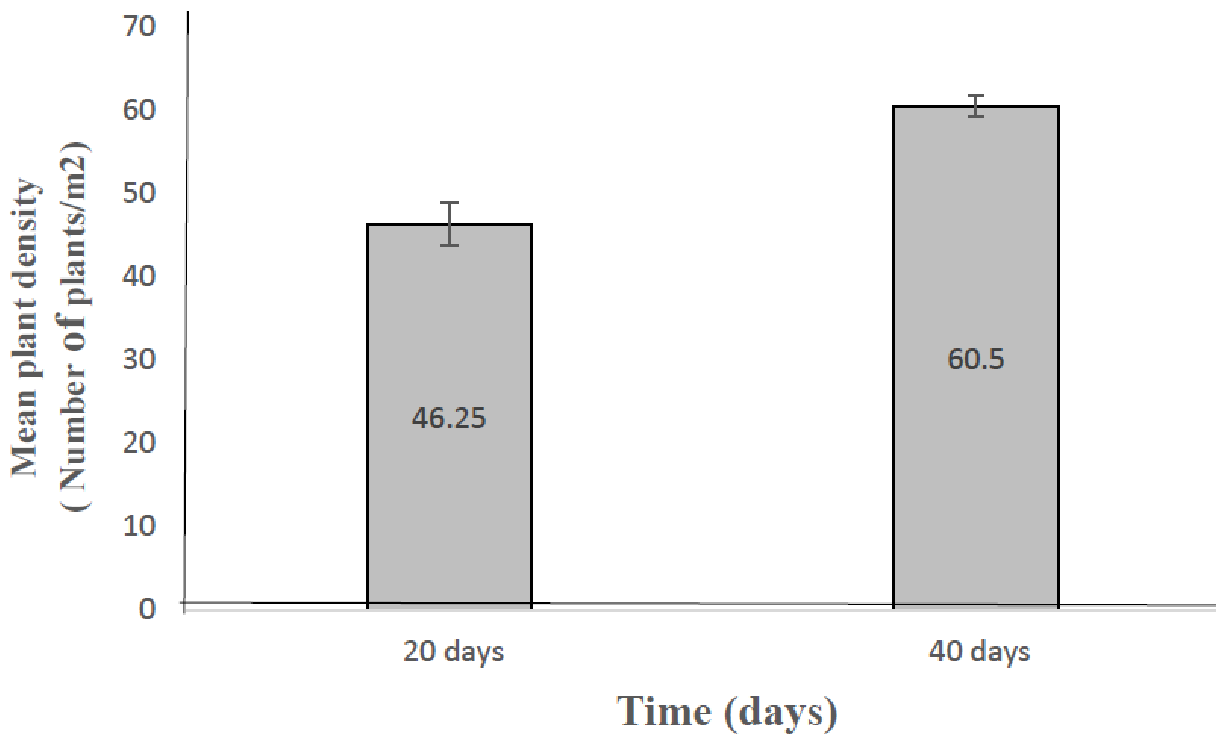


Figure 9: The mean with the standard error of plant density of jack bean evaluated at two different periods at the second field experiment in Gurabo. Plant density evaluation was conducted after twenty and fourty days of sowing for the effect of time.



Discussion

How did jack bean and sunn hemp grow and perform after two growing periods in an agricultural field in Gurabo? My expectation was these plant species would have high percentage of germinated seeds, a uniform seedling vigor, a large canopy cover estimation, growing denser and higher. As I expected, both plant species had a high percentage of germinated seeds due to the high viability of the seeds and field condition was adequate to facilitate the germination process. Brunner et al. (2009) stated sunn hemp germinates, develops rapidly and has a habit of dense growth suppressing weeds, reducing the population of nematodes in the soil, and fixing atmospheric nitrogen and produces abundant organic matter.

A further reason, sunn hemp was sown at high density in the field it was due their physiological structure. For instance, sunn hemp is a shrub with a small leaf while jack bean is a bushy plant with large leaf that can rapidly cover the soil. Therefore, the density of sunn hemp was greater to have a considerable coverage. In addition, a study reported a higher seeding rate of 49-58 lb/acre has been used to establish thick stands for green manure (Sheahan, 2012). In terms of seedling vigor evaluation, jack bean was lightly more vigorous based on the criteria (rapid germination, seedling growth rate and homogeneity); however, both plant species were homogeneous after emergence.

Studies reported jack bean is a fast-growing cover crops and has large leaves to produce a considerable cover in short period. For instance, jack bean can establish 85% cover 60 days after emergence (Sheahan, 2012). Moreover, at flowering (102 days after planting), jack bean had a mean of 60 % canopy coverage (Acosta, 2009). While in my study, jack bean covered 93.25 percent of the plot at the first planting round and 52.5 (at the flowering date) during the second planting. Both planting rounds showed jack bean have the most considerable soil cover in the field, but the period of planting and density in the plots may influence the percentage of coverage. In addition, sunn hemp grew higher than jack bean but is due to its physiological structure.

The performance of the studied variable such as: percentage of germinated seeds, seedling vigor, and canopy cover estimation decreased during the second round for jack bean. One main reason is the planting regime which is the effect of growing jack bean in two different periods. Therefore, planting period and/or season needs to be considered and planting strategy like number of seeds per hole, spacing between rows and would be a critical option to influence the variables in a short period. Furthermore, the flowering dates were noted between 93 and 109 days after planting which was mostly approximating to the findings the study of Acosta (2009) conducted in Puerto Rico where jack bean sunn hemp flowered about 106 days after planting. The resistance to pest attack was between the rank 1-5 % based on the modified Cobb scale. This means 1-5% of leaf area were damaged or infected by pests which was not considerable.

My study is consistent to the findings of Acosta (2009) a study conducted several legumes cover crop in the island. For instance, results on canopy cover of jack bean were close from both studies.

Based on a study conducted in Paraguay where jack bean had 85 percent of soil coverage 60 days after emergence whereas sunn hemp had 40 percent of cover (Florentín et al., 2010). Moreover, biomass accumulation and coverage at 90 after days of sowing was higher in jack bean in the first and second years of cropping according to study conducted in Yucatan, Mexico (Bernardino and Arturo, 2014). In addition, seedlings emergence was homogeneous and plant shown excellent vigorous growth at the beginning of the plantings (Bernardino and Arturo, 2014). My findings are also coherent to the findings of these studies and follows the same pattern where canopy cover of jack bean species was significantly greater than sunn hemp three months after planting.

Conclusions

I have demonstrated the performance of jack bean and sunn hemp in agricultural field during two planting rounds. Plant species have significant difference on the density, canopy cover, and plant height. I found jack bean and sunn hemp have a high percentage of germinated seeds during the first growing cycle. Sunn hemp had grew denser and taller than jack bean. While jack bean had a greater canopy cover than sunn hemp. In terms of planting periods, the mean of all the studied variables on jack bean decreased in the second growing round with the exception of plant height. In addition, the mean plant density of jack bean after fourty days was higher than the mean observed at twenty days at the second growing period. In terms of performance, jack had a higher percentage of germination, seedling vigor, and canopy cover. From my field observations, jack bean spread out in a short period to obtain a considerable canopy cover, resist and bounce back faster after natural disasters. Jack bean did better in the field than sunn hemp; however, the physiological structure needs to be considered while evaluating the performance of the plant species.

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Chapter III. Effects of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp on soil properties in two different growing periods.

Abstract

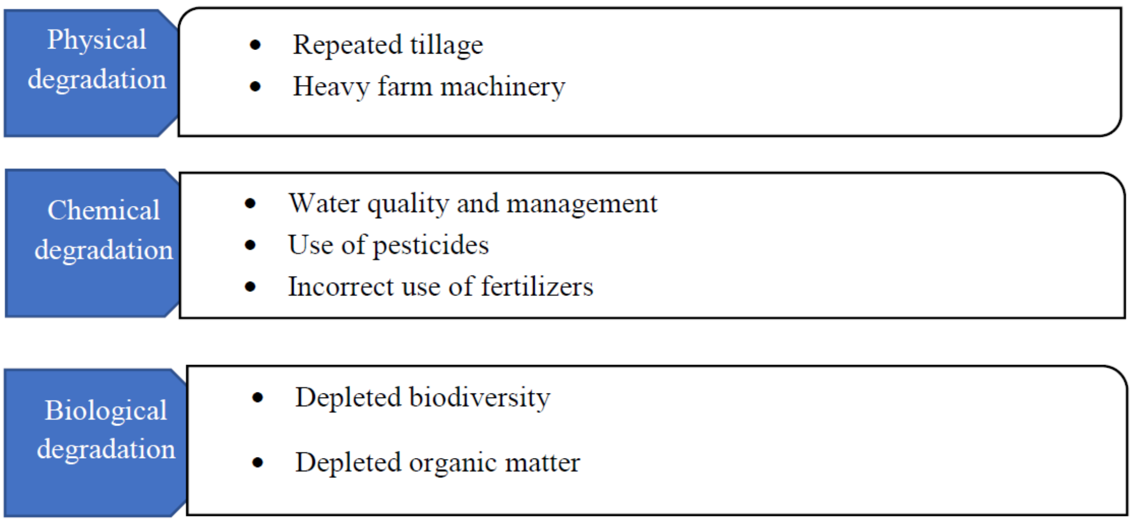
Soil degradation is one of the major threats facing the environment which weakens their productivity and affects ecosystems services. Unsustainable practice of conventional agriculture in the farming systems is one of the majors causes. For instance, the use of large synthetic inputs, heavy machinery, monoculture, and overexploitation of groundwater have led to heavy environmental consequences. Mitigation practices are highly considerable to maintain soil fertility. Among the mitigation practices, cover crops can reduce the impacts and maintain soil fertility through the benefits they provide to the soil. Therefore, jack bean and sunn hemp were planted to assess electrical conductivity, dehydrogenase activity, dielectric permittivity, soil aggregate stability, soil bulk density, soil pH, soil strength, soil temperature, soil texture, total Carbon and Nitrogen, volumetric water content and water-holding capacity as soil attributes. MANOVA and ANOVA were performed for the effect of jack bean and sunn hemp on the measured soil properties during two different periods. The findings had shown jack bean and sunn hemp had no significant effect on the evaluated variable at the growing period. During the second period, treatment jack bean 2 had significant effect on soil temperature. Soil temperature was greater in jack bean 2 than jack bean 1 and control plots.

Key words: Conventional agriculture, Jack bean, soil attributes, soil degradation, soil fertility, sunn hemp.

Introduction

During the 20th century, the rising demand globally for food was met by the conversion natural and semi-natural habitats into agricultural land and the intensification of farming methods, including mechanization and use of synthetic fertilizers (Edmondson et al., 2014). This unsuitable uses of conventional agriculture technology such as heavy machinery and synthetic inputs and practices such as monoculture and overexploitation of groundwater for additional irrigation have led to unprecedented environmental consequences including serious declines in soil quality (Daryanto et al., 2018). Continuous monoculture and use of extensive farming have led to degradation of soil and other natural resources (Farooq and Siddique, 2015). Soil degradation is one of the most important threats facing mankind which not only weakens the productive capability of an ecosystems but also affects overall climate (Alam, 2014). Noted that soil erosion and compaction, salinization, water contamination, water holding capacity, soil aggregate stability are common characteristic of degraded soil (Daryanto et al., 2018; Edmondson et al.,2014; Farooq and Siddique, 2015).

Figure 1: Physical, biological and chemical degradation of the land (Alam, 2014).



In addition, soil degradation describes ongoing processes that generally limit agronomic productivity, result in undesirable or deteriorating physical, chemical or biological properties (Stewart and Texas, 2015). A direct implication of soil degradation is that soil scarcity will become a critical issue for global agricultural production (Delgado and Gantzer, 2015). The phenomenon of soil degradation is often linked to the unsustainable management practices that farmers are applying in the agricultural farming system. To conserve soil better agricultural methods that sustain the soil are need (Delgado and Gantzer, 2015). Therefore, a shift towards nature-based solution practices as an alternative to conventional agriculture has been recommended (Daryanto et al., 2018). For instance, the food and agriculture organization of United Nations proposes practices that can reduce mechanical soil disturbance and allow permanent soil cover with crop residues and increase species diversity.

The systemic introduction of legumes cover crops into the farming system because they can provide benefits to the environment and the terrestrial ecosystems could be an important strategy (Amede and Kirkby, 2001). Legume cover crops could be a long-term solution to the environmental degradation caused by the modern agricultural practices. For instance, the use of cover crops could help to reduce the need to apply synthetic fertilizer and pesticides during the growing season in the farming system (Lichtenberg et al., 1994). Previous studies have already shown that chemical inputs have provoked harmful effects on the environment such as contaminations of water bodies, eutrophication and consequences. The integration of legumes as cover crop into the farms systems is an option to improve soil fertility as well as weed competition due their capacity to fix atmospheric nitrogen as well as to improve soil physical and chemical properties (Bernardino and Arturo, 2014).

In this study, two legume cover crops were used to evaluate the effects on some key soil properties. Legume cover crops have a long history of use in various cropping systems to prevent erosion on steep slopes and as an alternative method of weed control (Mulinge et al., 2017). Some leguminous prevent leaching of nitrate into groundwater (Lichtenberg et al., 1994). Apart from the environmental and ecosystem services they provide, legume cover crops are also now viewed as possible sources of agronomic service to the ecosystem (Durairaj and Stute, 2018). For instance, cover crops increase arbuscular mycorrhizal fungi, inoculation, reduced incidence of certain soil pathogens, and suppression for early-season weeds (Daryanto et al., 2018). In addition, the use of legume cover crops is an economically feasible and ecologically sustainable practice that plays an essential role in the recovery of soil fertility (Mulinge et al., 2018).

In addition, few studies in Puerto Rico promote the use of leguminous cover crops related to the benefits provided in the soil (Acosta, 2009; Valencia, 2016; Rico, 2017). Acosta (2009) reported cover crops could bring plenty of benefits such as erosion drops down and reduce fertilizers and pesticides costs to Puerto Rican Farmers. In this chapter, wireless sensors were installed in the field to record daily data on volumetric water content, electrical conductivity (EC), dielectric permittivity (Ka) and ambient soil temperature (Ts). Moreover, additional measurements on soil attributes were conducted such as soil texture, strength, pH, bulk density, aggregate stability, total Carbon and Nitrogen, water holding capacity, and dehydrogenase activity (DHA). The objective of this chapter was to determine the effect of jack bean and sunn hemp on the listed soil properties.

Materials and methods

Site description

This experiment was established at the Experimental Agricultural Substation at Gurabo. Acosta (2009), reports this station was created in 1953 by an official of the Experimental Agricultural. The geographical coordinates are: 18˚15,03 96̎ N and 65˚59 25 W. It has a semitropical climate and receives 1869 mm average annual precipitation. The average annual temperature in Gurabo is 25.4˚C. Gurabo is in the central eastern part of the island and covers 72.1 square kilometers of land. It is bounded by San Lorenzo on the north; Trujillo Alto on the south, east of Caguas; and Carolina and Juntos on the west. The highest point in Gurabo is La Silla peak, in the Masa sector, at 367 meters above sea level.

Since June to December 2017, a system with sixteen wireless sensors (CWS665) were installed in the field to record daily data on volumetric water content, soil temeprature, electrical conductivity and dielectric permittivity. Each experimental unit had a wireless sensor to monitor data on those measured variables. Sensors were stayed in the field experiment to record data at the second experiment from December 2017 to June 2018.

Aggregate stability measurements were conducted during both growing periods. Kemper and Rosenau method were used to determine the soil aggregate stability. This method stimulates the strengths that are naturally on the soil while water enters the aggregate. Soil samples were air-dried and sieved to maintain the sample which was between 2-4 mm. Moreover, soil sample was divided in two portions of 15 grams, one was placed directly in the oven to evaluate the moisture content. The other 15 grams was placed on a sieve #20 (1.65 mm) which in turn was placed on a # 10 sieve (0.68 mm). These sieves were sufficiently shaken or agitated in water to guarantee that the aggregates go down for fifteen minutes. The retained aggregates in the sieves were placed in oven at 105 ˚C for 24 hours. The percentage of aggregate stability was calculated by using this equation: (Sieve #20 + Sieve # 10) *100/ Original weight.

Soil bulk density assessment was conducted to measure the level of soil compaction. Soil samples were collected in every single experimental unit using a metal core sampling cylinder of known volume and oven-dried at 105 ^{0}C for 48 hours to calculate the level of soil compaction (bulk density). In addition, soil penetration resistant measurements were conducted by using a digital Soil Compaction Tester manufactured by Dickey John Corporation. Repeated measurements were conducted at the end of the growing cycle before the incorporation of those cover crops into the soil.

Table 1: Interpretation of penetration strength or resistance measurements when using digital Soil Compaction Tester.

Percentage of measuring points (PSI)	Compaction rating	Subsoiling recommended
< 30	Little to none	No
30 -50	Slight	No
50 -75	Moderate	Yes
> 75	Severe	Yes

S**ource**: Penn State College of Agricultural Sciences research and extension program (extension.psu.edu).

Water holding capacity

Water-holding capacity in the soil was assessed to determine the effects of jack bean and sunn hemp. Water-holding capacity assessment was conducted in three different periods in the study. The method of Beaker Protocol was used to evaluate water-holding capacity of the field. First, baker was weighted and tared to weight ten grams of fresh soil sample. Afterward, beaker and weighted fresh soil sample were placed in the oven at 105 ºC for twenty-four hours. Baker and dried soil were weighted to calculate the water content of the soil sample. Moreover, dried soil was soaked until the water film was observed on top of the soil sample is evident inside the beaker. Lastly, beaker and the saturated soil were weighted to determine the amount of water holding capacity of the soil.

Total carbon and nitrogen and soil pH

Soil samples were collected in three different periods in the field to compare the effects of those two cover crops between times. First soil sampling was conducted in June 16th, 2017 before planting jack bean and sunn hemp in the field. The second collection was conducted on November 21th, 2017 after the first growing round of the cover crops. The ultimate collection was conducted on April 5th, 2018 at the end of the second experiment. Total nitrogen and carbon were determined using TruSpec CN Analyzer and a modified version of the Leco Corp was used during the process. In addition, soil pH was also evaluated in three different periods to determine the effect of those two cover crops. Soil pH was determined in a solution 1:1 (soil: water/soil: KCL).

Soil texture

The study of soil texture of a given area is useful to make decision on type of crops and activities which are appropriate to the area. Therefore, initial soil samples were collected in the field experiment in Gurabo to determine the type of soil texture. Furthermore, the simplified Bouyoucos hydrometer method was used to conduct the analysis of soil texture. To determine the percentage of every class, this formula was used:

Sand %= (R_{40s}* R_L) = 100/oven-dried soil weight in g

Clay %= (R_{7h} * R_L) = 100/ oven-dried soil weight in g

Silt % = 100 – (Sand % + Clay %)

Dehydrogenase activity (DHA)

Determination of dehydrogenase activity in the soil samples gives us large amount information about biological characteristic of the soil (Wolinska and Stepniewsk, 2012). The method of the reduction 2,3,5- triphenyltetrazolium chloride (TTC) has been used to determine microbial activity in the soil. Soil samples were collected at the field experiment in sixteenth plots in three different periods to conduct analysis on enzyme activities in the soil. Soil samples were air-dried for two days, grounded and passed through sieves of 20 and 10 mm meshes. Six grams of air-dried soil and 0.06 gram of calcium carbonate (CaCO2) were weighted and mixed during a few minutes in a crystal tube. Moreover, one ml of aqueous solution of triphenyl tetrazolium chloride (TTC) and 3.5 ml distilled water were added to the mixing. The tube was shaken for a few minutes until the soil sample is wet enough inside the tube. The mixed were incubated into an oven at 37 ˚C for 24 hours to determine the concentration through the calibration curve. The following equation has been applied to determinate the DHA.

$$DHA\left(\mu g\,\frac{TPF}{g.h}\right)=\frac{CTPF\times V\times H}{P\times 0.99\times t}=\frac{CTPF\times H\times 2.10}{P}=$$

Figure 2: A) Wireless sensor (CWS665), B) Installing field equipment and C) CWS665 probe installed and identified with the plot number.

Results

Table 2: Mean and standard error of volumetric water content (VWC), electrical conductivity (EC), soil temperature (ST), and dielectric permittivity (Ka) in plots planted with jack bean and sunn hemp.

Treatments	VWC (m^2 water/m^3 soil)	EC (dS/m)	Soil temperature (^{o} C)	Dielectric permittivity
Jack bean	0.265 ± 0.02	0.259 ± 0.04	27.45 ± 0.25	15.546 ± 1.81
Sunn hemp	0.270 ± 0.06	0.283 ± 0.09	27.60 ± 0.23	16.815 ± 4.22
Control	0.306 ± 0.05	0.331 ± 0.03	28.05 ± 0.22	19.063 ± 1.11

Table 3: Multivariate analysis of variance for the effect of jack bean and sunn hemp on volumetric water content, electrical conductivity, soil temperature and dielectric permittivity at the first planting period.

Coefficient	Df	Pillai	Approx. F	nun Df	den Df	P (>F)
Plant species	1	0.37	0.63	8	22	0.74

Figure 3: A) Mean and standard error of volumetric water content, B) soil temperature, C) electrical conductivity, and D) dielectric permittivity in jack bean, sunn hemp, and control plots at the first experiment.

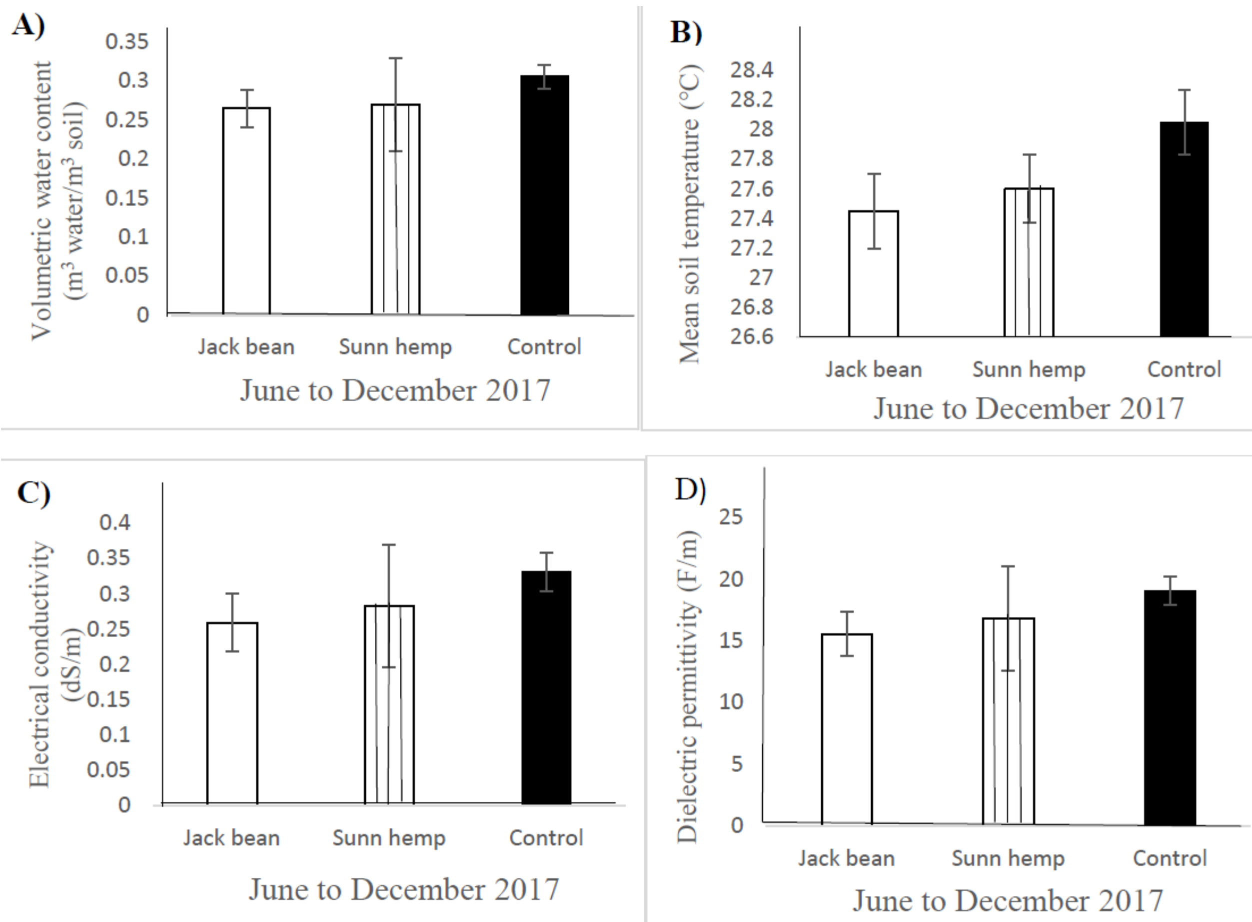


Figure 4: Mean air temperature during the two planting rounds in Gurabo.

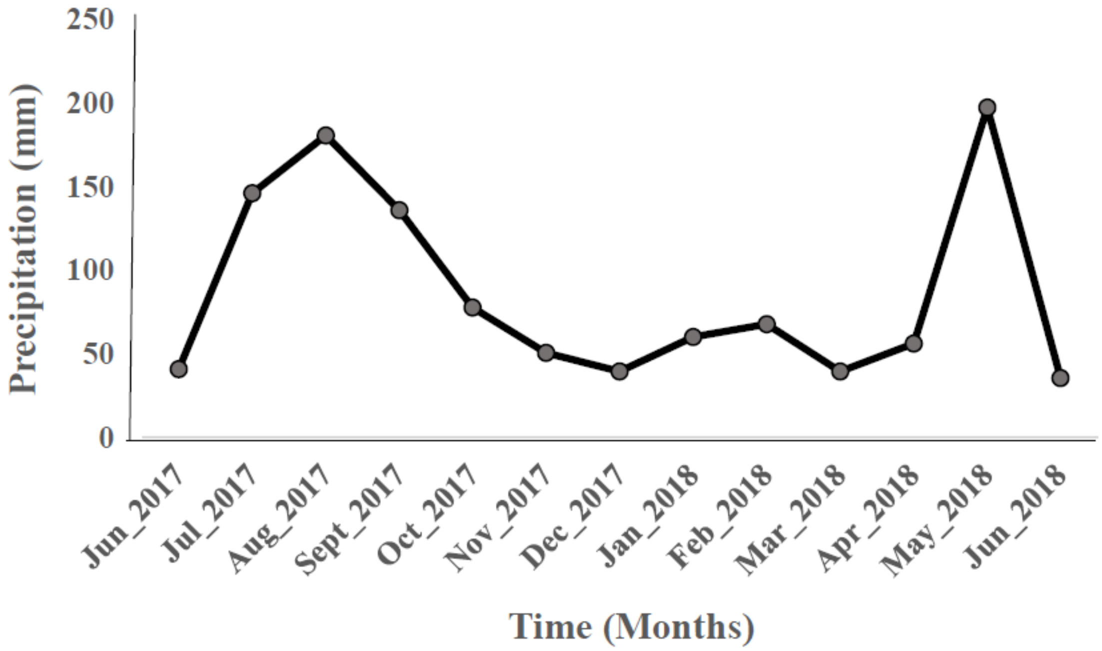
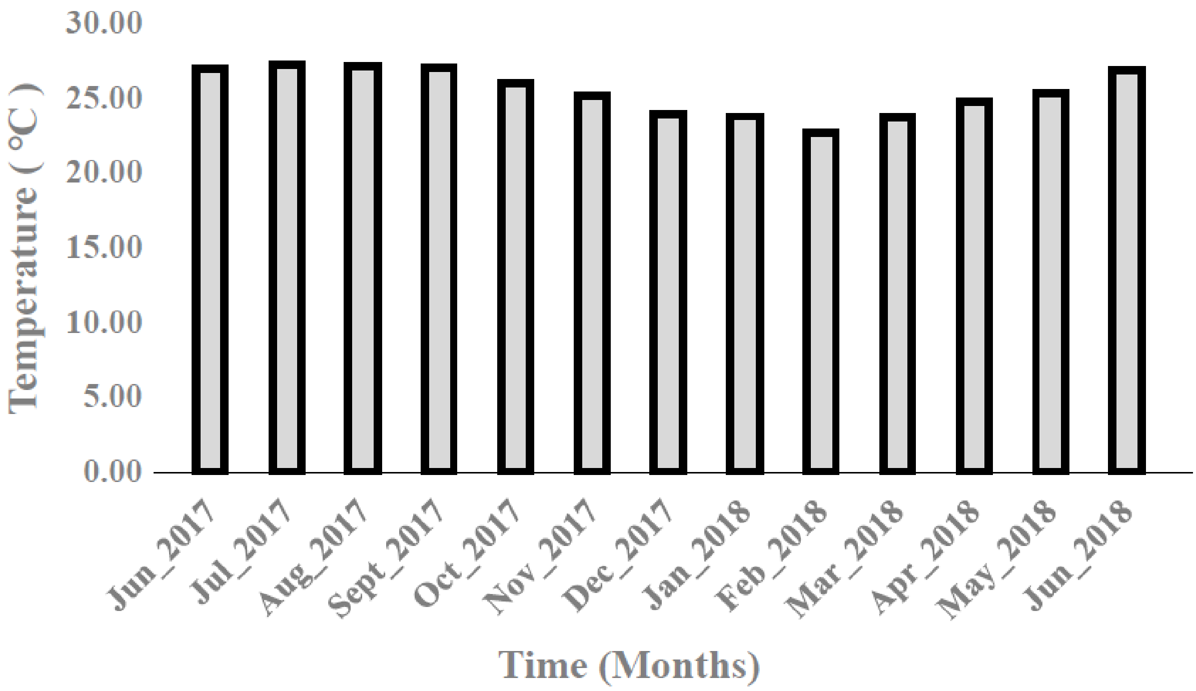


Figure 5: the average precipitation during the two growing cycles in Gurabo.



When comparing the results of the studied variables during the first planting round, I found the mean of volumetric water content in jack bean (0.265 m^3 water/ m^3 soil) was not significantly different to the mean in sunn hemp (0.270) and the control plots. In addition, the mean electrical conductivity in jack bean was not significantly different than the mean of sunn hemp plots. Afterward, there was no significant difference in the mean soil temperature of jack bean = 27.45 ^{o} C and sunn hemp = 27.60 ^{o}C, meanwhile the mean air temperature decreased to 26.57 ^{o} C. Jack bean and sunn hemp have no difference on the mean dielectric permittivity during the growing cycles. The multivariate analysis of variance in Table 3 shows that plant species have no significant difference on volumetric water content, electrical conductivity, soil temperature and dielectric permittivity during the first planting round. Meanwhile, plant species have significant difference only on soil temperature (Table 6) during the second growing cycle.

Table 4: Mean and standard error of volumetric water content (VWC), electrical conductivity (EC), soil temperature (^{o} C), and dielectric permittivity (F/m) in jack bean and control plots at the second planting round.

Treatments	VWC (m^3 water/m^3 soil)	EC (dS/m)	Soil temperature (^{o} C)	Dielectric permittivity (F/m)
2nd Jack bean	0.171 ± 0.02	0.147 ± 0.03	25.57 ± 0.22a	9.452 ± 0.08
1st Jack bean	0.187 ± 0.01	0.160 ± 0.01	25.05 ± 0.05	10.299 ± 0.45
Control	0.190 ± 0.02	0.158 ± 0.02	25.23 ± 0.19	10.474 ± 1.17

Table 5: Multivariate analysis of variance (MANOVA) for the effect of jack bean on volumetric water content, electrical conductivity, soil temperature and dielectric permittivity at the second planting period.

Coefficient	Df	Pillai	Approx. F	nun Df	den Df	P (>F)
Plant species	1	0.64	1.29	8	22	0.3

Table 6: The summary of the analysis of variance for the effect of jack bean on the four soil attributes: volumetric water content, electrical conductivity, soil temperature, and dielectric permittivity.

	Df	Sum Sq.	Mean Sq.	F Value	P (>F)
Volumetric water content	1	0.0008	0.0004	0.41	0.67
Electrical conductivity	1	0.0005	0.0002	0.15	0.86
Soil temperature	1	0.74	0.37	4.084	**0.042**
Dielectric permittivity	1	2.54	1.27	0.40	0.68

Figure 6: Mean and standard error of volumetric water content (m^3water/m^3soil), electrical conductivity (dS/m), soil temperature (ºC), and dielectric permittivity in jack bean and control plots. Jack bean2 represents plots replanted with jack bean for a second time meanwhile Jack bean 1 are plots planted with jack bean for the first time.

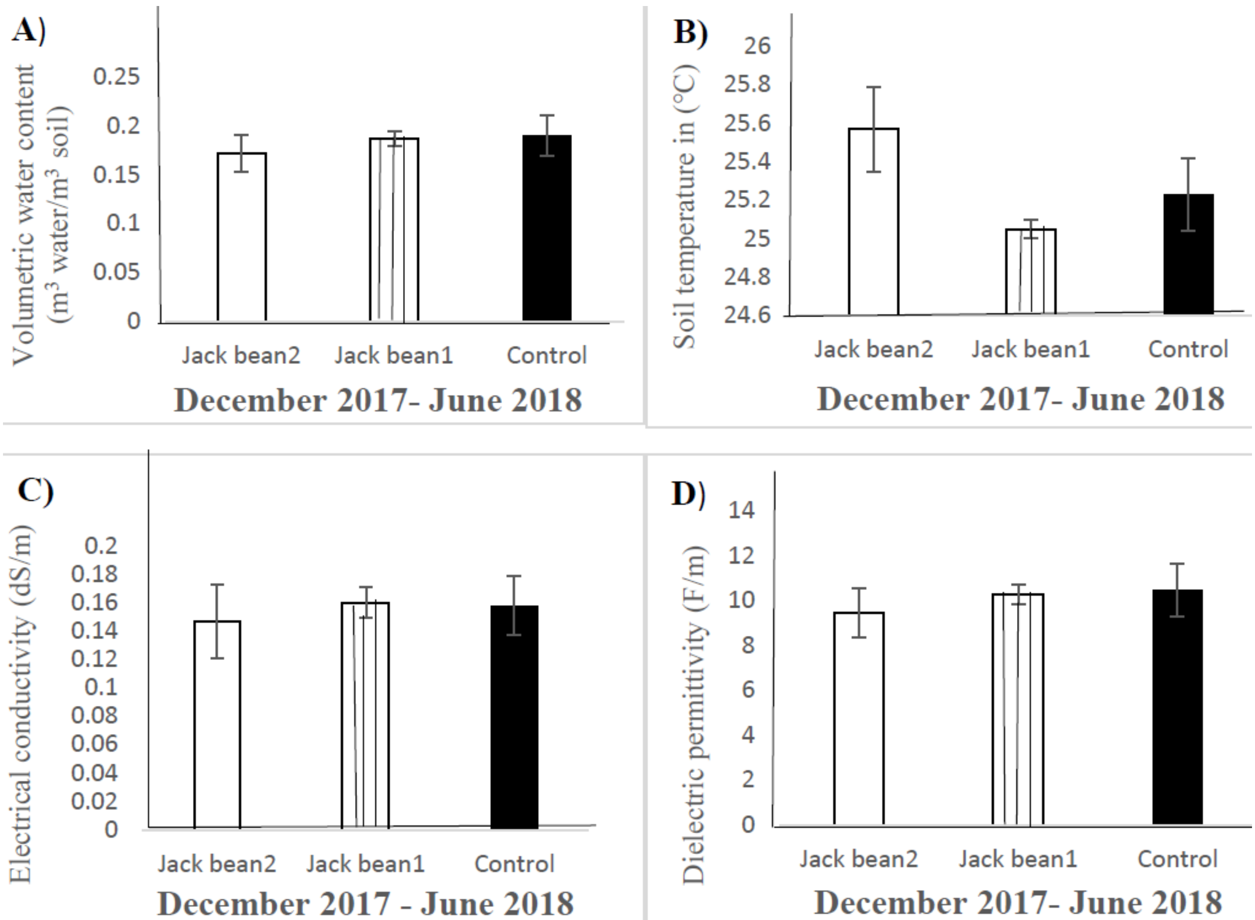


Table 7: Mean and standard error of air temperature and precipitation during the planting rounds. from December 2017 to June 2018 during the second planting round.

Periods (Times)	Air temperature (º C)	Precipitation (mm)
June to November 2017	26.57 ± 0.34	70.9 ± 0.72
December to June 2018	24.38 ± 0.51	105.37 ± 0.92

The results of these measurements: soil aggregate stability, bulk density, texture, strength, and water-holding capacity.

This experiment was conducted on a silt soil based on the soil texture analysis conducted during the study. The analysis has shown that silt is greater than 85 percent whereas Sand and Clay was less than 10 percent. Besides, the average of water-holding capacity of the soil is mostly similar during the both planting rounds. Soil bulk density analysis conducted at the end of the second planting round has shown a high level of soil compaction in the field. I assume that repeated tillage operation in the field may explain this considerable level of soil compaction. In addition, soil resistance or strength measurements penetration resistance was between the rank 50-75 based on the interpretation of penetration resistance established by Penn state Agricultural Sciences. This result indicates also the level of soil compaction is considerable which is similar to outcome of soil bulk density analysis. Lastly, the assessment conducted on soil aggregate stability was high (Table 8) during both planting rounds. The average of soil aggregates stability decreased at the second planting which may be originated by the presence of the cover crops in the field.

Table 8 : Mean and standard error of soil aggregates stability in jack bean, control plots, and initial soil samples collected before planting.

Dates	Treatments	Soil aggregates stability
30-Jun-17	Zero-treatment	80.03 ± 1.88
11-Apr-18	2nd Jack bean	72.80 ± 3.33
	1st Jack bean	70.66 ± 3.40
	Control	68.70 ± 4.59

Table 9: Soil texture analysis conducted in soil samples collected in June 16th, 2017 in the field experiment. The percentage and mean of Sand, Silt, and Clay was given for all plots.

Plot number	**Before planting**	**Percentage of Sand (%)**	**Percentage of Silt (%)**	**Percentage of Clay (%)**	**Soil Texture**
1A	06/30/2017	7.5	86.2	6.3	silt
2A	06/30/2017	6.6	87.7	5.7	silt
3A	06/30/2017	6.6	88	5.4	silt
4A	06/30/2017	6.6	88	5.4	silt
5B	06/30/2017	6.6	88.3	5.1	silt
6B	06/30/2017	7.2	87.1	5.7	silt
7B	06/30/2017	6.9	87.7	5.4	silt
8B	06/30/2017	6.6	88	5.4	silt
9C	06/30/2017	6.9	87.4	5.7	silt
10C	06/30/2017	7.2	87.1	5.7	silt
11C	06/30/2017	6.3	88	5.7	silt
12C	06/30/2017	7.2	87.1	5.7	silt
13D	06/30/2017	6.6	88	5.4	silt
14D	06/30/2017	5.7	88.6	5.7	silt
15D	06/30/2017	7.2	87.4	5.4	silt
16D	06/30/2017	7.5	87.3	4.8	silt
		6.82	**87.62**	**5.53**	

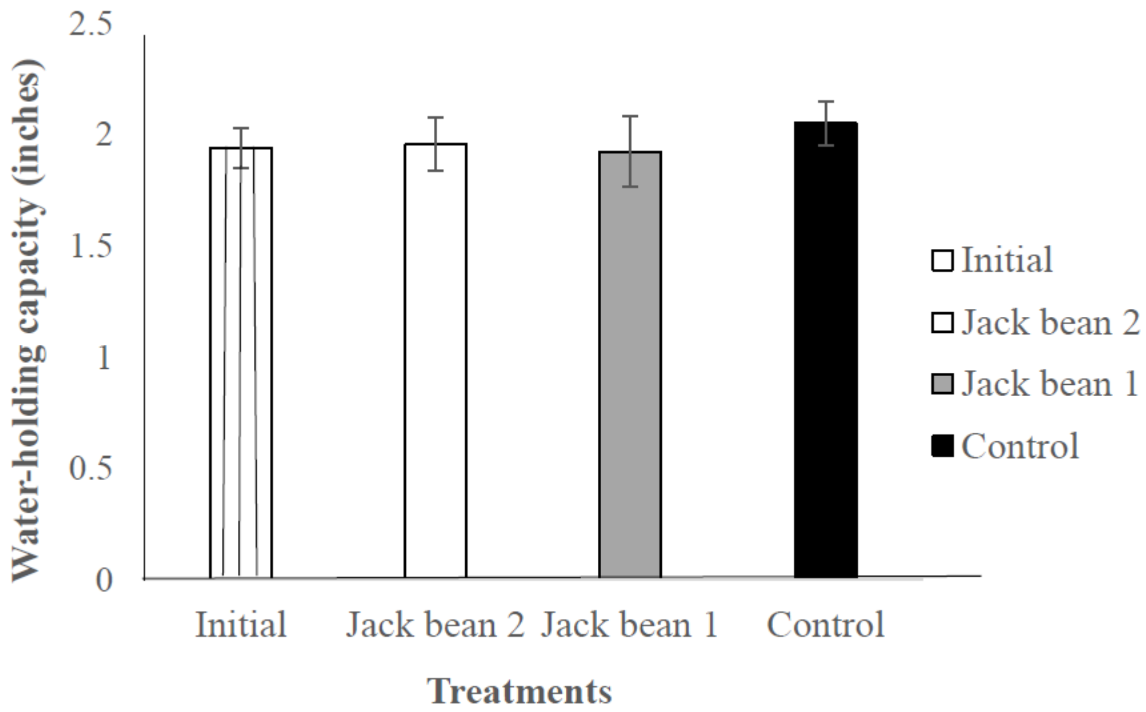
Table 10: Average of soil penetration resistance measurements using a Digital Soil compaction Tester or Penetrometer at two different periods during the study. Measurements were conducted with a penetrometer using a tip of ¾ inch diameter. Depth was recorded in psi and compacting rate of the soil (< 30= little to none compaction level, 30-50 = Slight; 50-75 = Moderate and > 75= Severe compaction level).

Dates	Treatments	Percentage of measuring points (PSI)	Compaction rating
30-Jun-17	Zero-treatment	74. 87 ± 4.22	Moderate
11-Apr-18	2nd Jack bean	110.75 ± 29.01	Severe
	1st Jack bean	84.75 ± 16.97	Severe
	Control	95.75 ± 33.28	Severe

Table 11 : Mean and standard error of water-holding capacity in jack bean, sunn hemp, control plots and initial soil samples collected before planting.

Dates	Treatments	Water-holding capacity (Inches)
30-Jun-17	Zero-treatment	1.939 ± 0.09
11-Apr-18	2nd Jack bean	1.956 ± 0.12
	1st Jack bean	1.924 ± 0.16
	Control	2.051 ± 0.10

Figure 7: Mean and standard error of water-holding capacity in initial soil samples collected before planting, jack bean 1 and 2, and control plots at the second planting**.**



Results of statistical tests performed on total carbon and nitrogen, soil pH, and dehydrogenase activity (DHA) in the soil.

Table 12: The mean and standard error of total carbon and nitrogen, and LOI in jack bean [*Canavalia ensiformis* (L.) DC.], sunn hemp (*Crotalaria juncea* L.), and control plots from soil samples collected at three different periods.

Dates	Treatments	Total Carbon (%)	Total nitrogen (%)	LOI
16-Jun-17	initial samples	2.78 ± 0.11	0.19 ± 0.01	13.09 ± 0.28
18-Nov-17	Jack bean	2.76 ± 0.22	0.19 ± 0.02	13.89 ± 0.36
	Sunn hemp	2.41 ± 0.11	0.16 ± 0.01	13.30 ± 0.58
	Control	2.41 ± 0.12	0.16 ± 0.01	13.79 ± 0.48
05-Apr-18	2nd Jack bean	2.91 ± 0.11	0.21 ± 0.02	13.01 ± 0.32
	1st Jack bean	2.61 ± 0.10	0.19 ± 0.01	12.73 ± 0.34
	Control	2.61 ± 0.06	0.19 ± 0.01	12.93 ± 0.64

Table 13: MANOVA for the effect jack bean and sunn hemp on total carbon and nitrogen, and LOI in the soil samples collected in November 2017.

Coefficient	Df	Pillai	Approx. F	nun Df	den Df	P (>F)
Plant species	1	0.5	1.35	6	24	0.27

Table 14: Multivariate analysis of variance for the effect jack bean on total carbon and nitrogen, and LOI in the soil samples collected in April 2018 at the second growing round.

Coefficient	Df	Pillai	Approx. F	nun Df	den Df	P (>F)
Plant species	1	0.097	0.43	3	12	0.73

Multivariate analysis of variance (Table 13 and 14) was performed for the effects of plant species on the total carbon and nitrogen during both planting rounds. There was no significant difference of plant species on total Carbon (C) and Nitrogen (N) and LOI during the two growing cycles. In addition, soil pH analyses were conducted on three different soil samples collected on June and November 2017 and April 2018, but there was no significant change in the soil pH (Table 15) within the plots of jack bean and sunn hemp during the study.

Table 15 : Summary of soil pH analyses conducted at three different periods in the study. Comparison of the soil pH in solution 1:1 (soil: water) and (soil KCl) in jack bean, sunn hemp and control plots. Comparison is made on results of the soil pH conducted in November 2017 and April 2018 between jack bean plots.

Dates of collection	Plant species	pH analysis	
		pH (1 :1) H_2O	pH (1 :1) KCl
June 16th, 2017	Initial	6.44 ± 0.04	5.28 ± 0.03
November 18th, 2017	Jack bean	6.60 ± 0.11	5.26 ± 0.09
	Sunn hemp	6.57 ± 0.08	5.17 ± 0.06
	Control	6.50 ± 0.04	5.18 ± 0.03
April 05th, 2018	2nd Jack bean	6.34 ± 0.05	5.12 ± 0.07
	First Jack bean	6.58 ± 0.04	5.26 ± 0.04
	Control	6.54 ± 0.08	5.22 ± 0.08

Table 16: Mean and standard error of dehydrogenase activity (DHA) in jack bean, sunn hemp, and control plots at three different periods.

Dates of collection	Plant species	Dehydrogenase activity (DHA) (µg TPF $g^{-1}mn^{1}$)
June 16th, 2017	Initial	0.947 ± 0.05
November 18th, 2017	Jack bean	0.941 ± 0.21
	Sunn hemp	0.943 ± 0.04
	Control	0.917 ± 0.19
April 5th, 2018	2nd Jack bean	1.471 ± 0.24
	1st Jack bean	1.265 ± 0.14
	Control	1.400 ± 0.18

Figure 8: Mean with standard error of dehydrogenase activity (DHA) in jack bean, sunn hemp and control plots during the first growing cycle.

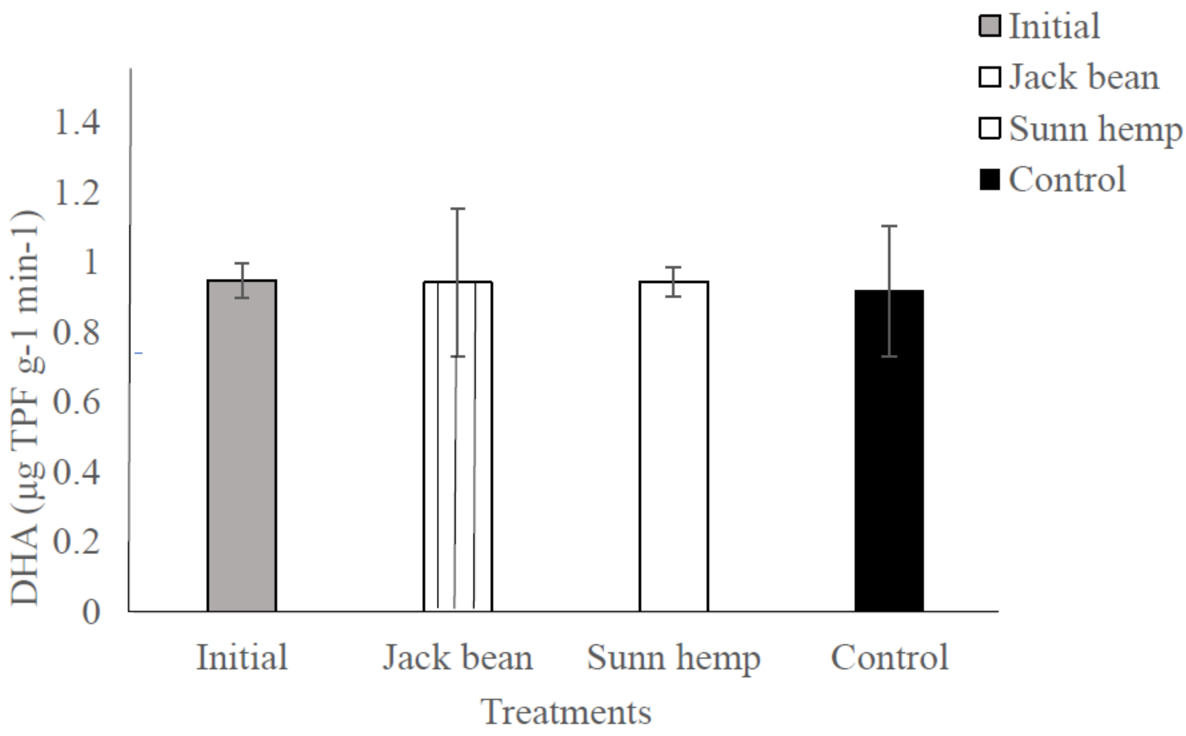
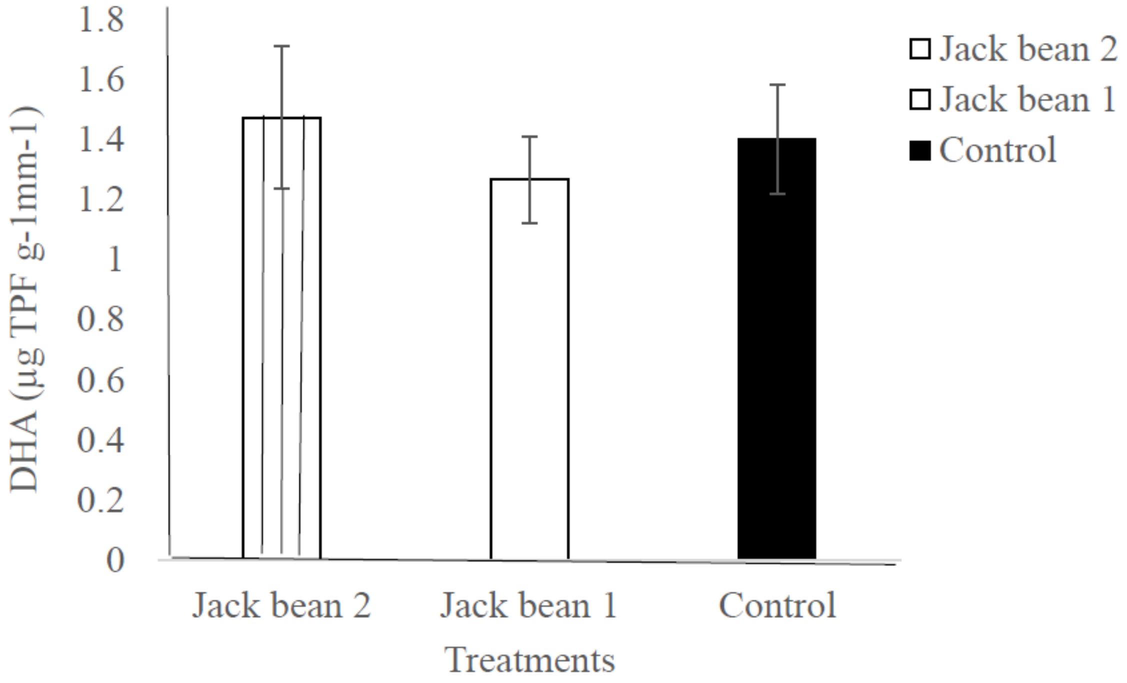


Figure 9: Mean with standard error of dehydrogenase activity (DHA) in jack bean and control plots during the second growing cycle.

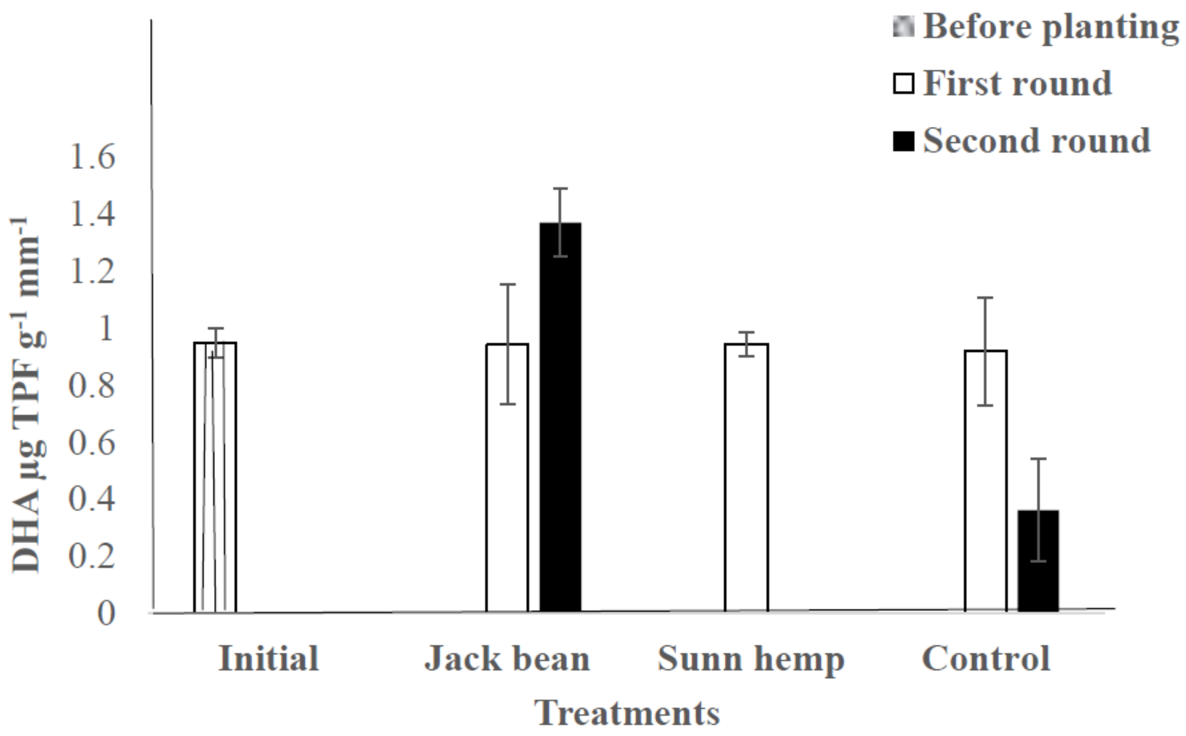


Dehydrogenase activity (DHA) in the soil was almost similar after the first growing cycle. During the second evaluation, the mean dehydrogenase activity has changed in all treatments (Table 16). For instance, the mean dehydrogenase activity in jack bean went from 0.941 to 1.471 µg TPF $g^{-1}mn^{1}$. Analysis of variance was conducted for the effect of plant species on dehydrogenase activity in the soil, but there was no significant effect of plant species during both planting rounds.

Table 17 : Analysis of variance for the effect of jack bean [*Canavalia ensiformis* (L.) DC.] and sunn hemp (*Crotalaria juncea* L.) on dehydrogenase activity in soil samples collected on November 2017 and April 2018

	Df	**Sum Sq.**	**Mean Sq.**	**F value**	**P (>F)**
Plant species	1	0.002	0.001	0.006	0.99
Plant species	1	0.13	0.063	0.37	0.70

Figure 10: Mean of dehydrogenase activity (DHA) in the soil at three different periods: June and November 2017, and April 2018.



Discussion

Do previous studies find significant effects of jack bean and sunn hemp on soil properties? Generally, planting crops may influence soil properties during the growing cycle because soil represents the primary source of nutrients to the crops. In my study, findings show that both plant species do not have significant difference on volumetric water content, electrical conductivity and dielectric permittivity based on the outcomes of p-values which are greater than significant level (α=0.05). Plant species may not directly influence these variables to explain my results. For instance, variation on electrical conductivity depends on the amount of moisture held by soil particles. In addition, soil moisture content, electrical conductivity is associated to soil salinity, clay content and cation exchange capacity, clay minerals, pore size and distribution, organic matter and temperature (José et al., 2011). However, plant species have significant difference on soil temperature during the second round. This is evident that plant species have significant effect on soil temperature because it is the main control of plant growth and development. In addition, jack bean can influence soil temperature given its large canopy cover which could reduce the amount of heat reaching directly the soil.

In this study, the compacting rate of the soil was between 50-75 psi at the initial measurements of soil strength. The compacting rate has been increased during the second growing cycle based on the ultimate measurements. Repeated tillage conducted during this study may provoke this increase of soil compaction level. In addition, plant species do not have significant effect on soil aggregates stability, water-holding capacity and soil bulk density during this study. Moreover, soil pH analysis was not different between plant species. In addition, the range of pH found in the field falls within the range of suitable soil for agriculture. Jack bean and sunn hemp can modify indirectly the soil pH when they influence soil temperature and soil organic matter which are two influential factors of soil pH.

Literature have shown jack bean and sunn hemp can increase total nitrogen and carbon in the soil; however, significant change cannot be attained in a short period. I also suppose the amount of biomass of jack bean and sunn hemp added into the soil would need to be considerable to see the change in total carbon and nitrogen. I observed the total carbon has changed after the second growing cycles. The rapid decomposition of the biomass of jack bean can be explained this change in the total carbon after the second planting.

Dehydrogenase activity in the soil is one of the indictors of good soil quality but it can be influenced by soil properties such as soil moisture content, seasonal variations, pH, temperature, soil organic matter, redox potential and others (Hanajík et al, 2017). Moreover, dehydrogenase activity is very sensitive to natural and anthropogenic disturbances (Kumar et al, 2013). In this study, plant species do not have significant difference on dehydrogenase activity in the soil. Dehydrogenase activity in the soil was low based on the results found during the study which may be explained by the lowest microbial activity in the soil. In addition, dehydrogenase activity was found greater in forest soils than grassland area and the highest DHA was reported in rainy season and lowest in winter (Kumar et al., 2013). It was shown in many studies that DHA is significantly influenced by water content and dropped with the decrease of soil humidity. For instance, higher DHA level was observed in flooded soil, rather than in non-flooded conditions (Wolinska and Stepniewsk, 2012). My findings agree with these results because they describe the conditions to have higher DHA in the soil.

Conclusions

In this study, I have demonstrated the effects of plants species on soil properties: electrical conductivity, dehydrogenase activity, dielectric permittivity, soil pH, soil temperature, total Carbon and Nitrogen, soil strength and water-holding capacity of the soil during two planting rounds. Jack bean and sunn hemp have no significant effect on the soil properties. Only, plant species have significant difference on soil temperature during the second planting round. I suppose this significant effect was due to the two growing cycles of jack bean and sunn hemp in the field. Based on the outcomes of this study, significant changes can be attained on these soil properties by planting jack bean and sunn hemp at different seasons.

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CHAPTER IV. The effects of jack bean and sunn hemp on earthworm populations.

Abstract

Earthworms are a major component of soil fauna communities in most natural ecosystems of the humid tropics and comprise a large proportion macrofauna biomass. Earthworms are recognized for their important role regarding the improvement of soil physical and chemical characteristics of soil and thus increasing its fertility. Earthworms have been classified in three primary ecological categories: Epigeic, Endogeic, and Anecic. Earthworms are also good biological indicators of soil fertility and promote plant growth. Two earthworm samplings were conducted in this study to evaluate the effect of jack bean and sunn hemp on the density and biomass of earthworms. Multivariate analysis of variance (MANOVA) was performed for the effect of jack bean and sunn hemp on the density and biomass of earthworms. In addition, a summary of analysis of variance was also performed to see if plant species had significantly different on the biomass and density of earthworm populations. The findings showed jack bean and sunn hemp had no significant effect on the density and biomass of the population of earthworms during both planting periods. I sampled *Pontoscolex* sp., *Pontoscolex corethrurus*, *Pontoscolex spiralis*, and *Amynthas or Polytheretima* but *Pontoscolex* sp. was the most abundant species with the highest the density (187 m^2). Lastly, more than 80 % of sampled of earthworms were immature in both planting rounds.

Key words: Earthworms, Tropical and Temperate ecosystems, soil fauna, Epigeic, Endogenic and Anecic.

Introduction

Earthworms have been described as soil engineers in tropical and temperate ecosystems because they change the structural properties of soil and thus influence soil microorganisms and plants growth (Kooch et al. 2008). They are also called ecosystems engineers, which means that they affect their environment (Babu Ojha and Devkota, 2014; Chauhan, 2014; Kanianska, et al., 2016). Ecosystem engineers are keystone species that modify their habitat so strongly that can have effects on other organisms (Kanianska, et al., 2016). In addition, these invertebrates are important for fragmentation and redistribution of plant materials and for the excretion of nutrient-rich faeces (Coupland and McDonald, 2008).

Earthworms are a major component of soil fauna communities in most natural ecosystems of the humid tropics and comprise a large proportion macrofauna biomass (Bhadauria and Saxena, 2010; Coupland and McDonald, 2008; Salehi et al, 2013). That's why earthworms are subject to physical, chemical and biological change in soil (Salehi et al, 2013). González (2017) reported earthworms often dominate the fauna of soil food webs in terms of biomass. Earthworms biomass is an appropriate biological indicator of soil fertility. humus quality, degradation, pollution and habitat productivity (Salehi et al, 2013). Earthworms are generally assumed to be beneficial soil animals which promote plant growth (Scheu, 2003).

Earthworms have been divided into three primary ecological categories that may contribute differently to ecosystem processes and thus ecosystems services (Blouin et al., 2013). The three major types found in soil ecosystem are classified as Epigeic, Endogeic, and Anecic (Babu Ojha and Devkota, 2014; Blouin et al., 2013; Barva and Rica, 1992; Shipitalo, 2004; Yadav et al., 2017; Singh et al., 2015; Kooch and Jalilvand, 2008). The Epigeic worms are litter-feeding worms with dorsal pigmentation and spasmodic movements that live in the litter or the first 5 cm in the soil (Barva and Rica, 1992; Babu Ojha and Devkota, 2014). Typical habitats include forest litter or manure piles; thus, they have a little direct effect on the structure of mineral soils (Shipitalo, 2004).

They are phytophagous worms and are efficient biodegraders (Yadav et al., 2017; Singh et al., 2015). Anecics worms were soil-burrowing species with antero-dorsal pigmentation and a flattened posterior end that live in the soil but feed on litter (Barva and Rica, 1992; Laossi et al., 2013). Worms in this category tend to make vertical tunnels into the ground, their primary food source is decaying matter on the topsoil and they are considered to be geophytophagous (Yadav et al., 2017). The endogeics were all those unpigmented soil-dwelling species with slow movements which feed on soil organic matter (Barva and Rica, 1992; Laossi et al, 2013), they are considered to be geophagus (Yadav et al., 2017; Singh et al., 2015). Endogeic earthworms may deposit 20-200 t dry soil ha^{-1} surface casts that contain a significant proportion of SOM yearly (Lavelle et al., 1998).

Importance of earthworms

Among the organisms with their living activity in soil, the earthworms are recognized for their important role regarding the improvement of soil physical and chemical characteristics of soil and thus increasing its fertility (Iordache and Borza, 2010). Earthworms play a major role in soil nutrient dynamics by altering the soil physical, biological and chemical properties (Kooch and Jalilvand, 2008). Earthworms are also known to be important regulators of majors soil process and functions such as soil structure, organic matter decomposition, nutrient cycling, microbial activity decomposition and activity, and plant production (Lemtiri et al., 2014; González, 2017). They also play role in nutrient cycling and dynamics along with mineralization due to their burrowing, casting and mixing actions (Chauhan, 2014). Earthworms bring up the nutrients from deep in the soil and deposit them on the soil surface as castings, hence counteract leaching nutrients (Chauhan, 2014).

Soil physical properties

Earthworms have typically been thought to improve soil physical properties through their burrowing and casting activities (González 2017; Shipitalo 2004). The two major activities of earthworms which results in influencing the soil structure are: eating soil through their mouth and breaking down and mixing the organic matter, then conclusively voiding the gut contents as subsurface casts and burrow inside sub-layers of soil, thus importing subsoil to the surface (Yadav et al., 2017). Earthworms also help to enhance the soil penetrability and reduce the soil compaction and enhance root distribution (Babu Ojha and Devkota, 2014).

Soil chemical properties

Earthworm activities have important effects on soil chemical properties, and they are benefits for organisms and agricultural purposes. The earthworms' gut is well known for the breaking down the organic matter into finer particles, so as to expose to greater surface area of organic matter to microbial decomposition and is egested in their casts, this result in rapid mineralization of organic matter resulting in nutrient liberation in feasible forms that can easily absorb by the plants (Yadav et al., 2017). Earthworms increase the mineralization of organic matter in soil and thus add to the amount of nitrogen in soil from the mineralization because of enhanced nitrification in earthworm casts (Chauhan, 2014).

Soil biological properties

The effects of earthworms are largely described on soil biological properties and they seem to be well considered or positive. For instance, earthworms have a complex inter-relationship with microorganisms. They are the microsites for millions of useful microbes including the nitrogen fixation bacteria in their gut (Yadav et al., 2017). Information to be considerable, Nitrogen fixation in casts is comparatively greater than that in soil due to the presence of Nitrogen-fixing bacteria in the gut of earthworm and in earthworm casts which increase nitrogenase activity (Chauhan, 2014).

Earthworm effects on plant growth

Earthworms are farmers' friends because they mix the upper and lower soil layers and bring the nutrients from lower soil layers to the layer of root penetration from where a plant can easily absorb nutrients (Yadav et al., 2017). In other way, earthworms provide the seedbed for plant growth by passing the soil through their gut and incorporate mineral and organic elements in the soil (Singh et al., 2015). They are known to affect plant growth through five main mechanisms: 1) the enhancement of soil organic matter mineralization 2) the production of plant growth regulators via the stimulation of microbial activity, 3) the control of pests or parasites, 4) the stimulation of symbionts and 5) the modifications of soil porosity and aggregation, which include changes in water and oxygen availability to plant roots (Laossi, Deca, and Jouquet, 2013). In addition, earthworms have been shown to increase the production of shoots and grain in a variety of field trials and greenhouse experiments (Kooch and Jalilvand, 2008).

MATERIAL AND METHODS

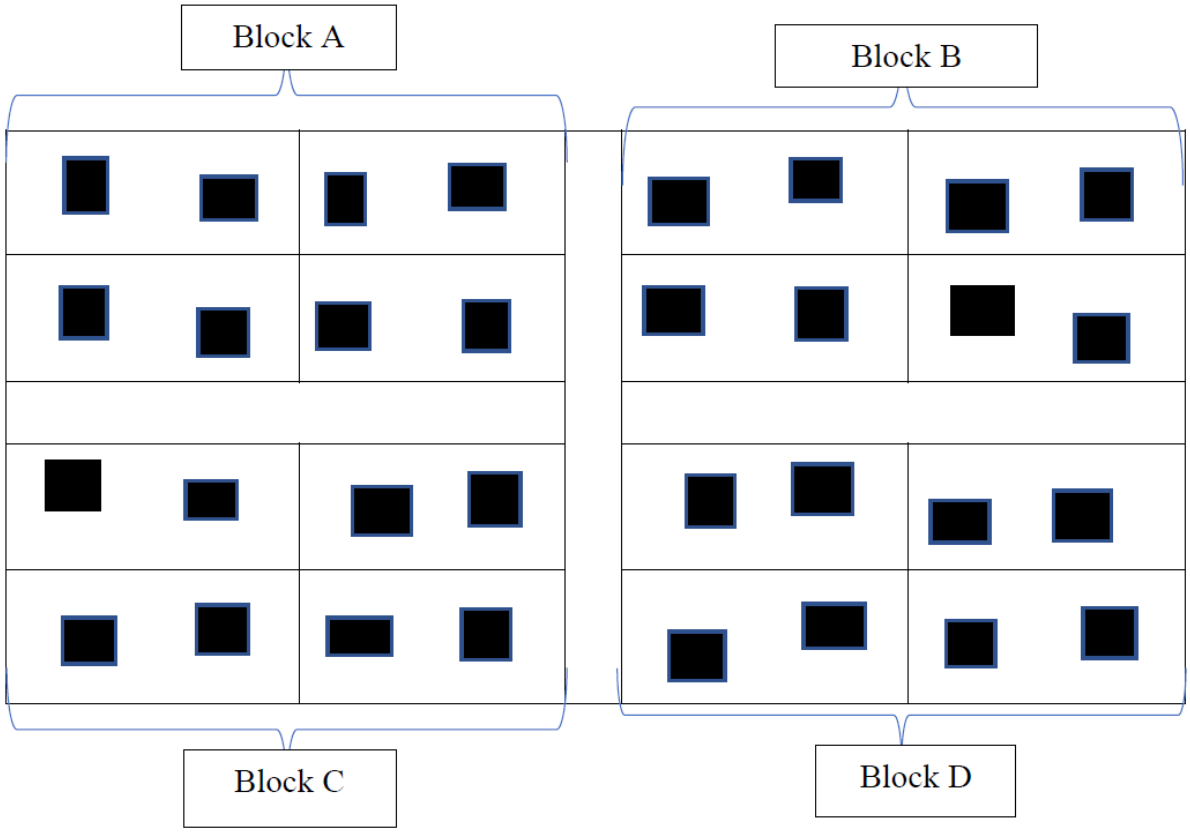
Description of the study site

This study was conducted at the Agricultural Experimental Substation at Gurabo of the University of Puerto Rico, Mayagüez campus. The geographical coordinates of the experimental station are 18˚15’ 03 96" N and 65˚59 25 W. Gurabo climate is semitropical and has 1869 mm yr^{-1} as mean annual precipitation and temperature is 25.2˚C. This area is uniform and a flat region at the altitude less than 10 m. The soil texture of the study area is formed on the alluvial fine texture and it is mainly silt soil. It was designated for Investigators and researchers to conduct academics studies. Acosta (2009) asserts that this substation is one of the most important ones for students in the fields of pre-veterinary and livestock industry. The highest point in Gurabo is at 367 m above sea level.

Experimental Design

This experimental design has been applied in the field experiment at the end of the first growing season of the cover crops in December 2017. This design contained 32 plots overall experiment in reason of 2 pits per plot. By April 2018, the same experimental design was repeated in the field to sampling the earthworms at the end of the second planting round. Pits area were selected randomly in the plots. Noted that control plots established through both growing seasons were to compare the effect of the cover crops on earthworm population in the plots.

Figure 1: The experimental design established at the field experiment to collect the earthworms contained 32 plots which were selected randomly in the field.



Materials and Methods

A solution which contains 10 percent of formaldehyde was prepared in a chemical laboratory to conserve collected worms in the field and small containers were used to store and preserve the worms. Every single container was labeled with plot number, date of collection and number of collected earthworms. Digging tools like handle shovel, trenching spade and post-hole digger were used to dig out the holes. In addition, a huge tarpaulin was used to put the removed soil from the plots to facilitate the collection.

Digging process

The standard plot size for the National Earthworm Recording Scheme is 25 cm by 25cm to a depth of 50 cm to sample earthworms but a 25 cm* 25cm * 30 cm has been applied in this investigation to collect earthworms in this experiment. Thirty-two holes or plots were established to collect the earthworms and two per each plot in the field experiment. Earthworms sampling were repeated twice during the two differences growing season. The first sampling was conducted on December 12th, 2017, and the second sampling was realized on April 11th, 2018.

Sorting earthworms

Studies reported that hand sorting and digging is the most widely used technique for quantitative sampling of earthworms (Singh et al., 2015). The hand-sorting method was used to remove earthworm species from the removed soil. Rhea-Fournier (2014) reports this method is well and broadly applied in studies seeking a relationship in environments with variation with earthworm abundance, communities' structure and diversity alone, or in correlation with other factors such as tree species and communities. In this study, hand sorting method was used to sample the earthworms.

The first 10 cm of soil from the pit was placed on a tarpaulin and worms were sorted by hands. Every clod was broken down in small parts to avoid missing worms. From ten to 20 cm the soil was removed from the pit and put on the batch to collect the worms. Finally, from 20 to 30 cm was put apart from the two previous layers to see if worms are available at that depth. Any earthworms that were found in the soil should be removed and collected into a container. Total of collected worms was counted from every layer from every single plot.

Storing earthworms' conditions and labeling

After earthworms were removed and collected, they have been stored in normal positions and conditions to make the process of identification of the specimens easy in the laboratories. Collected earthworms were stored in a solution of ten percent of formaldehyde since collected to relax and straighten. The purpose of using this solution was to serve as a killing agent and preserve the earthworms as it was collected in the field. A label was placed on the container with plot number, date of collection, the quantity of collected worms, investigator's name and the study site.

Earthworm identification process

Both worm samplings were transported to the laboratory of University of Puerto Rico Mayagüez campus for the identification process by Dr. Sonia Borges. Microscopes were used to identify the specimens. Generally, some characteristics which are reasonably visible to naked eyes including colors, size, head, number of segments, and details regarding the clitellum were used to help to identify the species of earthworms. Other academics literature was used as support to confirm identification. In addition, all the scientific criteria were strictly followed to during earthworm identification process.

Statistical analysis

All Statistical analyses were carried out by using R packages**.** The observed data was checked out to determine the distribution pattern and homogeneity. Multivariate analysis of variance (MANOVA) was performed to determine the effect of plant species (jack bean and sunn hemp) on the density and biomass of earthworms. Simple linear correlation was conducted between the density and biomass of earthworms, soil pH, soil temperature, soil aggregate stability, bulk density, soil resistant, volumetric water content, dielectric permittivity and electrical conductivity. Statistical decision to reject or not was taken at the significant level $\alpha = 0.05$.

Results

Table 1: Density of earthworm/m^2, fresh and dry biomass (g/m^2) of worms sampled in jack bean, sunn hemp and control plots during two different periods in Puerto Rico. The area sampled per plot was 1* 0.5 m^2. Four plots were planted with jack bean, 4 plots with sunn hemp, and 8 plots were controls during the first planting round. During the second planting 12 plots were planted with jack bean 4 others were controls.

		Earthworm density	Earthworm biomass	
Dates	Treatments	(Density/m^2) ± SE	Fresh (g/m^2) ± SE	Dry (g/m^2) ± SE
12/18/2017	Jack bean	18 ± 0.85	3.58 ± 0.26	1.42 ± 0.12
	Sunn hemp	44 ± 1.55	8.14 ± 0.31	2.62 ± 0.12
	Control	40 ± 0.78	7.23 ± 0.18	1.80 ± 0.045
04/11/2018	Jack bean 2	94 ± 4.68	9.74 ± 0.52	1.033 ± 0.15
	Jack bean 1	74 ± 1.65	5.61 ± 0.22	1.037 ± 0.042
	Control	32 ± 2.27	5.22 ± 0.40	0.58 ± 0.059

Table 2: Earthworm density (per m^2) per species in jack bean, sunn hemp and control plots sampled in December 2017 in Gurabo, Puerto Rico. Zero means species was not found and the area sampled per plot was 1* 0.5 m^2.

Dates	Earthworm species	Density of earthworms/m^2 with standard error		
		Jack bean	Sunn hemp	Control
12/18/ 2017	*Pontoscolex* sp.	28 ± 0.88	52 ± 2.19	72 ± 0.73
	Ponstoscolex corethrurus	16 ± 0.5	0 (0)	2 ± 0
	Pontoscolex spiralis	0 (0)	32 ± 3	2 ± 0

Table 3: Earthworm density (per m^2) per species in jack bean 1 and 2 and control plots at the second growing period in April 2018 in Gurabo, Puerto Rico. Zero means species was not found and the area sampled per plot was (1 x 0.5 m^2) to a depth of 30 cm.

Dates	Earthworm species	Density of earthworm/m^2 with standard error		
		Jack bean 1	Jack bean 2	Control
04/11/ 2018	*Pontoscolex corethrurus*	8 ± 0	2 ± 0	0
	Pontoscolex sp.	136 ± 1.61	176 ± 4.64	64 ± 2.60
	Amynthas or Polypheretima sp.	8 ± 0	2 ± 0	0

During the first earthworm sampling in November 2017, collected earthworms were greater in sunn hemp plots (44 per m^2) than jack bean (18 per m^2). I also found the density of earthworm per meter squared (40 per m^2) in control plots was higher than jack bean (Table 1) during the first collection. Besides, I observed that more than 80 % of sampled earthworms were immature at the first earthworms sampling. Moreover, more earthworms were colleceted during the second sampling in April 2018. Therefore, the density of sampled earthworms in jack bean 2 increased to 94 m^2, and 74 m^2 in jack bean 1, but collected earthworms in control plots decreased to 32 m^2 at the second sampling. In addition, few earthworms were not able to identify during the samplings because they got damaged during sampling operation in the field and they were immature.

Figure 2: Density of *Pontoscolex* sp, *Pontoscolex corethrurus* and *Pontoscolex spiralis* as three worms species collected in Jack bean, sunn hemp and control plots at the first sampling in December 2017.

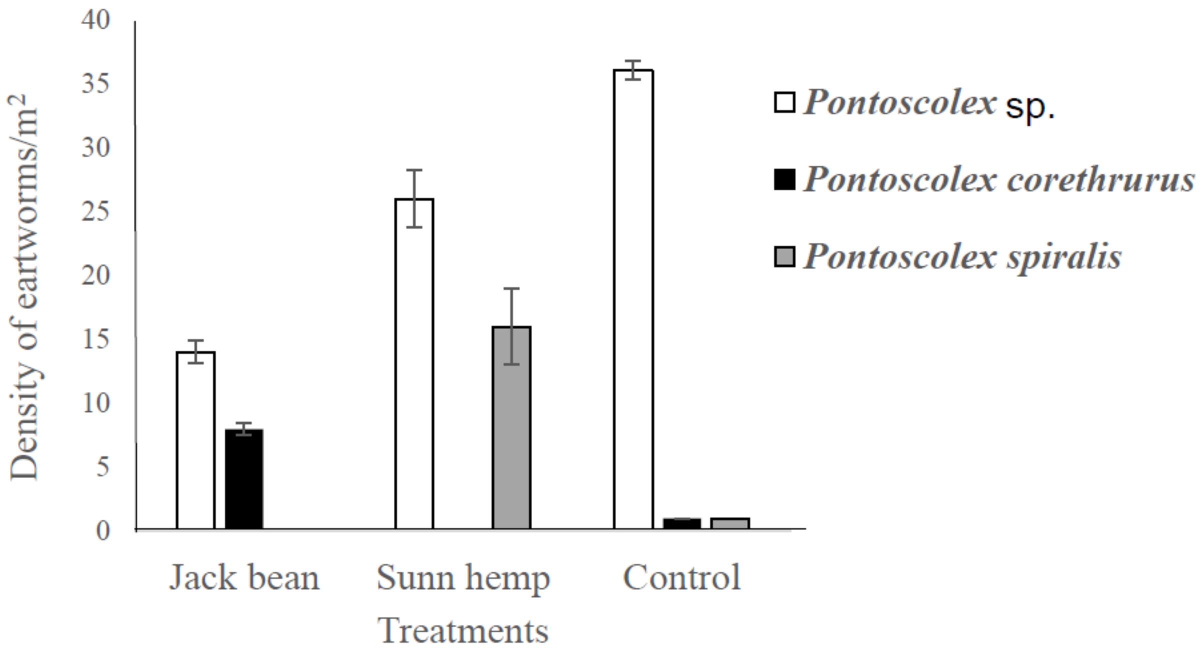
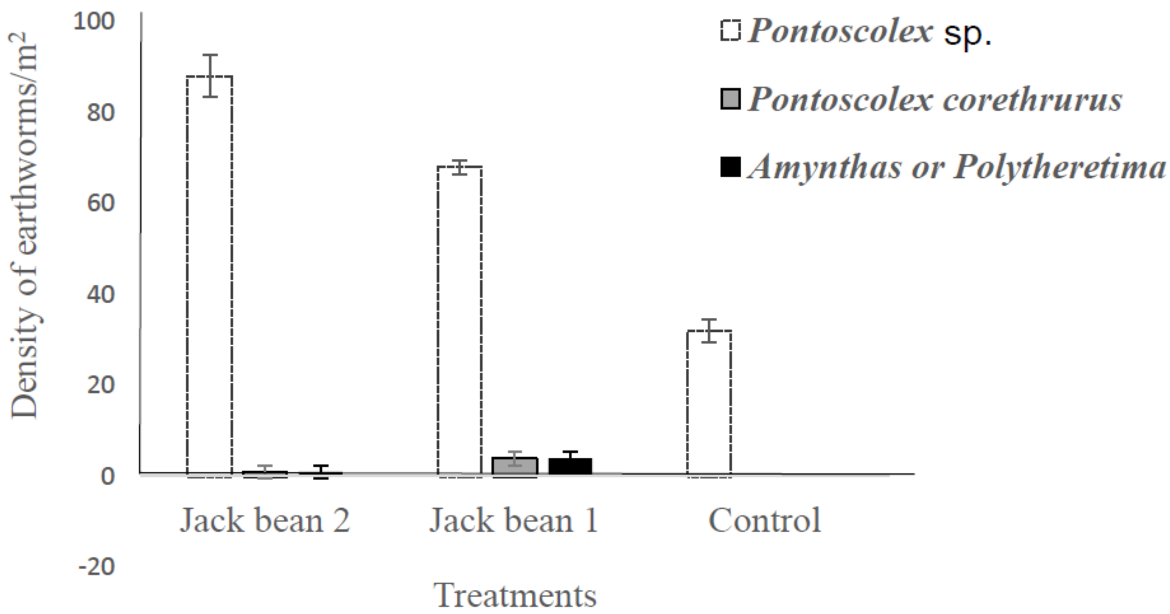


Figure 3: Density of *Pontoscolex* sp, *Pontoscolex corethrurus* and *Amynthas or Polytheretima* as three worms species collected in Jack bean 1 and 2, and control plots at the second sampling in April 2017.



Three species of earthworms were collected: *Pontoscolex corethrurus, Pontoscolex spiralis,* and *Pontoscolex* sp. during the first sampling in December 2017. *Pontoscolex* sp. was the most abundant species encountered in the field; and it was mostly found in every plot. *Pontoscolex* sp. had a density of earthworms (28 m^2) in sunn hemp, (52 m^2) in jack bean and (72 m^2) in control plots. I also sampled three species of earthworms: *Pontoscolex* sp, *Pontoscolex corethrurus* and *Amynthas* or *Polypheritima* during the second earthworm sampling conducted on April 11th, 2018. However, *Pontoscolex spiralis* was not collected at the second earthworm sampling but *Amynthas* or *Polypheritima* (Table 3) was found as a new earthworm species. The density of *Pontoscolex* sp. increased in jack bean 1 (136 m^2) and jack bean 2 (174 m^2) but decreased in control plot in Table 3. Jack bean and sunn hemp have no significant diffference on the population of sampled earthworms based on the multivariate analysis of variance during the first sampling in December 2017 in Table 3. Moreover, there was no significant effect of plant species on the population of earthworms during the planting round.

Table 4 : MANOVA test performed for the effect of jack bean and sunn hemp on the density and biomass of earthworms at the first growing period.

Coefficient	**Df**	**Pillai**	**Approx. F**	**nun Df**	**den Df**	**P (>F)**
Plant species	1	0.54	1.49	6	22	0.22

Table 5 : Summary of the multivariate analysis of variance for the effect of jack bean and sunn on the density and biomass of sampled earthworms at the first sampling in December 2017.

	Df	**Sum Sq.**	**Mean Sq.**	**F Value**	**P (>F)**
Density	1	25.69	12.84	2.15	0.16
Fresh biomass	1	0.97	0.48	1.70	0.22
Dry biomass	1	0.12	0.061	1.76	0.21

Table 6 : Multivariate analysis of variance for the effect of plant species on the density and biomass of earthworms during the second sampling in April 2018.

Coefficient	**Df**	**Pillai**	**Approx. F**	**nun Df**	**den Df**	**P (>F)**
Plant species	1	0.15	1.73	3	12	0.55

Table 7 : Summary of the analysis of variance for the effect of plant species on the density and biomass of earthworms.

	Df	**Sum Sq.**	**Mean Sq.**	**F Value**	**P (>F)**
Density	1	28.52	28.52	0.65	0.43
Fresh biomass	1	0.14	0.14	0.23	0.63
Dry biomass	1	0.030	0.030	0.91	0.35

Table 8 : Soil properties (soil pH, H_2O and KCl), soil texture (Sand %, Clay %, and Silt %), Soil temperature (ST), water-holding capacity (WHC), soil density (SD), volumetric water content (VWC), electrical conductivity (EC), and dielectric permittivity (Ka)) and linear correlation coefficients (r) and significant level (p=0.05) with earthworm density and fresh biomass during two different periods (November 2017 and April 2018)

	Soil pH		Soil Texture								
Earthworms	H_2O	KCl	Sand	Clay	Silt	ST	VWC	EC	SD	Ka	WHC
Density	-0.229	-0.225	-0.432	-0.074	0.428	0.102	-0.017	-0.124	0.303	-0.111	-0.444
P-values	0.272	0.401	0.094	0.785	0.098	0.706	0.949	0.646	0.254	0.681	0.084
Biomass	-0.137	-0.063	-0.293	-0.066	0.304	0.077	0.157	0.159	0.460	0.114	-0.386
P-values	0.613	0.817	0.270	0.807	0.251	0.778	0.561	0.556	0.073	0.673	0.140
Density	-0.539	-0.705	0.130	0.684	-0.449	0.652	0.052	-0.154	0.435	0.070	0.284
P-values	**0.031**	**0.002**	0.631	**0.003**	0.080	**0.006**	0.848	0.567	0.092	0.795	0.287
Biomass	-0.544	-0.692	-0.132	0,539	-0.158	0.491	-0.099	-0.264	0.474	-0.113	0.241
P-values	**0.029**	**0.003**	0.625	0.031	0.560	0.053	0.715	0.323	0.063	0.677	0.368

Correlation analysis between the density and fresh biomass of earthworms and soil parameters such as soil temperature, percentage of clay and soil pH was performed during this study (Table 8). Density of earthworms showed significantly correlation with soil temperature (r= 0.006, p= 0.006), percentage of clay (r = 0.684, p = 0.003), and soil pH (soil : water p = 0.002, soil : KCl p= 0.031). Earthworms' biomass were significantly correlated with soil pH (soil : water p = 0.029, soil : KCl p = 0.003) during the second sampling. Based on the results in Table 8, density of earthworms and fresh biomass were not significantly correlated with soil parameters during the first earthworms sampling.

Discussion

Is there any difference or positive effect of jack bean and sunn hemp on the population of earthworms while growing in agricultural land? In Puerto Rico, earthworms ecological research has been mostly conducted in the Luquillo Experimental forest (González et al., 2007; González et al., 1999; Lugo et at., 2006; Larsen et al., 2012; Zou and González, 1997; Zou and Borges, 1996; Dechaine et al., 2005). Most of the findings indicate significant species richness and abundance /density and high fresh biomass with the forest types. My expectation was to find less density and biomass, species richness and abundance due to the land use type in the area this study was conducted. However, I was expected that planted cover crops could have a positive effect on the population of earthworms by adding biomass in the soil.

Scientists indicates the most important factors determining earthworm abundance and biomass are the quantity and the quality of the food (González et al. 1999). In addition, earthworm population density at a specific site is the result of the interaction of a number of factors: soil texture, moisture, pH and organic matter content (Himalaya, 2015). Results from this study indicate that there is no species richness, but *Pontoscolex* sp. *was* only the abundant one among the four earthworms' species sampled during the study. *Pontoscolex* sp. has dominated the density and fresh biomass during both planting rounds. For instance, twenty- four *Pontoscolex* sp. were sampled from one plot of jack bean species. I also collected an important number of *Pontoscolex* sp. in the control plots.

In addition, this considerable density and fresh biomass of this earthworm species may be explained by the abundance of the food and lack of predators in the area. For instance, the main source of food for earthworms is the organic wastes such as agro-horticultural crop wastes, weeds, forest leaf litter and agro-industrial wastes and others (Thyug and Kakati, 2018). Therefore, I assume this increase in number of collected worms was due to the previous cover crops incorporated in the soil during the first growing round. I found *Pontoscolex* sp. had the highest density and fresh biomass during both growing periods in this study.

In contrast, a study in Mexico and Costa Rico was dominated by *Pontoscolex corethrurus* in terms of density and biomass (Barva and Rica, 1992). *Pontoscolex corethrurus* is native to South America and has successfully corroborated itself in disturbed tropical soil around the world (León et al., 2003). Moreover, in mature forest *Pontoscolex corethrurus* is the most abundant species (Larsen et al., 2012). In Puerto Rico, earthworms were dominated by the endogeic species *Pontoscolex corethrurus* Muller (Dechaine et al., 2005; León et al., 2003). In my study, *Pontoscolex corethrurus* has been sampled during both planting round with a small density. In addition, *Pontoscolex spiralis* and *Amynthas* or *Polypheritima* were collected during the study; however, both species were not sampled in the two samplings. It might be the consequence of the two hurricanes which have been struck the island during the study. Therefore, other study would need to take into consideration these variables that can influence the community of earthworms.

In terms of earthworms' species, my findings are comparable to most of the earthworms' ecological research conducted in Puerto Rico, meanwhile the study was conducted in different land use type. In this study, earthworms sampled were similar to the previous studies conducted in Puerto Rico. Generally, earthworms' density and species richness in forests would be higher due to food availability and less disturbances. However, in agricultural land, frequencies of field operation may influence the population of earthworms. In addition, agrochemical inputs and unsustainable crop management practices may impact the earthworm community. For instance, finding from a study conducted in Hawaii indicates that earthworms were not present in sugarcane fields and increased gradually with age of eucalypt plantations and secondary communities and reached 400 individuals m^{-2} and 136 gm^{-2} by 10 year old (Zou, 1997).

Conclusion

Based on the outcomes of the performed statistical tests, jack bean and sunn hemp had no significant effect on the density and fresh biomass of the population of earthworms in the field. Four species of earthworms were found in my study: *Pontoscolex* sp., *Pontoscolex corethrurus and Pontoscolex spiralis. Pontoscolex* sp. was the most abundant species with the highest density and fresh biomass per m^2 in jack bean and sunn hemp at the growing rounds. I conclude that these are common species of this area and adapt well in agricultural land and resist to environmental and anthropogenic disturbances. I suggest other studies to be conducted with the same approach of studying the effects of those cover crops on the community of earthworms in different seasons as a confirmation of this study. Future studies would need to take into consideration anthropogenic and natural factors that can affect or influence species richness and distribution in the area.

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General conclusions and recommendations for future direction

Previous studies have already shown some benefits provided by jack bean and sunn hemp in tropical regions but the evaluation of the performance of jack bean and sunn hemp is important to better integrate into the farming systems in Puerto Rico and other regions. My findings showed that plant species had significant effect on plant density and height, and canopy cover. Sunn hemp grew taller and denser in plots than jack bean meanwhile jack bean had greater coverage than sunn hemp. Plant species had no significant effect on percentage of germinated seeds and seedling vigor. Second, jack bean and sunn hemp had no significant effect on soil properties: electrical conductivity, dehydrogenase activity, dielectric permittivity, soil aggregate stability, soil bulk density, soil pH, soil strength, soil temperature, soil texture, total Carbon and Nitrogen, volumetric water content and water-holding capacity. Except treatment jack bean 2 had significant effect on soil temperature during the second growing round. My expectation was to see greater changes in the soil attributes evaluated in this study based on the findings of previous studies on jack bean and sunn hemp. I assume that climate condition, soil texture, study period and time spent in the field could influence the findings. Lastly, findings showed jack bean and sunn hemp did not have significant effects on the density and biomass of earthworm populations. *Pontoscolex* sp, *Pontoscolex corethrurus*, *Pontoscolex spiralis and Amynthas* or *Polypheritima* were sampled in this study but *Pontoscolex* sp was the most abundant species. I suppose that these three species are resistant to environmental and anthropogenic disturbances. In addition, jack bean and sunn hemp were adapted and performed well in the field; however, jack bean showed few important advantages. Jack bean was more resistant to disasters and attains rapidly an important cover at initial growth. Therefore, jack bean could be a better option due to its important biomass and cover produced in a short period.

Recommendations and future direction of this study

Based on the findings of the current study, I suggest forthcoming studies on jack bean and sunn hemp emphasize these three recommendations: (1) jack bean and sunn hemp need to be planted at different seasons to evaluate its long term effects or changes on the soil properties, (2) jack bean and sunn hemp need to be sowed at different densities in the field to assess the effect of density on canopy cover and soil properties and (3) the seasonal effect of jack bean and sunn hemp planting need to be investigated on earthworm populations. I observed during the second growing cycle the number of collected earthworms have increased even in control plots. There is also a need to further this research on these two plants species to determine its effects on soil respiration (CO_2), Cation exchange capacity (CEC), erosion control and other ecosystems services.

Glossary

1. **Experimental design** is a plan for assigning experimental units to treatment levels and statistical analysis associated with the plan. The design of an experiment involves a number of inter-related activities
2. **Experimental unit** is the person or object that will be studied by the researcher. This is the smallest unit of analysis in the experiment from which data will be collected.
3. **Factor** is a controlled independent variable ; a variable whose levels are set by the experimenter. A factor is a general type or category of treatments. Different treatments constitute different levels of a factor.
4. **Treatment** is something that researchers administer to experimental units. For example, a cornfield is divided into four parts, and each one is treated with different fertilizer to see which produces most corn.
5. **Randomization** is the process by which experimental units (the basic objects upon which the study or experiment is carried out) are allocated to treatments; that is, a random process and not by any subjective and hence possibly biased approach.
6. **Randomized Complete Block Design** is a design in which the subjects are matched per a variable which the experimenter wishes to control. The subjects are put into a group or block of the same size as the number of treatments.
7. **Blocks** are groups of experimental units that are formed to be as homogeneous as possible with respect to blocks characteristics.
8. **Soil sampling** is defined as a technique that combines several discrete samples collected from a body of materials into a single homogenized sample for analysis. The objective of composite soil sampling is to represent the average conditions in the sampled body of material.
9. **Surface runoff** refers to the water leaving an area of drainage and flowing across the land surface to a point of lower elevation.

10. **Infiltration rate** is the velocity at which the water can seep into the soil. It's commonly measured by the depth (mm) of the water layer that the soil can absorb in an hour.

11. **Soil strength** is the capacity of the soil to resist deformation and refers to the amount of energy required to break apart aggregates or move implements through the soil.

12. **Soil fertility** is referred to the ability of the soil to supply essential plant nutrients and soil water in adequate amounts and proportions for plant growth and reproduction in the absence of toxic substances which may inhibit plant growth.

13. **Biomass** is biological material derived from living or recently living organisms. In the context of biomass for energy, this is often used to mean plant-based material, but biomass can equally apply to both animal and vegetable material**.**

14. **Nutrient cycling** describes how nutrients move from the physical environment into living organisms and subsequently are recycled back to the physical environment.

15. **Soil respiration** is a measure of carbon dioxide (CO_2) released from the soil from the decomposition of soil organic matter by soil microbes and respiration from plant roots and soil fauna.

16. **Water holding capacity** refers to the amount of water held between the field capacity and the wilting point.

17. **Soil temperature** is the measurement of warmth in the soil. It is the factor that drives germination, blooming, composting, and a variety of other processes.

18. **Soil productivity** is the inherent capacity of the soil to support the growth of specified plants, plant communities or a sequence of plant communities. Soil productivity may be expressed in terms of volume or weight/ unit area/year, percent plant cover, or other measures of biomass accumulation.

19. **Soil bulk density** is an indicator of soil compaction and soil health**.** It affects infiltration, rooting depths/restrictions, available water capacity, soil porosity, plant nutrient

availability, and soil microorganism activity, which influence key soil processes and productivity. It is the weight of dry soil per unit of volume typically expressed in gram/ cm^3.

20. **Soil moisture** is the water that is held in the spaces between soil particles. Particularly, it is the water content in the unsaturated zone of a soil profile.
21. **Cover crop** is a crop planted mainly to control soil erosion, soil fertility, soil quality, water, weeds, pests, diseases, biodiversity, and wildlife in an agroecosystem (Lu, 2000).
22. **Jack bean (*Canavalia ensiformis*)** belongs to the family of the Leguminosae and has its origin in the western part of India and Central America. Jack bean is an annual legume with climbing or bushy growth forms. As a cover crop, jack bean produces phytochemicals that act as a pesticide, bactericide, and a fungicide (Akande, 2016).
23. **Sunn hemp (*Crotalaria juncea L.*)** is a tall growing (1-3m) herbaceous annual, that is probably native to Indo-Pakistan subcontinent. Sunn hemp is widely grown in tropical regions as a cover and /or green manure crop (Mojidis et al., 2011).
24. **Seedling vigor** is a measure of the increase of the plant growth or the foliage volume after planting or sowing.
25. **Plant Density or plant spacing** describes the amount of space left between plant when planting field experiments or garden and another landscaping plant.
26. **Canopy cover estimation** is the percentage of ground surface covered by the vegetation or other coverages including rocks, litter, moss or bare ground. It also estimates the area of influence of the plant.
27. **Weed control** is the botanical component of pest control, which attempts to stop weeds, especially noxious or injurious weeds, from competing with desired flora and fauna, this includes domesticated plants and livestock, and in natural settings, it includes stopping non-

local species competing with native, local, species, especially so in reserves and heritage areas.

28. **Percentage of germination** is an estimate of the viability of a total of seeds. The equation to calculate germination percentage is GP = seeds germinated/total seeds x 100.