

**Plant diversity and vegetation structure as factors correlated with parasitoid wasp
(Hymenoptera: Braconidae and Ichneumonidae) diversity in a degraded dry forest
ecosystem in Puerto Rico**

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ABSTRACT

Tropical dry forests worldwide have a long history of human use, invasion by non-native species and degradation. In Puerto Rico, while overall forest cover has increased due to socioeconomic and land use changes over the past decades, recovery of tropical dry forest areas has been slower. Within the Laguna Cartagena National Wildlife Refuge, a site of critical habitat for migrating waterfowl, recovery of forest cover following agricultural abandonment has occurred both through natural secondary succession (primarily non-native trees) and active restoration (primarily native trees). The next step in this restoration process is the evaluation of the implemented strategies. For this reason, the aim of my research was to evaluate the relationship between the diversity of two families of parasitoid wasps (Braconidae and Ichneumonidae, which are insects that might be sensitive to changes in habitat) with the diversity of plants and plant architecture in three cover types: native trees, non-native trees and grasslands without trees. Three plots were established in each of the three cover types. In each of the plots, I installed one Malaise trap for insect sampling. For the plant sampling, I measured plant diversity and structural variables such as the number of vegetation layers in the canopy and understory, percent canopy cover percent and percent understory plant cover, and tree diameter. Analyses showed differences in parasitoids species composition, abundance and richness/individual among the different canopy cover types. In addition, partition analysis showed that beta diversity (species turnover between canopy cover types) is the most important component for parasitoids regional diversity. I also found differences in plant species composition among the types of coverage as well as average tree diameter. Meanwhile, canopy percent cover, and vegetation percent cover in the understory were also different among treatments. With non-metric multidimensional scaling I found that most parasitoids are associated with forested sites,

particularly with the areas having native tree canopy cover. The variables that best explained this result were: number of layers of trees, tree percent cover and tree species richness. These results indicate the importance of tree coverage for the community of parasitoid wasps. This coverage may be providing a variety of shelters and hosts in contrast to grasslands. In addition, these results point to the relevant role of the studied fragment in maintaining the parasitoid wasp community and I recommend an extension of active restoration efforts in the remaining grasslands in order to stimulate the movement of these insects between large areas of the refuge.

RESUMEN

Los bosques secos tropicales del mundo tienen una larga historia de uso humano, invasión por especies no nativas y de degradación. En Puerto Rico, mientras que en general las áreas de bosque han incrementado debido a cambios socioeconómicos y cambios del uso de la tierra en la isla, la recuperación de las áreas de bosques secos tropicales ha sido más lenta. Entre éstas, el Refugio de Vida Silvestre Laguna Cartagena es un hábitat crítico para aves acuáticas migratorias que ha recuperado cobertura de bosque después del abandono de actividades agrícolas, posterior sucesión secundaria (principalmente por árboles no-nativos) y restauración activa (principalmente por el cultivo de especies nativas). El siguiente paso en este proceso de restauración es la evaluación de las estrategias implementadas. Por esta razón, el objetivo de este trabajo de investigación fue evaluar la correlación entre la diversidad de dos familias de avispas parasitoides (Braconidae e Ichneumonidae, que son insectos que pueden ser sensibles a cambios en el hábitat) con la diversidad de plantas y la arquitectura vegetal en áreas con cobertura de árboles nativos y no nativos, además de pastizales. Para esto, se establecieron tres parcelas en cada uno de los tipos de cobertura. En cada una de estas parcelas se instaló una trampa Malaise para el muestreo de insectos. Por otro lado, en el muestreo de plantas se midió la diversidad de las plantas, además de variables estructurales como la cantidad de estratos de plantas en dosel y sotobosque, porcentaje de cobertura de plantas en dosel y sotobosque, además de DAP (diámetro a la altura del pecho). Los análisis indican que hay diferencias en la composición de especies, y en la abundancia y riqueza/individuo de parasitoides entre los diferentes tipos de cobertura vegetal. Por otro lado, el análisis de partición de la diversidad mostró que el componente más importante para la diversidad regional de parasitoides es la diversidad beta (recambio de especies

entre los diferentes tipos de cobertura). Las plantas presentaron también diferencias en la composición de especies entre los tipos de cobertura y, en términos de estructura se encontraron diferencias en el DAP y en la cobertura de herbáceas entre algunos de los tipos de cobertura. Con un “NMDS” (escalamiento no-métrico multidimensional) se encontró que la mayoría de los parasitoides se encuentran asociados con las parcelas con cobertura de árboles, en particular con las parcelas con cobertura de árboles nativos. Las variables que mejor explican esta asociación son: la cantidad de estratos de árboles, porcentaje de cobertura de árboles y la riqueza de especies de árboles. Se discute la importancia de la cobertura para el mantenimiento de la comunidad de avispas parasitoides, debido que ésta puede estar otorgando una variedad de refugios y hospederos que no ofrecen los pastizales. En adición, se remarca la importancia del fragmento estudiado para el mantenimiento de esta comunidad de avispas parasitoides y se recomienda una continuación de los esfuerzos de restauración activa para estimular el movimiento de estos insectos entre diferentes áreas del refugio.

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DEDICATION

I dedicate this work to my grandfather, my mother Liliana, my brother Jonathan, my aunt
Adriana, my uncle Diego and my beautiful cousins.

I also dedicate this to Dani and all the people who have offered me their friendship and support
in this process.

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1. Introduction

Tropical dry forest (TDF) is a threatened ecosystem type that sustains high diversity of mammal, birds and major insect groups (butterflies, moths, wasps and ants) and a substantial number of plant species. Despite being less rich and less floristically complex than wet forests, dry forests have elevated values of endemism and support a considerable diversity of life history strategies and species interactions (Murphy and Lugo 1986, Janzen 1988, Portillo-Quintero and Sánchez-Azofeifa 2010), attributes that make this type of ecosystem an important target in conservation. Furthermore, TDF is one of the most suitable areas for human settlements, consequently these habitats have suffered high rates of deforestation. Therefore, any management strategy to prevent or diminish deforestation in TDF will contribute to the overall mitigation of deforestation and loss of biodiversity in the tropics (Portillo-Quintero and Sánchez-Azofeifa 2010).

At the end of the 1980's, TDF covered approximately 42% of the total area of earth's tropical and subtropical forests (Murphy and Lugo 1986). However, these habitats have been associated with dense human populations and have been susceptible to different types of land exploitation due to their climate, fertile soils and the suitability for livestock (Murphy and Lugo 1986, Janzen 1988). Consequently, by the beginning of this decade, this biome had the third highest estimated area of gross forest cover loss, with an estimate of 204,000 km² of the total cover removed mainly by agro-industrial scale clearing and a remaining area of 1,048,700 km² (Miles et al. 2006, Hansen et al. 2010). Of this area, approximately 54% can be found in the Americas. Of these Neotropical dry forests, 39% are located in North and Central America, 51%

are found in South America, and 9% are located in the Caribbean islands (Portillo-Quintero and Sánchez-Azofeifa 2010).

On the island of Puerto Rico TDF occupies approximately 1200 km² (Brandeis et al. 2007). These forests, like others in the West Indies, have gone through a process of disturbance by human activities since 16th century (Ramjohn 2012). In Puerto Rico in particular, forest areas were lost as a consequence of cane sugar production, cattle grazing, logging and the establishment of subsistence crops as shade coffee, tobacco, and some ornamentals (Weaver and Schwagerl 2008). However, between 1950 and 1990 there was approximately a 60% decrease in agricultural activities on the main island, and a considerable recovery of some types of forest areas (more than 20%) by the end of the same century (Rudel 2000, Helmer 2004).

Among the different types of habitats on the island, tropical dry forests had a slower rate of recovery for two main reasons: 1) they have more urban development and smaller proportion of protected areas (Helmer 2004); and 2) dry forests have stressors such as invasive non-native species, in particular several species of grasses (Thaxton et al. 2012). This type of vegetation may limit native plant regeneration by promoting fires and enhancing water stress on native species (Wolfe and Van Bloem 2012). Despite these limitations, TDF in Puerto Rico retains a significant percentage of plant diversity and structure and currently is composed of numerous small fragments, which are critical to help mitigate effects of fragmentation and deforestation (Lugo and Helmer 2004, Ramjohn et al. 2012).

Laguna Cartagena National Wildlife Refuge (LCNWR) is a dry forest reserve in Puerto Rico that passed through the described disturbance and recovery process. At the time of the arrival of Spanish explorers at the end of the 15th century, this place was an important waterfowl

habitat surrounded by native vegetation typical of tropical dry forests. However, at the beginning of the 20th century this forest began to be reduced as agricultural activities increased, transforming all the cultivable areas to sugar cane and pastures for cattle grazing. By the 1950's extensive irrigation systems were constructed, and the land was enriched with external nutrients leading to the extensive establishment of the highly competitive species such as *Typha dominguensis* (Weaver and Schwagerl 2008, Díaz 2011) and reduction of open water in the lagoon. Nevertheless, in the last 60 years agriculture activities have decreased throughout Puerto Rico due to industrialization and its consequent socioeconomic changes, which have led to the abandonment of agricultural lands and recovery of forest cover through secondary succession (Grau et al. 2003, Brandeis et al. 2007).

In 1989, the US Fish and Wildlife Service established Laguna Cartagena as a refuge as an initiative to help rehabilitate the Lagoon's avian fauna, and restore and preserve this forest ecosystem (Díaz 2011). Since then, passive and active rehabilitation strategies have been employed to improve the habitat quality. Passive restoration has meant promoting secondary succession through control of recurrent fires and livestock grazing, while active restoration has focused on the removal of exotic species and native tree planting in open areas (DellaSala 2003, Weaver and Schwagerl 2008). As a result, LCNWR is currently covered with sections of exotic (e.g. *Urochloa mutica* (Forssk.) T.Q. Nguyen) and native grasses (e.g. *Paspalum conjugatum* P.J. Bergius; Más and García-Molinari 2006), and patches of secondary forest composed of exotic and native species and patches of native tree plantings (Weaver and Schwagerl 2008, 2009).

The augment of forest cover in this ecosystem has been a key step to recover ecosystem processes and habitats (Grau et al. 2003) and is part of the overall restoration process, which aims to retrieve the ecological integrity in the target site. An important next step in the

restoration process is the evaluation of success in the strategies employed (DellaSala et al. 2003) in Laguna Cartagena NWR. This can be done through the measurement of different ecosystem attributes such as the diversity of different taxa (e.g. plant and arthropods) and vegetation structure (Ruiz-Jaen and Aide 2005a, b). These attributes are the ones most commonly used in the assessment of restoration success (Ruiz-Jaen and Aide 2005b) and are intimately related, since changes in vegetation structure can generate consistent changes in diversity of some groups through bottom-up forces. This occurs due to the fundamental role of plants in food webs, because they can affect higher trophic levels through their biomass, primary productivity, secondary compounds and architecture (Power 1992, Denno et al. 2002), consequently influencing the assembly of different taxa, such as arthropods. Within these arthropod assemblages, herbivores will be affected by plants because they depend directly upon them as a source of food and shelter. In turn, if the density of herbivores varies due to resource availability, this will consequently affect the survival and reproductive success of predators and parasites that rely upon this type of prey (Lassau et al. 2005).

The fact that the variation of different plant community attributes (diversity, vegetation structure or biomass) could generate a cascading effect on arthropods, makes them useful as habitat dependent model organisms (Lassau et al. 2005, Schaffers et al. 2008). Within this phylum, insects have been widely used in conservation work, particularly in the characterization of habitats and the assessment of restoration success (Andersen et al. 2002, Pik et al. 2002, Ruiz-Jaén and Aide 2005b). There are several attributes that make them attractive for such an approach: (1) they constitute a significant proportion of all the diversity and biomass on the planet, (2) play an important role in ecosystem processes, and (3) some of these groups of organisms are susceptible to changes in habitat (Kim 1993, Samways 1993, McGeoch 1998,

Samways 2007). As a result, measures of habitat complexity joined with biodiversity surveys of some insect taxa may provide land managers with useful perspectives for making more accurate decisions involving biodiversity and conservation (Pik et al. 2002, Lassau et al. 2005).

Among insects, Hymenoptera is one of the richest orders, together with Coleoptera, Lepidoptera and Diptera (Mason and Huber 1993). This order contains a great diversity of life strategies, and consists of organisms like ants that dominate tropical ecosystems and influence the dynamics of soil and trees, as well as pollinators, predators, herbivores and parasitoids (Sharkey and Fernández 2006). Besides, many of the species in this order are sensitive to disturbances in the environment and consequently susceptible to extinction (Shaw and Hochberg 2001). For example, parasitoid wasps are more susceptible to habitat fragmentation compared to their herbivore hosts. In particular, the extent of isolation between fragments exert negative effects on their diversity and parasitism rates (Kruess and Tscharntke 1994), factors that can lead to smaller populations with increased risk of extinction.

Parasitoids are classified as consumers of the third and fourth order (Elzinga 2004), because they require a host (an arthropod) to meet short-term nutritional needs and complete their life cycle (Lewis et al. 1998). In general, their life strategy is based on finding a host on which to lay their eggs. When those eggs hatch, the emerged larvae will feed on its host upon killing it (Shaw 2006). Having this type of life strategy, parasitoids depend directly on the availability of hosts which in turn depend on the availability of plants (Haddad et al. 2001). This group can be considered one of the best indicators for biodiversity studies, since their diversity tends to reflect the host diversity. In addition, they are sensitive to ecological disturbances such as habitat fragmentation (Kruess and Tscharntke 1994) and the use of insecticides (Price and

Schuster 1991). As a result, it is sometimes possible to detect population fluctuations before they occur in the host populations (Sharkey & Fernández 2006).

According to the mentioned characteristics, parasitoid wasps are good candidates to provide useful information about the restoration process implemented in Laguna Cartagena Wildlife Refuge. Consequently, the aim of this research project was specifically to assess how the changes in plant species richness or structural diversity (in terms of the number of leaf layers in each strata and tree diameter) could be correlated with the diversity of two families of parasitoid wasps (Braconidae and Ichneumonidae) in sites with native and non-native tree canopy cover (either native or non-native), or without canopy cover, through one year of sampling with malaise traps.

1.1 Literature Review

1.1.1 Disturbance and restoration history in Laguna Cartagena

The Laguna Cartagena Wildlife Refuge (LCNWR) has passed through a process of recovery of vegetation after an agricultural and cattle grazing history prior to the 1980's. This recovery has resulted both from an ecological succession process (Horn 1974) and restoration activities (Weaver & Schwagerl 2008). As a result of a history of intensive degradation, non-native species dominate the plant community presently on the site. In order to jumpstart succession of native species it has been necessary to apply restoration strategies such as invasive species removal and planting of native trees. Consequently, during the 1990's a grassland management plan and a reforestation plan were developed for this site (Schaffner 1995a, b, cited by Weaver & Schwagerl 2009). From 1980 to 2001, more than 9000 trees representing 78 species were planted on the refuge for habitat restoration (Weaver & Schwagerl 2004, cited by Weaver & Schwagerl 2009). At present, 30 years of natural succession and restoration plantings have resulted in a site with areas of complete canopy cover (native and/or non native), with a dense understory and a complex vegetative structure (in terms of height, foliage layers and basal area).

After a restoration process has been initiated, it is necessary to evaluate the success of the management plan implemented through the assessment of biotic diversity, vegetation structure, and ecological processes (e. g. biotic interactions and population dynamics) that reflect the recovery trajectory and identify the level of sustainability of the restored ecosystem. Ruiz-Jaen & Aide (2005a) indicate that the most commonly used measures for the recovery of diversity are

plant and arthropod richness. In addition, to assess the changes in vegetation structure, the most commonly used measures are plant cover, density, biomass, and height.

1.1.2 Restoration process and evaluation of restoration success

In the tropics, deforestation and expansion of agricultural activities are among the main sources of anthropogenic disturbances. These transform the landscape, generating a mosaic of patches that alter the habitat available for most of the species, isolating them and usually leading to a reduction in biodiversity and changes in species composition (Dobson et al. 1997, Lugo & Helmer 2004, Samways 2007). However, the induced changes may be either slowed or reversed partially through ecological restoration, i.e. the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (Society for Ecological Restoration 2004). A variety of restoration approaches may be employed to overcome specific barriers that may include altered soil conditions, lack of native propagules, and competition with exotic species, among others. All of these approaches have as their final objective to increase structure and function of the disturbed landscape (Dobson et al. 1997).

The forests of Puerto Rico provide a test case of the capacity for passive restoration (i.e. recovery through natural secondary succession) following anthropogenic disturbance to return an ecosystem to its pre-disturbance state. This process has occurred on forest lands that have experienced a cycle of deforestation, agricultural use and abandonment (Lugo & Helmer 2004). In both mesic and wet zones, forest structure recovers after <50 years of natural succession, although these forests differ in species composition from old forest (Aide et al. 2000). In drier areas, forest recovery is marked by dominance of non-native pioneer species and a high prevalence of invasive grasses that can delay succession (Wolfe and Van Bloem 2012).

1.1.3 Effect of habitat on insects and other arthropods

Insect diversity and abundance are often positively correlated with taxonomic and functional diversity of plants. However, these vegetation attributes may have differential effects on the arthropod community, depending upon the trophic group. Siemann et al. (1998) found that the taxonomic diversity of plants influenced directly herbivores and predator richness, but it had an indirect effect on the parasitoid species richness. Similar results were found by Haddad et al. (2001) with respect to the positive relation of plant richness with herbivore and predator richness. However, these authors additionally found that parasitoid abundance was positively related to plant biomass and the richness of functional plant groups.

On the other hand, insect diversity and distribution could also be influenced by other characteristics of plant communities, such as structural complexity, nutrition, or plant productivity (Wenninger & Inouye 2008). Previous experimental and field studies have evaluated the correlation among habitat complexity and the presence of different arthropod taxa, including spiders and insects (e.g. beetles, herbivores and parasitoids). Schaffers et al. (2008) compared the predictive values of various factors for the species composition of arthropod assemblages (insects and spiders) within 47 sites in the Netherlands. They measured environmental conditions, plant species composition, vegetation structure and flower richness as determinants at both the local and landscape level. They found that the plant composition was the best predictor of all studied arthropod groups, rather than the vegetation structure or environmental conditions. In contrast, for ground beetle species, a powerful predictor of richness was the complexity of habitat, in terms of five habitat characters: tree canopy cover, ground herb cover, amount of leaf litter and the soil moisture (Lassau et al. 2005).

Corcuera et al. (2008) evaluated the influence of plant architecture on the spider community in a tropical dry forest in Mexico. They analyzed the abundance of foliage spiders and four plant attributes (foliage area, number of leaves and number of branchlets and plant cover). The foliage spider species were positively influenced by small-leaved trees and shrubs with a higher number of leaves and branches, and negatively by broad-leaved plants with a high foliage area. In other organisms, like trap nesting bee and wasps, the vegetation structure and the heterogeneity of specific attributes of habitat structure generate strong responses in terms of species richness and abundance on a local scale. The most effective predictors for the richness in these groups of Hymenoptera were heterogeneity in trunk circumference, abundance of wood logs and shrub height (Loyola and Martins 2008).

On the other hand, the spatial arrangement of the habitat can influence activity of functional groups like parasitoid insects. Roland (2000) argues that this characteristic, as well as the arrangement of patches and the quality of the matrix that separate the community of parasitoid insects, could explain the spatial differences in the rate of parasitism in forest stands. Another habitat attribute generating effects in the structure and functionality in insect communities in disturbed sites, besides the vegetation structure and heterogeneity, is the level of isolation. To prove what type of response generates this property of the landscape, Kruess & Tscharrntke (2000) analyzed the effects of fragmentation on endophagous insects (herbivores and parasitoids) inhabiting the pods of bush vetch (*Vicia sepium*) in terms of the abundance of species and the interaction between the two groups. They found that species diversity from the herbivores and the parasitoids increased with habitat area. This was supported by the field experiment, in which the isolation was a relevant factor negatively influencing the abundance of both groups and the interactions between them.

1.1.4 Parasitoid wasps

The Hymenoptera represent one of the most diverse and abundant insect orders, with more than 120,000 described species and an estimate of 300,000 species worldwide (Campos 2001). Members of this group have a profound and often highly specialized interaction with other organisms, resulting in a predominant role in the maintenance of the diversity of other insects and plants (Shaw & Hochberg 2001). Among the different lineages within Hymenoptera, the parasitoids (Orussoidea, Stephanoidea, Ichneumonoidea, Cynipoidea, Proctotrupeoidea, Chalcidoidea, Trigonoidea, Megalyroidea, Ceraphronoidea and Evanoidea; Whitfield 1998) are one of the most diverse groups with exceedingly high levels of species richness. They comprise 75% of all species of the suborder Apocrita, accompanied by an equally high level of diversity in life-history strategies.

These insects are dependent on other organisms to complete their life cycle and are located in the third and fourth trophic level (Shaw & Hochberg 2001; Townsend et al. 2003). Specifically they develop as parasitoids of other arthropods during their immature stages, but are free-living as adults. The female wasp starts the cycle laying her egg or eggs inside, outside, almost always attached to, or in close proximity to the egg, larva, pupa, nymph, or even the adult of the particular prey that her larvae need to develop. The parasitoid larva proceeds to consume its host in various ways that are usually fatal to the host. When fully grown, the parasitoid larva pupates and finally emerges as another adult parasitoid (Mason & Huber 1993; Strand 2000).

Depending on the particular group of parasitoid wasps and the manner in which they parasitize their hosts, they can be classified as having one of two different life strategies, koinobiont or idiobiont. Koinobiont species generally attack mobile and growing host stages,

thereafter developing endoparasitically over a prolonged period within the live host. In contrast, idiobiont adult females usually paralyze or kill immobile host stages (i. e. eggs and pupae), and their larvae subsequently exploit the host as an ectoparasitoid (Strand 2000). However, it is relevant to note that this dichotomy has exceptions regarding the development of the wasp larvae in the host, since endoparasitic idiobionts wasps and ectoparasitic koinobionts can be found (Shaw 2006).

Due to their complex life strategy and the dependence of the presence of hosts to complete their life cycle, adult parasitoid activity and survivorship is strongly dependent on factors like the availability of hosts for oviposition and availability of energetic resources (nectar resources) (Tscharnkte 2000). These characteristics make them vulnerable to changing conditions in the habitats (in terms of plant diversity and structure) (Fraser et al. 2008b) and prone to affect the diversity of other insect taxa from a top-down perspective. Hence, it is important to point out that destroying one parasitoid species could have large and unpredictable impacts on the number and abundance of phytophagous insects with possible cascade effects on the ecosystem (Lasalle 1993, cited in Sharkey & Fernández 2006). The use of insects has been common in conservation approaches. Due to their short life cycle, insects can serve as focal indicator taxa for rapid assessment of changes in Neotropical forest systems (McGeogh 1998, Lobry 1999, Vasconcelos 2000, Brown 2004, Samways 2007, New 2009).

2 Materials and methods

2.1 Study site

Laguna Cartagena National Wildlife Refuge (18° 0'7.73"N and 67° 6'16.43"W, Google Earth 2013) is a subtropical dry forest (Ewel & Whitmore 1988) located in the municipality of Lajas in Puerto Rico (Figure 1). It has been established as a protected area since 1989 for the conservation and management of fish and wildlife resources according to 16 U.S.C.742f (a)(4), and is managed by the U.S. Fish and Wildlife Service (Earsom et al. 2004, cited by Weaver & Schwagerl 2009). The site is partitioned into two major tracts: Lagoon and Tinaja (acquired in 1996 by U.S. Department of Agriculture Farm Service) (Weaver & Schwagerl 2009). It has different types of vegetative cover, including wetlands (41.92%), grasslands (29.71%), forest (9.97%), shrublands-woodland (9.21%) and shrublands (9.14%) (Weaver & Schwagerl 2008).

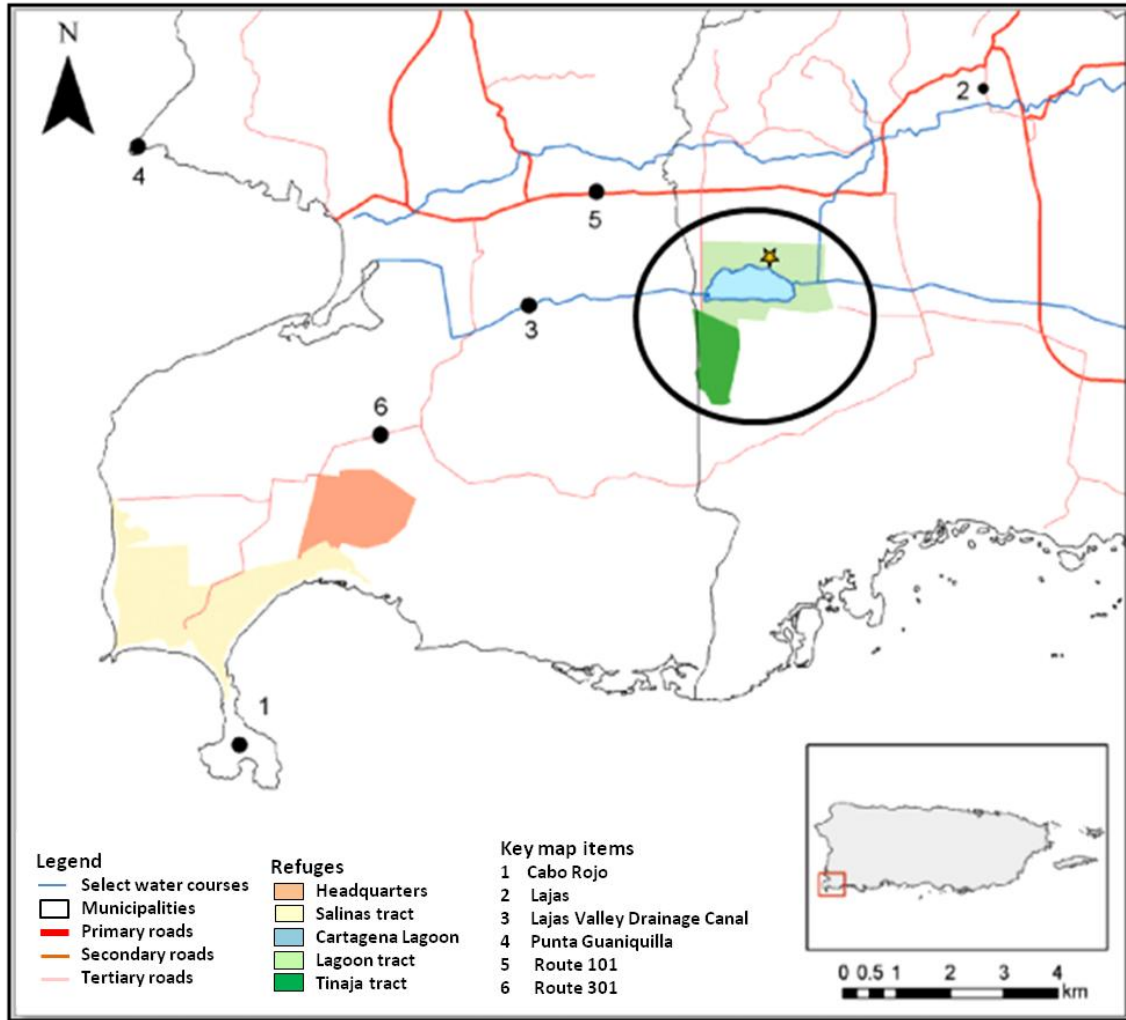


Figure 1. Location of the Laguna Cartagena U. S. Wildlife Refuge in Lajas Municipality (Puerto Rico) (figure modified from Weaver and Schwagerl 2008). The refuge is circled and the study area inside the refuge is marked by a star.

The Lagoon tract (Figure 1) contains 317 ha across an elevation range of 11 m to 40 m. It is composed of a freshwater lagoon, grass and scattered trees, with a total of 189 plant species (Weaver & Schwagerl 2004, cited by Weaver & Schwagerl 2008). The climate is characterized by a bimodal annual rain cycle, with the driest months in February and March and the wettest in

May and September (Figure 2, Source: Lajas Substation Puerto Rico) and a total annual rainfall average of 1958 mm/yr (Weaver & Schwagerl 2009).

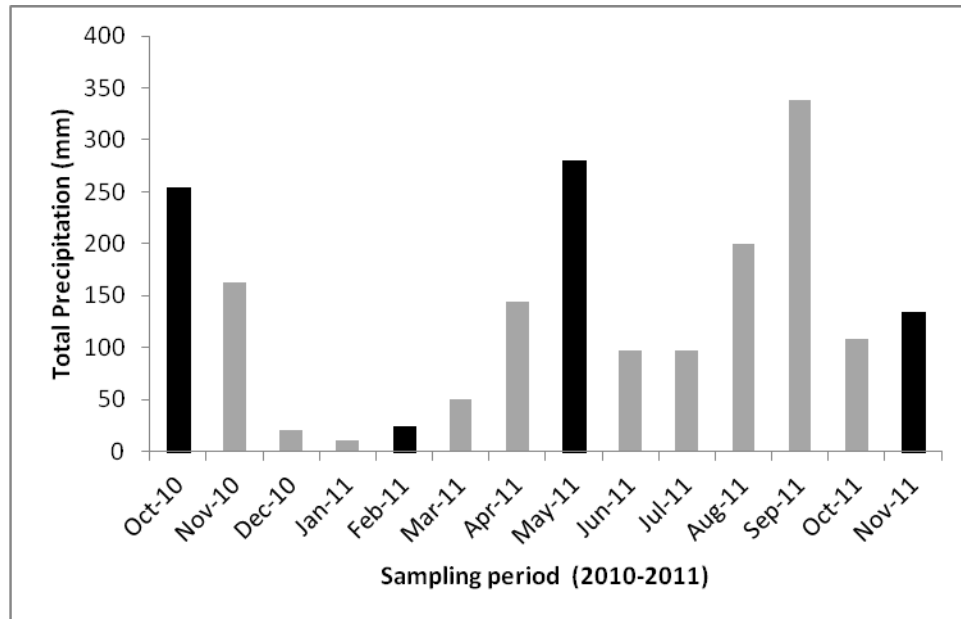


Figure 2. Total precipitation per month during the sampling period (between October 2010-November 2011). Black bars represent the specific sampling months.

The current landscape is a result of past disturbance and subsequent recovery. The Lagoon tract in the 1920's supported heavy grazing and continuous cropping, mainly with sugar cane (Weaver & China 2003, Weaver & Schwagerl 2008). In the present time, this place is undergoing secondary succession and a restoration process due to the planting of native tree species in open sites and the control of fire (Weaver & Schwagerl 2008). As a result, the current habitat is a combination of pastures interspersed with native and exotic trees, patches of secondary forest, and tree plantings of various species (Weaver & Schwagerl 2009).

The Lagoon tract has three distinct areas: (1) the lagoon, centrally located, with its aquatic vegetation, (2) a recently abandoned sugar cane plantation, now planted with trees, and

(3) an abandoned pasture with naturally occurring trees and several patches of planted trees. The highest plant diversity corresponds to dicotyledonous plants with 252 species, including 42 of nitrogen fixing species, followed by 60 species of monocotyledonous species and 43 species of planted trees (Weaver & Schwagerl 2009).

2.2 Sampling design

Sampling was carried out in areas with three different types of canopy cover: (1) native trees canopy cover, (2) non- native trees canopy cover and (3) absence of canopy cover (grasslands) (Figure 3). In each of these sites, I established three plots of 20 m x 20 m, being careful to locate them at least 10 m from an edge, due to the particular conditions that this portion of the fragment may have and the biases that these could represent in the parasitoid wasps collection (Fraser et al. 2008a).

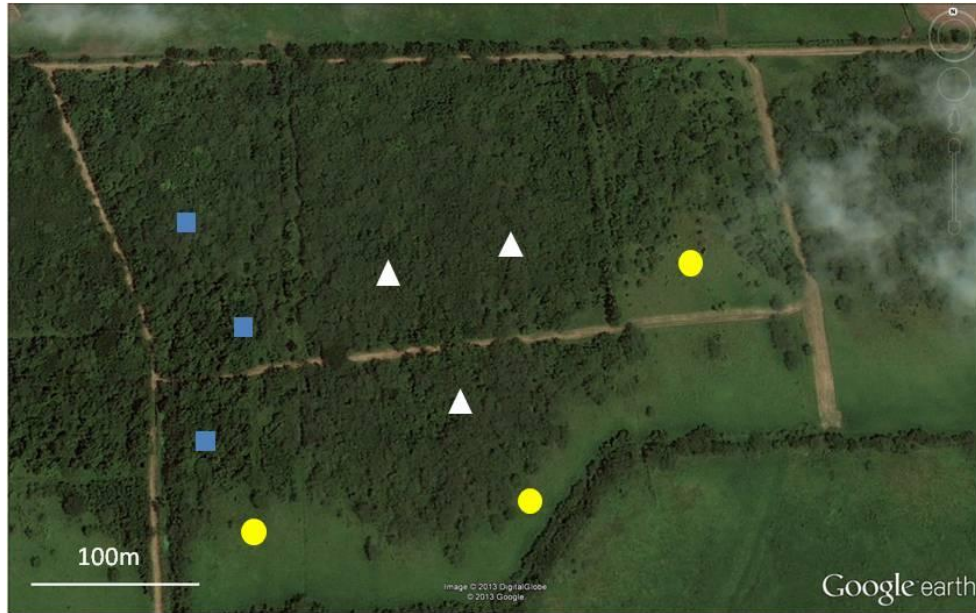


Figure 3. Sampling area in the lagoon tract of Laguna Cartagena Wildlife Refuge. The area consisted of three different cover types, with 3 plots within each type: forested areas with restored native trees (squares), non- native trees canopy cover (triangles) and grasslands (circles).Vegetation and parasitoid wasp sampling were performed on these plots. This image was modified from Google Earth (2013).

2.3 Materials and methods: wasps

Malaise traps were employed to collect parasitoid wasps. This is a type of interception trap designed to capture flying insects. It is the most widely used for Hymenoptera surveys, because it is an efficient method that permits large catches of individuals (Noyes 1989, Sarmiento 2006, Fraser et al. 2008a). The traps employed (model 2875AG, www.bioquip.com) were green, with a square configuration (2.13 m tall and 1.21 m on the sides) and four central

vanes to stop the flight of insects. The collector head was about 21.59 cm high x 15.24 cm in diameter, and adjusted into the socket on the top of the traps. The head contained approximately 1 liter of alcohol.

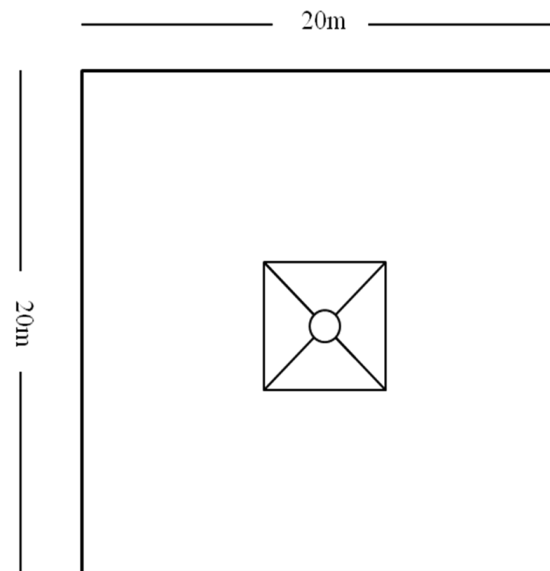


Figure 4. Model of the plots established in each of the three types of canopy cover. All the plots covered an area of 20m x 20m. In the center of each plot, a Malaise trap was located to collect the parasitoid wasps.

I used one Malaise trap per plot (Figure 4), and each trap was maintained in the field permanently. Samples were collected from the traps for two week periods in each of the following dates: October 2010, February 2011, May 2011 and November 2011, comprising approximately 56 days/trap as the total field collection effort (Table 1). During the 2 week sampling period, I changed the heads every seven days. During this period I planned to cover two dry seasons and two rainy seasons to have a representation of the climatic conditions in the

area throughout the year. However for the data analysis I did not take into account this division because no significant differences were found in the total precipitation on the sampling months (comparison based on a t-test, $p = 0.0607$).

Table 1. Sampling dates for parasitoid wasps collection with malaise traps.

Sample	Date
1	October 1st-10 th
	October 15th-24th
2	February 11th-24th
3	May 6th-13th
	May 27th-June 3rd
4	October 28th- November 12th

All the trap samples were collected in 95% alcohol, and stored in a freezer for identification at the UPRM Invertebrate Collection (UPRM-INVCOL; see Franz & Yusseff Vanegas 2009). I sorted all insect samples to extract all the parasitic Hymenoptera, and then selected the Braconidae and Ichneumonidae wasps. For each family I identified individuals to the level of morphospecies based on: 1) body shape, 2) body coloration patterns, 3) wing venation, 4) sutures, 5) shape and arrangement of the antennae segments.

2.3.1 Data analysis

Richness estimators and species accumulation curves

To evaluate the efficiency of sampling I made species accumulation curves with EstimateS 7.5 ® (Collwell 2005). The curves were constructed with several estimators, including the nonparametric estimator Chao1. This estimator calculates the absolute numbers of species present in an assembly taking into account species abundance, and it is a function of singletons (unique species) and doubletons (species with two individuals) ratio. In addition I employed Chao 2, which is adequate for presence-absence data; this richness estimator is quite robust and it is not biased by small samples (Lewis and Whitfield 1999, Villareal et al. 2006). I also added the estimation curves for doubletons, singletons in addition to the observed richness (Sobs) (Magurran 2004).

Richness, abundance and diversity

I calculated the number of species (richness) and the number of individuals (abundance). To avoid the effect of abundance in the calculation of richness, I calculated richness/individual dividing the richness by the number of individuals. To compare richness between canopy cover types I employed a permutation test. On the other hand, I made the comparison of abundance values and richness/individual among treatments with a non-parametric ANOVA (Kruskall Wallis test) and a post-hoc Pairwise-comparison. Additionally, I calculated the diversity for each one of the sampling plots (spatial units) with Fisher's alpha (α) (Williams 1944, Magurran 1988). This index is widely used to compute richness and characterized by low sensitivity to sample size. It is suited for a Log-series distribution in the data, which reflects a community of species

where the number of abundant species is small and the amount of 'rare' species is large (Magurran 2004). If Fisher's α is large then it indicates that the community is more heterogeneous and has a more equitable distribution of individuals. On the other hand, if alpha is small, then it means that individuals are divided into few species and that some of these species have a greater number of individuals (Williams 1944). To compare statistically the computed Fisher's α values between the three types of canopy cover, I additionally performed a permutation test (Crist and Veech 2006).

To find out which species share the different types of canopy coverage (native, non-native tree cover and grasslands), I calculated beta diversity with the Whittaker index (β_w) (Wilson and Shmida 1984) which indicates how different is the species composition between areas of alpha diversity (Wilson and Schmida 1984, Magurran 2004). To graphically explore the differences in species composition between different treatments, I performed a Jaccard's cluster using UPGMA distance (unweighted pair-group method using averages) as a grouping algorithm. This graph is constructed using the Jaccard distance as similarity measure, and it only requires presence-absence data. This distance in particular is not influenced by the abundances of species between samples, so species with very large abundances are not going to have a greater importance in the analysis (James 1990, Kindt and Coe 2005).

The nonparametric ANOVA (Kruskal Wallis) was performed in Infostat® (Di Rienzo et al. 2011). The analyses to calculate alpha and beta diversity (Whittaker's index) were executed with the software PAST ® version 2.17 (Hammer et al. 2001). I performed the two- way Jaccard Cluster in PC-ORD version 6.0 (McCune and Mefford 2011).

Partition

The additive partition of diversity decomposes γ diversity (regional diversity) in the components α (average number of species present in a particular spatial unit) and β (species shared between different α components at the same scale), and can be expressed as $\gamma = \alpha + \beta$ (Lande 1996). Such diversity partitioning has several advantages over traditional partition ($\gamma = \alpha * \beta$). The first one is that α and β components have the same units which makes it easier to compare. Additionally, this approach is flexible because γ diversity can be partitioned based on any categorical factor such as habitat guilds, host or resources. Therefore, it can be potentially used to analyze any determinant factor of species diversity (Veech et al. 2002, Crist et al. 2003)

PARTITION ® (<http://www.users.muohio.edu/cristto/partition.htm>) is a statistical program that allows the user to assess whether the observed diversity partition differs or not from one randomly generated (Crist et al. 2003, Veech and Crist 2009). In other words, the null hypothesis states that α and β components of diversity may have been a result of the random distribution of individuals between the samples across different hierarchy levels (the lowest level would correspond to samples collected and the landscape would be the highest level) (Crist et al. 2003). To assess this hypothesis, the statistical program performs a simulation based on individual-based randomization on each of the samples found at the lowest hierarchical level, which allow the user to make comparisons between the observed diversity components on each hierarchical level (Crist et al. 2003). This randomization finally generates a distribution null data and is compared with the distribution observed (Chandy et al. 2006).

For this analysis I assumed additive partitioning of diversity instead of multiplicative. I considered 3 levels of diversity (Table 2): 1) α_1 corresponded to the diversity within each plot, 2)

β_1 represented the diversity shared between the plots (spatial units at α_1 level) and 3) β_2 (equal to $\alpha_1 + \beta_1$) indicated the turnover of species between the three types of canopy cover (Table 2). Furthermore, I employed an individual based randomization with 10000 randomizations.

Table 2. Diversity component and sample size for each of the levels taken into account for partition analysis.

Diversity component	Sample size
α_1 (alpha diversity on each plot)	3
β_1 (turnover of species between α_1 units)	9
β_2 (turnover between canopy cover types)	9

Whittaker plots or dominance/diversity curve

I generated Whittaker plots for insect species within canopy cover types. This type of graph is performed to visualize the distribution of species abundance in a community. On this representation, the horizontal axis corresponds to a range of species going from the less abundant to the more abundant ones, and the vertical axis corresponds to the relative abundance of each species. The resulting shape is an indicator of species evenness and can be also employed to infer the best species abundance model describing the data. Therefore, when the curve has a steeper slope this is an indicator of high dominance of certain species, and the data are probably more fitted to a log series or geometrical series distributions. On the other hand, if the graph has a shallower slope that means that there is a greater evenness and the data are distributed according to a broken stick model or log normal (Magurran 2004)

2.4 Materials and methods: plants

Sampling of plant community diversity and structure took place in the same 20 x 20 m plots used for insect sampling. For this part of the sampling, a grid was created within each of the previously established plots (Figure 5). This was done by locating flags in the entire plot, starting from 2m from the corner and then every 4m until the total sampling area was covered. The grid was employed to make the vegetation diversity and vegetation structure measurements.

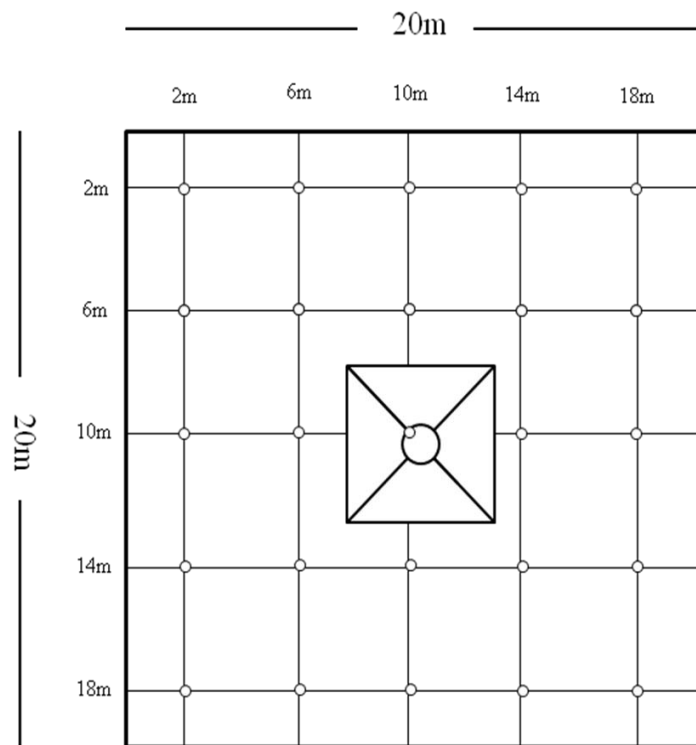


Figure 5. 20 m x 20 m grid employed for the plant sampling on each of three types of canopy cover. Each blank circle inside the grid represents a flag and a sampling point. I located a malaise trap in the center.

Understory (<2 m) plant diversity and vegetation structure were quantified using a point-intercept method. To perform this, I dropped a narrow pole at each of the 25 grid points (Figure 5), then registered each plant species that made contact with the pole within height increments (0-0.5 m, 0.5-1 m, 1-1.5m, 1.5-2 m). To sample the plants above the 2m height stratum, I used the same 25 sampling points employed for the understory plants. At each point I used a telescoping pole that was extended until it made contact with the canopy. I recorded this height and every other stratum that the pole touched until it reached the limit of the canopy or until the 12m limit (maximum height that the instrument reached). Only in the native tree canopy cover plots this limit was exceeded at 12 points (out of 75 total points in this canopy cover type). At these points, I registered the tree layer as >12m regardless of the number of layers of vegetation that were above this height.

From these data I generated an estimate of the number of leaf layers of different types of plants at different elevation strata. Taken over the entire plot, these data were used to estimate percent cover. In addition to point-intercept measures, all trees with diameter at breast height (DBH) >2 cm were counted, measured for diameter and identified to species. Together the leaf layers, cover percentage and tree diameters and density constituted the vegetation structure information. All unknown species were collected and identified by Dr. Gary Breckon (retired director at the UPRM herbarium). All the species names were defined according to Axelrod (2011).

2.4.1 Data analysis

I calculated plant species richness for each canopy cover type. These values were compared using a permutation test. On the other hand, percent cover was calculated as the percentage of sampling points of the grid (25 in total, see Figure 5) that had one or more plant species (100% - All the sampling points had plants, 0 % - No plants registered on the plot). This calculation was performed on each plot per canopy cover type for the understory (<2m), the canopy (> 2m) and for trees (>2m stratum, excluding the vines).

2.5 Correlation between parasitoid wasps and vegetation diversity and structure

To evaluate the correlation between vegetation variables (diversity and structure) and parasitoid wasp community composition, I performed a nonmetric multidimensional scaling (NMDS). This non-parametric ordination method aims to build a parsimonious representation of individuals in a low-dimensional space. This analysis initially locates objects (or individuals) randomly in an ordination space with a predetermined amount of dimensions. This action is iteratively performed, until reaching a configuration in which the stress is minimal (i.e. the best goodness of fit) (Table 3). In the final representation (the one with the lowest value of stress), the distances between objects somehow correspond to experimental dissimilarities between them (Kruskal 1964, Matthews 1978, Kenkel and Orloci 1986, Ramette 2007).

Table 3. Stress values to assess the goodness of fit of the final configuration generated by NMDS (classification according to Kruskal 1964).

Stress value	Goodness of fit
20%	Poor
10%	Fair
5%	Good
2.5%	Excelent
0%	Perfect

I performed NMDS analysis in PC-ORD (McCune and Mefford 2011), using the Sorensen (Bray-Curtis) distance measure, which is calculated based on the differences in abundance of each species (Kindt and Coe 2005). The starting coordinates were randomized and 500 iterations were performed. In addition, 250 runs were performed with real data and 250 with randomized data. The variables included in the analysis were richness of plants above 2m, richness of plants below 2m, vegetation richness (above and below 2m height), DBH, amount of vegetation strata, amount of vegetation layers above 2m, amount of vegetation layers below 2m, percent cover of herbs and trees.

3. Results

3.1 Results: Parasitoids

Richness estimators and species accumulation curves

The sampling efficiency of wasps varied between the three types of tree cover. The native tree canopy cover plots were the sites with the lowest sampling efficiency (46.98%). This can be appreciated in species accumulation curves (Figure 6-a) for several reasons: 1) there is an increasing trend in observed richness curves (Sobs) and the estimator curves for CHAO 1, ACE and CHAO 2 (expected richness), 2) these Sobs curves and the estimator curves do not approach to each other, so the observed richness is lower than that estimated by the index, 3) there is an increasing trend in the number of species with a single collected individual (singletons) and stabilization of the number of species with two individuals collected (doubletons).

On the other hand, the non-native trees plots had a greater sampling efficiency compared with the native trees plots (62.77%) (Figure 6-B). In this case, Sobs and estimator curves followed the same pattern as in the Native trees plots, so the observed richness is lower than the richness estimated by Chao 1, Chao 2 and ACE. The singletons were stabilized and doubletons followed the same pattern as native trees plots.

The highest sampling efficiency was calculated on the grassland plots (72.24%) (Figure 6-C). In this case, Sobs had an increasing tendency but Chao1 and Chao2, unlike in the other treatments, were increasing until the eighth sample, then these curves started to decrease. Additionally, on this graph the singletons reached stability and the doubletons tended to increase.

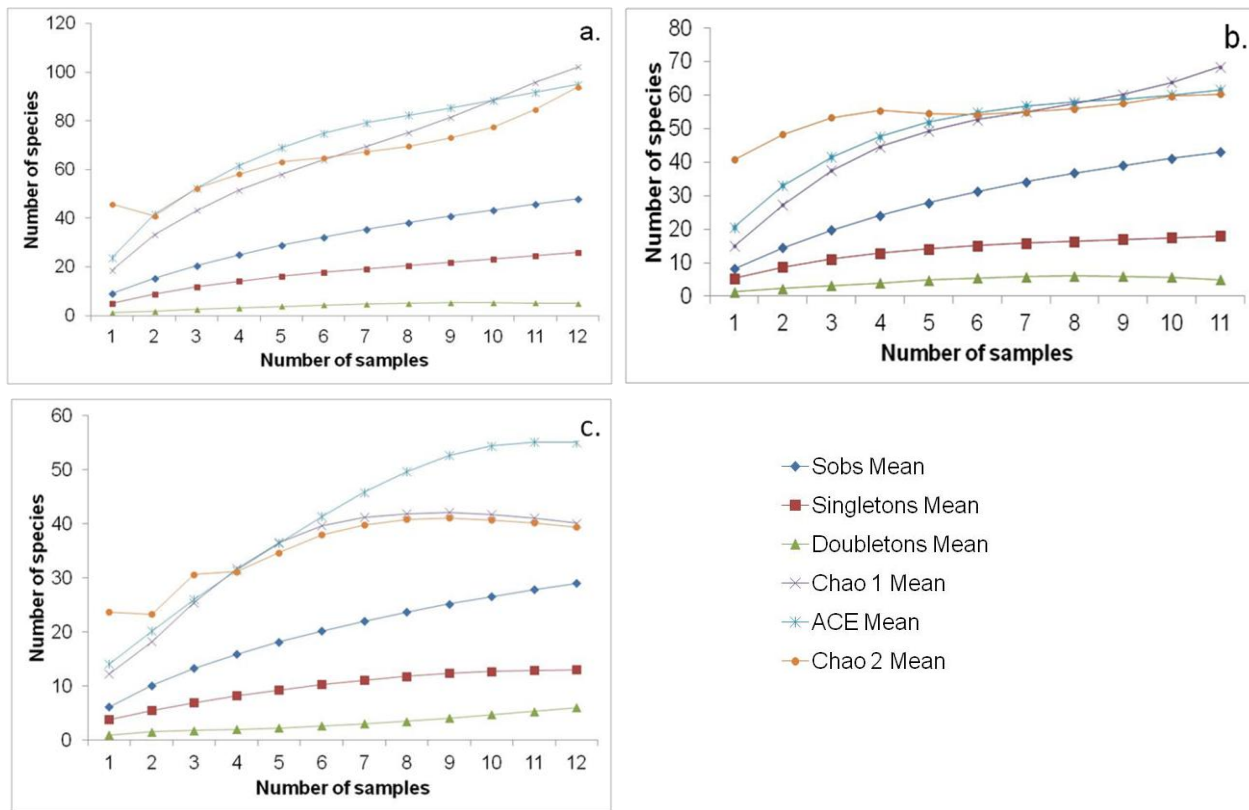


Figure 6. Species accumulation curves for the three types of canopy cover: a) Native trees canopy cover; b) Non native trees canopy cover; c) grasslands.

Richness, abundance, α and β diversity

A total of 760 individuals were collected in all the samplings; these were grouped into 85 morphospecies belonging to the families Braconidae and Ichneumonidae. Braconidae was the most common with a total of 557 individuals (73%) and 62 morphospecies, while Ichneumonidae had 203 individuals (27%) and 23 morphospecies. Most of the individuals from

the family Braconidae were collected at the forested plots. In this particular family, 60% of the total individuals were collected in the native trees canopy cover plots, 24% were collected in the non-native tree canopy cover plots, and the remaining 16% of the total amount of wasps were collected in the grasslands plots (Figure 7). Of the Ichneumonidae, 35% of individuals were collected from native tree cover plots, 35% were collected from non-native tree canopy cover and 30% from grasslands (Figure 8).

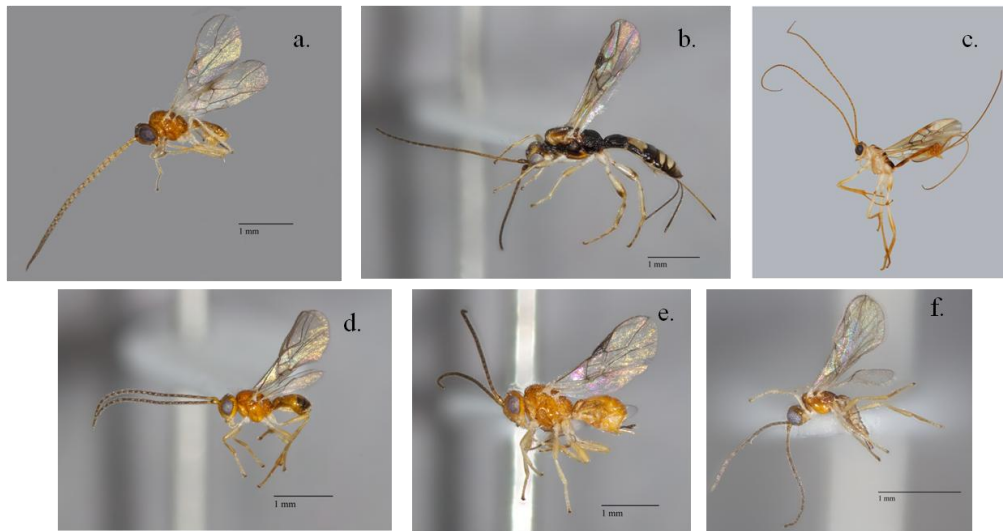


Figure 7. Most abundant morphospecies from Braconidae (>20 individuals) collected in the three types of canopy cover.



Figure 8. Most common specimens from Ichneumonidae (>20 individuals) collected on the three types of canopy cover.

Although overall species richness did not differ among cover types, differences in wasp abundance contributed to significant differences in richness/individual among cover types. Average wasp species richness in native tree cover (9 ± 2.64 species per plot) tended to be higher than that of non-native tree cover (8 ± 3.82 species per plot) and grasslands (6 ± 2.78 species per plot) but these differences were not significant (Table 4, Figure 9). Abundance differed between the three cover types ($p = 0.0028$, Table 5). The highest abundance was found in the native trees plots (33.9 ± 21.3 individuals), followed by the non-native tree canopy cover plots (18.7 ± 11.1 individuals); while the lowest abundance was found in grasslands (12.2 ± 8.1 individuals). When performing post-hoc pair wise comparison to compare these values, I only found significant differences between the plots of native trees and grasslands (Table 5, Figure 9). On the other hand, average richness/individual was 0.33 ± 0.14 for native trees canopy cover plots, 0.52 ± 0.21 for non-native canopy cover and 0.61 ± 0.24 for grasslands. These values were significantly different, specifically between plots with native tree cover and the other two types of tree cover (Table 5, Figure 10).

Table 4. Permutation test to compare parasitoid wasps richness among canopy cover types. The richness values per canopy cover type are indicated in the shaded area (NatTr: Native trees canopy cover, NonNatTr: non-native trees canopy cover, Grass: Grasslands).

	NatTr	NonNatTr	Grass
NatTr	48	0.966	0.827
NonNatTr		43	0.123
Grass			29

Table 5. Non parametric ANOVA (Kruskall Wallis) to compare abundance of parasitoid wasps among the three different types of canopy cover.

Variable	Canopy cover type	Mean \pm standard deviation	P-value
Abundance	Native trees	33.92 ± 21.31^a	0.0028*
	Non native trees	18.73 ± 11.08^{ab}	
	Grasslands	12.25 ± 8.06^b	
Richness/individual	Native trees	0.33 ± 0.14^a	0.0115*
	Non native trees	0.52 ± 0.21^b	
	Grasslands	0.61 ± 0.24^b	

*Significant differences in the variables (pvalue ≤ 0.05).

* a,b: different letters represent different mean values

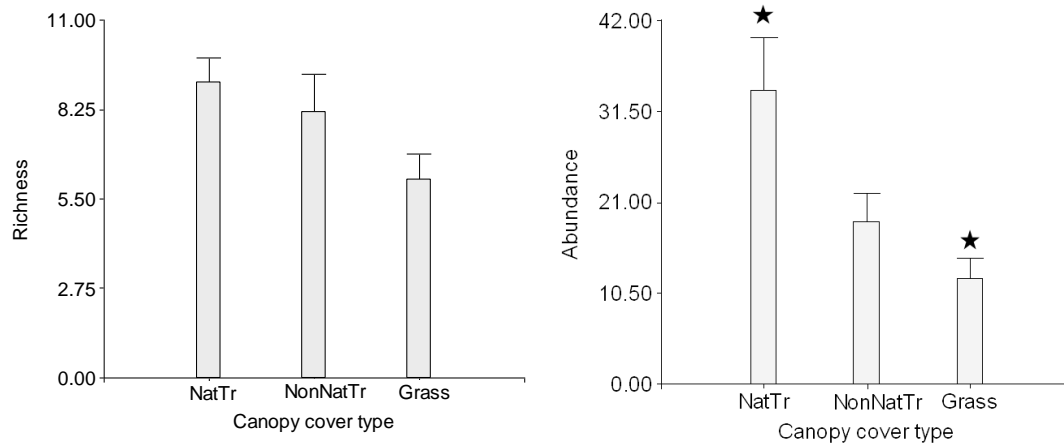


Figure 9 . Richness (number of species) and abundance (number of individuals) per each canopy cover type (NatTr: native trees canopy cover; NonNatTr: non-native trees canopy cover; Grass: grasslands). Black stars indicate significant differences among the canopy cover types (pvalue < 0.05).

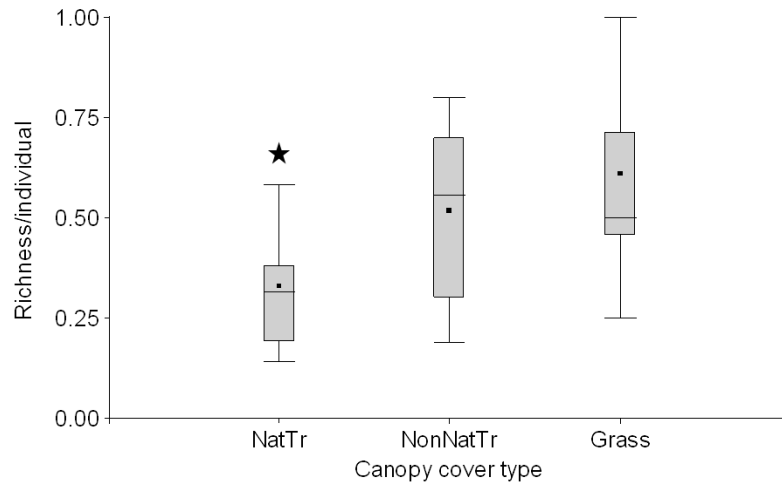


Figure 10. Richness/individual of parasitoid wasps for the three types of canopy cover (NatTr: Native trees canopy cover, NonNatTr: Nona-native trees canopy cover, Grass:grasslands). The black star in the figure indicate significant differences among canopy cover types (pvalue <0.05).

In the Whittaker curves it can be seen that overall assemblage of species for the three canopy cover types was characterized by the presence of dominant species and a large number of unique species (Figure 11). The species abundance model that best describes the data is the log series distribution, therefore, the adequate index to calculate alpha diversity corresponded to Fisher's alpha (Magurran 2004). Fisher's α (index to measure local diversity) for native tree canopy cover plots was 14.14, for non-native trees canopy cover plots was 16.54, and for grasslands was 10.82. None of these values were significantly different according to the permutation test (Table 6) indicating that each treatment was similar in their heterogeneity and evenness.

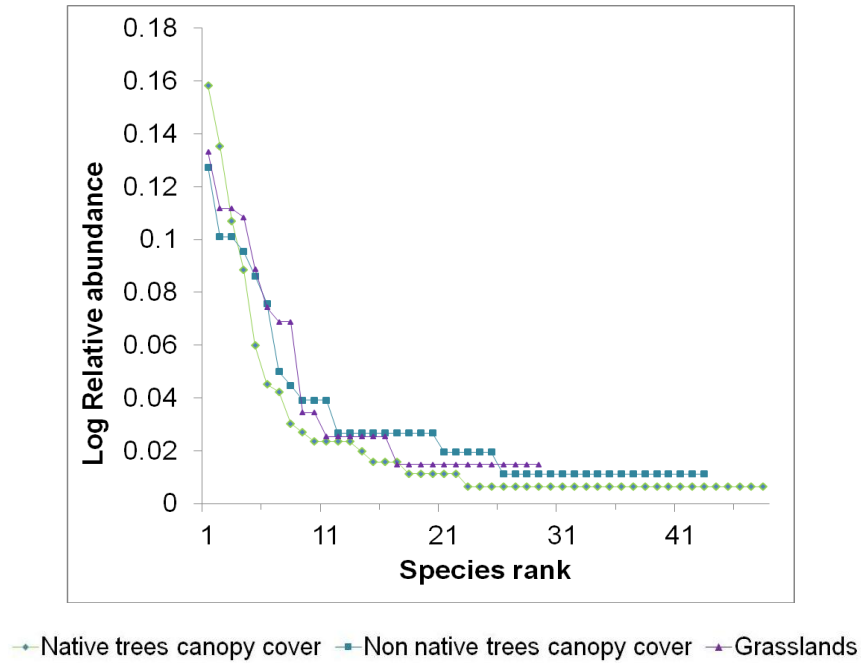


Figure 11. Whittaker dominance curves for the three types of canopy cover.

Table 6. P-values for the permutation test to compare Fisher's α of parasitoid wasps among the three types of canopy cover. The Fisher values per each type of canopy cover are indicated on the shaded area (NatTr: native trees canopy cover, NonNatTr: non-native trees canopy cover, Grass: grasslands).

	NatTr	NonNatTr	Grass
NatTr	14.14	0.538	0.603
NonNatTr		16.54	0.17
Grass			10.82

Beta diversity, on the other hand, reflected that the highest similarity (in terms turnover rate of species) was between native and non-native trees canopy cover plots (Whittaker index: 0.4505). The second highest similarity was between non-native trees canopy cover and grasslands (Whittaker index: 0.7778). The lowest similarity was between grassland plots and native trees plots (Whittaker index: 0.8182). I found similar results with Jaccard's cluster (Figure 12). On this graph two groups were formed based on the species composition: the forested plots and the grasslands. Among the forested plots, there was no clear division between those with native tree cover and those with non-native canopy cover.

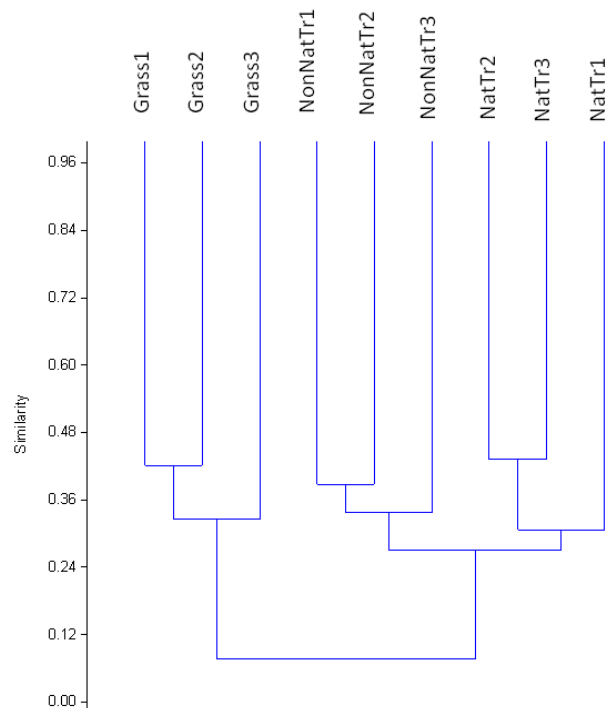


Figure 12. Jaccard cluster for the three types of canopy cover (three repetitions per type). (NatTr: native trees canopy cover, NonNatTr: non-native trees canopy cover, Grass: grasslands)

In the two- way Jaccard cluster (Figure 13), two large groups were formed (as in Figure 11). The first group is composed by all grassland plots (except NN3), and it does not have any

pattern related with the sampling dates. The second group includes most of the forested plots. Within this group two additional subgroups were formed, the first group includes plots with native and non-native trees canopy cover, and it does not have a clustering pattern based on sampling date, but it has a pattern related with the specific type of canopy cover (native or non-native). The second subgroup is a mixture of native and nonnative trees canopy cover plots, and unlike the others, it mainly clustered together plots with different canopy cover type in the same sampling date (May 2011).

In the Presence / Absence matrix it can be seen that most of the parasitoid wasps were found in the forested plots. Inside this group, three distinct subgroups are formed according to the species composition, which correspond to the groups that were formed in the cluster in the right. On the other hand, on the left of the same matrix it can be seen that there is a group of morphospecies in grasslands that is almost exclusive to this cover type (Figure 13).

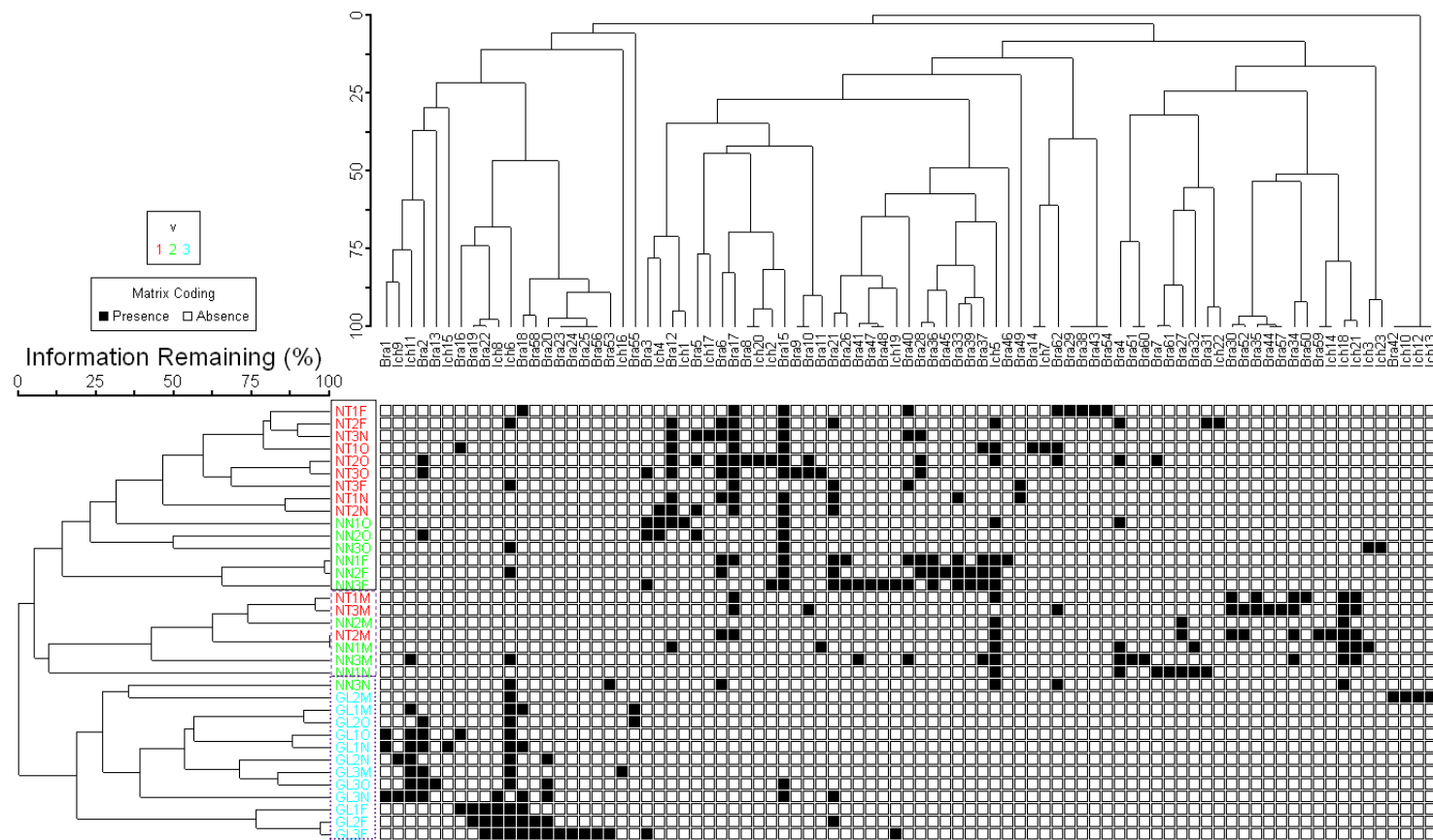


Figure 13. Two way Jaccard cluster (based on group average linkage). The matrix above illustrates the presence and absence of morphospecies in the different treatments. The cluster on the left shows the grouping of the different types of plots (NT: native trees canopy cover, NN: Non-native trees and GL: grasslands) for each type of canopy coverage and each sampling date according to species composition (sampling dates: O: October/2010, F: February-2011, M: May/2011, N: November/2011). The cluster at the top shows the grouping of the parasitoid wasps morphospecies.

Partition of diversity components

For regional diversity (γ), the most important component was β_2 ($p < 0.05$). This level of diversity is related to species turnover among different types of cover (native trees canopy cover, non-native trees canopy cover and grasslands). This suggests that movement of species has great relevance for regional diversity. In contrast, the other two levels α and β_1 (related with the diversity on each plot and the species turnover among plots) did not generate diversity values greater than those generated by the random distribution of the data ($p > 0.05$), hence, they were not significant for the total diversity in the landscape (Figure 14).

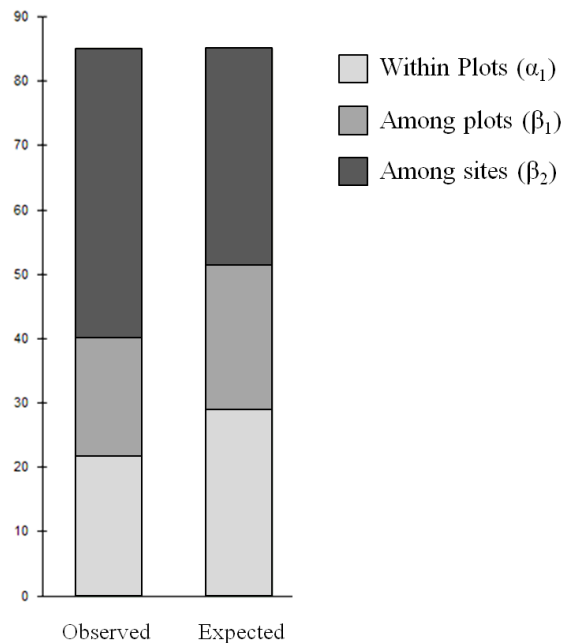


Figure 14. Partition of α and β diversity components and comparison between observed and expected values. The components of diversity were divided according to different levels in the landscape (those considered for this test were α , β_1 and β_2 , see Materials and Methods for details). The observed values of diversity (bar graph on the left) for each of these levels were compared with randomly generated diversity values (bar graph on the right). Each of these

comparisons generated a p-value that determines whether the observed diversity values were higher or lower than expected by chance.

3.2 Results

Plant diversity

In total, 41 morphospecies were collected, 27 of them were identified to species level and 5 of them corresponded to species used in restoration plantings (Appendix 3). The number of species recorded was similar between native trees canopy cover plots and non-native trees canopy cover plots. In particular, in the native trees plots I registered a total of 21 with an average of 14.33 ± 3.79 species. Of these 21 species, 11 were unique (5 native species and 6 unidentified to species level). In plots with non-native tree cover I also registered 21 species, the average was 14.67 ± 1.53 species and I found 10 exclusive species (1 endemic species, 2 native species, 2 possibly native species, 1 introduced and 4 unidentified). On the other hand, grasslands plots had the lowest value of richness with 13 species recorded, with an average 6 ± 3.46 species and 9 species exclusive species recorded (3 native, 2 introduced and 4 unidentified) (Figure 15 , Appendix 1-3). When I compared the richness among canopy cover types, I did not find significant differences (Table 7).

In the forest layer above 2 m, the most common species was *Bucida buceras*. This native tree species was most frequently found in plots with canopy cover of native trees, it was not recorded in the plots with non-native tree cover and there was a single individual in grasslands. The second most abundant tree species in the sample was *Prosopis juliflora* that was only found in plots with no native canopy cover. Other common woody species in this forest stratum were *Trichostigma octandrum* and *Jasminum fluminense*, both species are lianas that sometimes were found in forest layers up to 9 m. The former is a native species in Puerto Rico that grows as a shrub or as a vine; the latter is an exotic vine.

In general, in the stratum below 2m the most abundant plant species was *Urochloa mutica* (only present on grasslands and most frequent species on this type of habitat), an introduced species from tropical Africa (Axelrod 2011). Other abundant plant species in the understory were *Trichostigma octandrum* and *Jasminum fluminense*. Both species were absent in grassland plots and were more frequent in the plots with non-native tree cover than in plots with native tree cover.

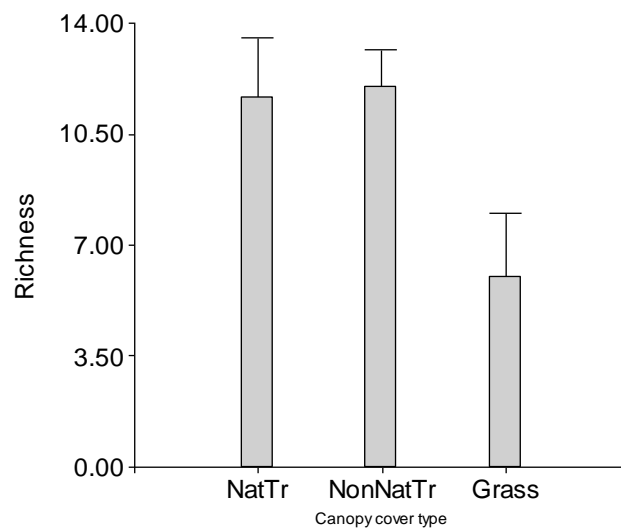


Figure 15. Plant species richness for the three different types of canopy cover. (NT: Native trees canopy cover; NN: Non native trees canopy cover; PP: Grasslands).

Table 7. Permutation test to compare plant richness among canopy cover types. Richness values per each canopy cover type are indicated in the shaded area (NaTr: Native trees canopy cover plots, NonNatTr: Non-native trees canopy cover trees, Grass: Grasslands)

	NatTr	NonNatTr	Grass
NatTr	21	1	0.12
NonNatTr		21	0.363
Grass			13

Overall assemblage of plant species for the three canopy cover types was characterized by high evenness (Figure 16). Thus, the species abundance model that best describes the data is the log normal distribution (Magurran 2004). Of the three types of coverage, non-native trees canopy cover had greater evenness compared with the native trees canopy cover plots and grasslands.

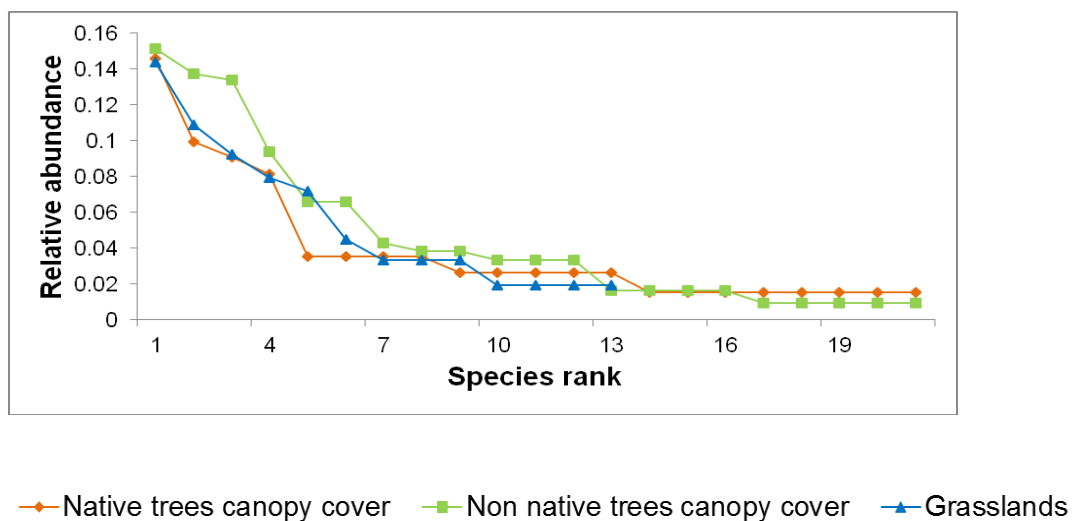


Figure 16. Whittaker abundance plot for the three types of canopy cover types.

Jaccard cluster (Figure 17) shows a splitting between tree canopy cover plots and grass plots. Inside the group formed by the plots with tree cover there are two subgroups, the first one corresponds to the non-native trees canopy cover areas and one plot with native trees canopy cover; the second groups the remaining native trees canopy cover areas. These differences in species composition between the different types of coverage can be seen in more detail in Appendices 1 and 2.

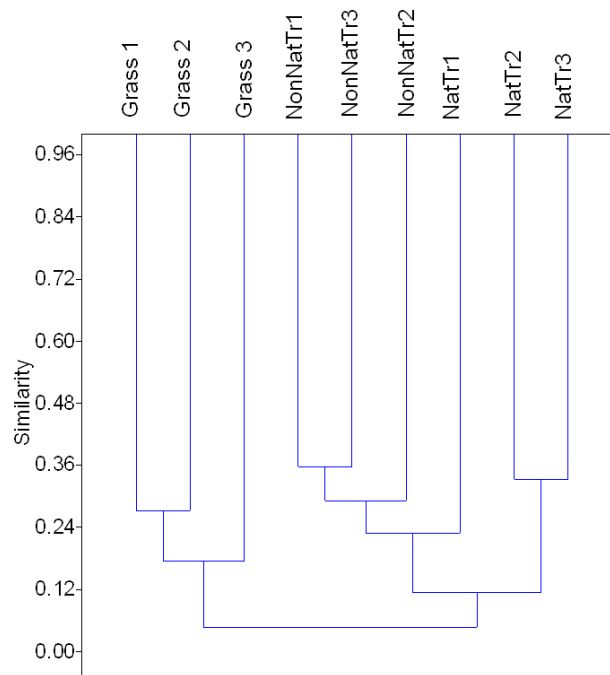


Figure 17. Jaccard cluster for the three types of canopy cover (NtTr: Native trees canopy cover, NonNtTr: Non-native trees canopy cover, Grass: grasslands).

Vegetation structure

In general, there were differences in vegetation structure among the three cover types. Grasslands were characterized by homogeneity in terms of plant architecture, due to their being dominated by grasses and lacking tree canopy cover. Plots with native tree cover had a dense canopy with little herbaceous cover in the understory. In contrast non-native tree plots had complete canopy cover, but the canopy was less dense and small gaps permitted the development of significant herbaceous cover in the understory.

In particular, vegetation strata were defined in terms of the number of layers of herbs and trees on each type of treatment. It was observed that native tree canopy cover plots had the lowest amount of layers with an average of 5.23 ± 0.58 strata. On the other hand, the non-native trees canopy cover plots had an average of 7.92 ± 0.83 strata, and the grasslands had 7.96 ± 2 strata in average. There were no significant differences among treatments, however non-native and grass covered plots tended to have more layers than native trees canopy cover plots (Table 8, Figure 18).

Table 8. Comparison of vegetation strata between canopy cover types.

Variable	Canopy cover type	Mean \pm standard deviation	P-value
Vegetation strata	Native trees	5.23 ± 0.58	0.0714
	Non native trees	7.92 ± 0.83	
	Grasslands	7.96 ± 2	

*significant differences among treatments (pvalue \leq 0.05)

The percent cover of vegetation was calculated for the understory (<2m stratum), canopy (>2m stratum, which includes trees and vines) and trees in canopy. Grasslands had the highest percent of vegetation cover below 2m ($100\% \pm 0$), followed by the non-native trees canopy cover plots ($98.67\% \pm 2.31$) and the native trees canopy cover plots ($45.33\% \pm 8.33$) (Figure 19). When I assessed canopy cover resulting from both trees and vines, canopy cover was 100% and did not differ among forested plots; however when I excluded vines, I found that tree cover percent was higher in native ($100\% \pm 0$) than in non-native canopy cover plots (74.67 ± 16.17) (Figure 20).

Average tree diameter (DBH) differed among plots with trees. Native tree plots, in particular, had an average of $14.12 \pm 13.84\text{cm}$, and a range of values ranging from 2.50cm to 84.3cm. On the other hand, the plots with non-native tree cover had an average of $16.35 \pm 13.13\text{cm}$, and a range of values ranging from 2.60cm to 87cm. These averages were significantly different (Kruskal Wallis test; $p = 0.047$) (Table 9, Figure 21)

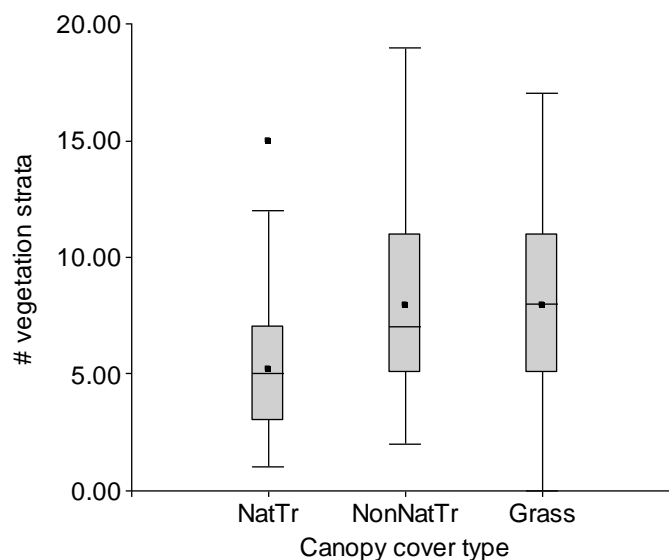


Figure 18. Number of vegetation layers for the three types of canopy cover. (NatTr: native trees canopy cover types; NonNatTr: Non native trees canopy cover; grass: grasslands).

Table 9. Kruskal-Wallis test (Non-parametric ANOVA) to compare DBH between the native and non-native canopy cover types.

Variable	Canopy cover type	Mean \pm standard deviation	P-value
DBH	Native trees	13.84 \pm 14.12	0.047
	Non native trees	13.13 \pm 16.35	

*significant differences among treatments (pvalue \leq 0.05)

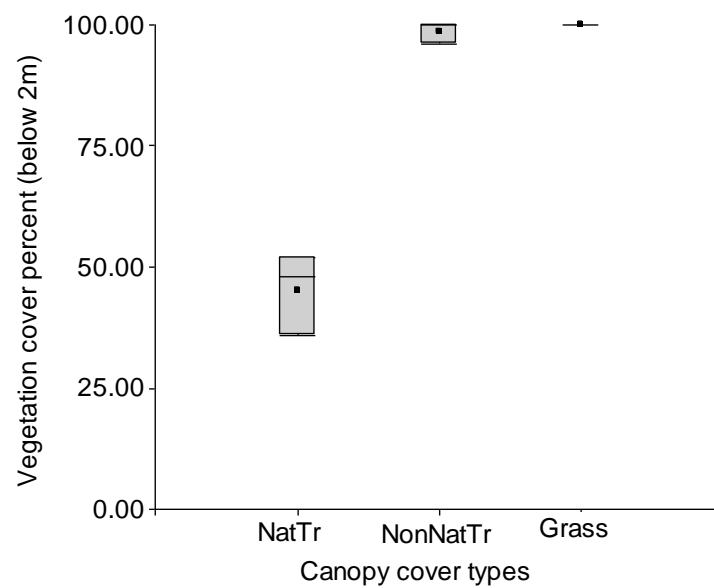


Figure 19. Percent of vegetation cover in the understory (below 2m height) for the three canopy cover types.

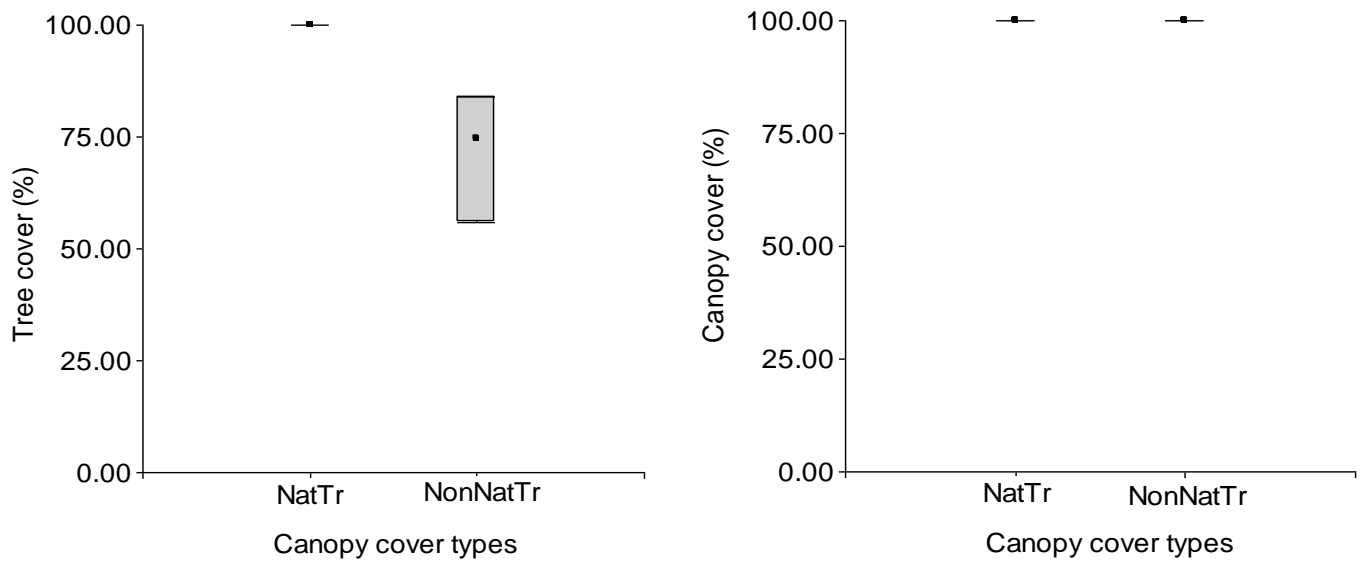


Figure 20. Percent canopy cover (vines and trees) for the forested plots and percentage of tree cover in the forested plots.

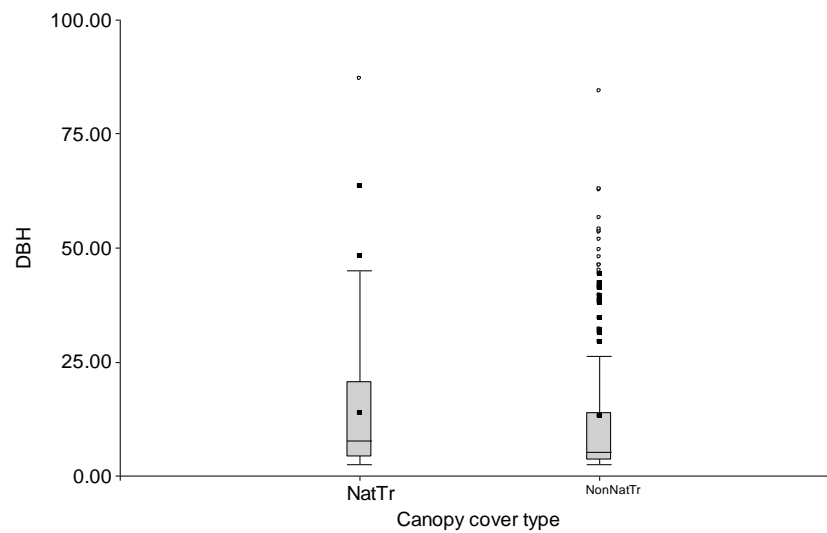


Figure 21. DBH measurements for the two types of tree canopy cover (NatTr: native trees canopy cover, NonNatTr: non-native trees canopy cover).

3.3 Correlation between parasitoid wasps diversity and vegetation diversity and complexity

To assess whether there is a correlation between the diversity of parasitoid wasps and vegetation variables, I performed a NMDS (Non-metric multidimensional scaling). In the resulting ordination (Final stress =11.27492) two groups were clearly differentiated. The first one corresponded to grassland plots on different sampling dates, and was mostly related to the number of layers in the understory strata and to a lesser extent to the vegetation cover in the understory. The second group was made up of the plots with tree cover; within this group there is a subgroup composed mostly of native tree plots at different sampling dates, this group is mainly explained by the number of vegetation layers in the canopy and the percent of tree cover, but to a lesser extent is explained by the diversity of plants in the canopy. The remaining plots outside this group belong to the native and non native trees canopy cover plots sampled in May 2011 (Figure 22).

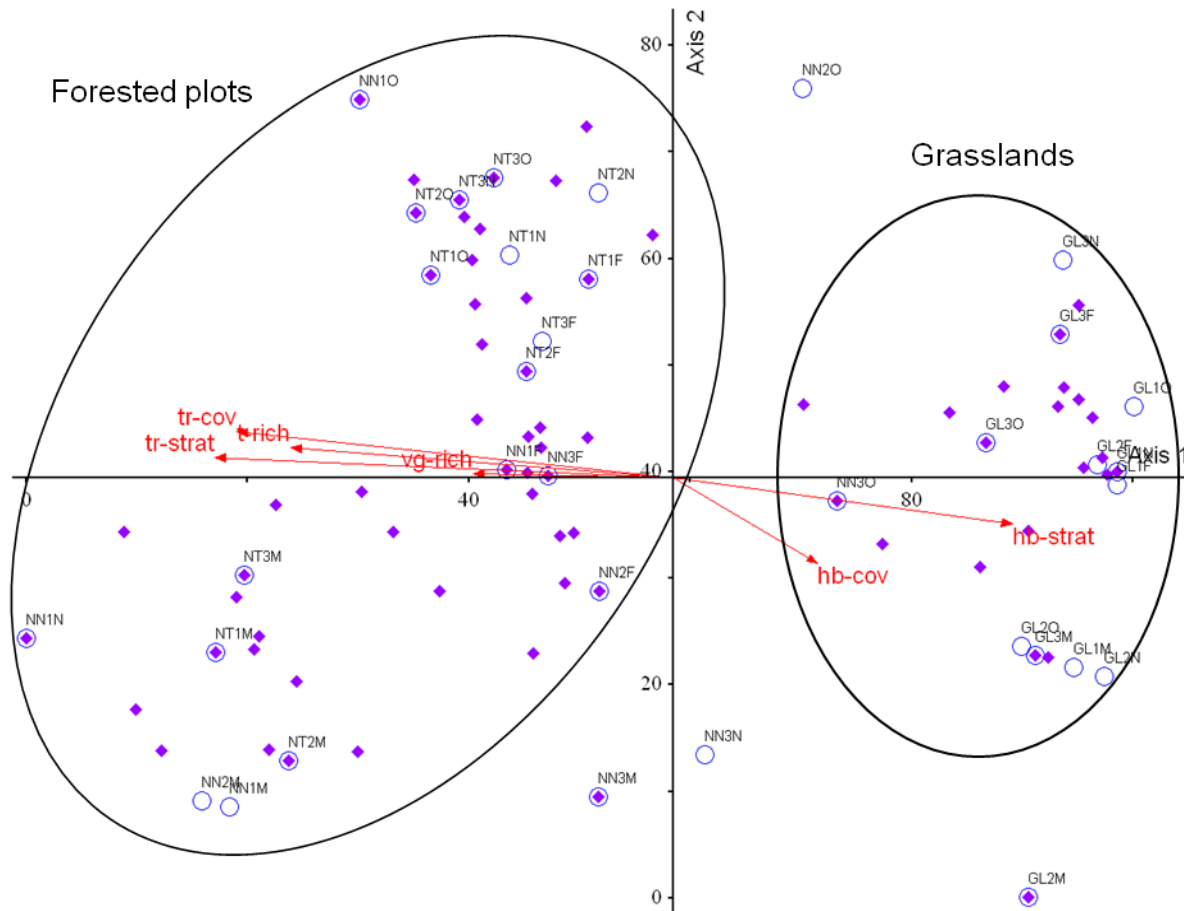


Figure 22. Non-metric multidimensional scaling. Blue circles represent the plots per each type of canopy cover in different sampling dates. Each of these circles has a four character code; in this code, the first two characters correspond to cover type (NT: native trees, NN: Non-native trees, GL: grasslands), the third character is the plot number (1, 2 or 3) and the fourth is the sampling date (O: October-2010, F: February-2011, M: May-2011, N: November-2011). Purple points in the ordination represent the parasitoid morphospecies and the red lines are the significant vectors for the ordination (hb-strat: vegetation layers in the <2m stratum, hb-cov: species cover percent at <2m stratum, vg-strat: vegetation layers at <2m and >2m stratum, hb-rich: vegetation richness in the <2m stratum; t-rich: tree richness, tr-cov: tree cover percent).

Two

4. Discussion

4.1 Discussion: wasps

Braconidae and Ichneumonidae are the largest families within Hymenoptera and some of the most studied in this order (Fernández 2006). Their hosts frequently are holometabolous insect larvae, especially those included in the orders Lepidoptera and Coleoptera (Wharton 1998, Palacio and Wahl 2006). Sampling efficiency of these taxa varied among the three types of coverage, but in general it was found that the sampling effort was on average less than 70%, indicating that the number of morphospecies recorded was less than the amount modeled by the estimators. The best sampling efficiency was found in grasslands (72.24% efficiency), while the lowest efficiency was found on the native tree coverage (46.98% efficiency). These efficiency values are mainly caused by the high rate of singletons and the presence of highly dominant species, specifically on the forested plots. Similar efficiency values were found by Lewis and Whitfield (1999).

The absence of canopy may contribute to a higher sampling efficiency in grassland areas compared to forested areas. It is possible that in native and non-native tree canopy cover plots, singletons may correspond to canopy associated species that normally use this vegetation stratum for oviposition and mating activities. Therefore, this species might be only collected accidentally by the malaise traps (Fraser et al. 2008a). Furthermore, the exclusive use of malaise traps in the study may be another factor influencing the sampling efficiency. Although this method is one of the most efficient methods for collecting a large number of species, it should be used in long-term sampling or in combination with complementary methods such as sweep nets and yellow trays in order to enhance the efficiency in the sampling (Wharton 1998, Fraser et al. 2008a).

Despite this shortcoming, it should be noted that the employment of malaise traps as the only sampling method can still provide a list of species that allows us to generate useful information for long-term monitoring and to detect the effect of changes in the environment on the diversity of these organisms (Fraser 2008a). In this study the exclusive use of malaise traps was deemed appropriate given that the primary goal of sampling was to compare relative effects of different vegetation cover types, rather than generate an exhaustive list of all species at the site.

The use of morphospecies rather than taxonomic species in diversity calculations may have affected the number of singletons. The use of morphospecies to group similar organisms depending on external morphological traits is called parataxonomy. This is a common practice in ecology, because it allows rapid classification of individuals by non-expert taxonomists. In Hymenoptera particularly, the use of morphospecies is considered a reasonable surrogate of taxonomic species, as long as the person carrying out the separation has minimal training in taxonomy (Derraik et al. 2010). Although this method facilitates the processing of samples for various purposes, it has been widely criticized due to the tendency to overestimate or underestimate the actual diversity (Majka and Bondrup-Nielsen 2006). In this study, classification to morphospecies was considered adequate due to the focus on relative differences in diversity among cover types rather than total diversity at the site.

Diversity partition showed that the most relevant component for global diversity is the beta diversity of parasitoid morphospecies. This shows that the gamma diversity of wasps is more dependent on the turnover of species in the landscape, than it is of local or alpha diversity. Apparently, alpha diversity patterns in each of the plots and each of the types of coverage have been affected by the high frequency of unique and dominant species, which generates diversity

observed values lower than expected (Crist et al. 2003). Beta diversity analysis showed a greater similarity in species composition between native and non-native canopy tree cover plots, reflecting higher turnover among them. These similarities were not that high among grasslands and the other forested plots. It is interesting that despite the short distance among these canopy cover types, I still found differences in species composition among the areas. This may be a consequence of the difference in available resources in the forested areas in comparison to grasslands, which apparently constitute a matrix with scarce resources, limiting the movement and establishment of parasitoids.

In general terms, species composition of wasps is dependent of habitat complexity. Another aspect to consider is that the total number of morphospecies collected in this study was lower than those reported by other authors. Lewis and Whitfield (1999) collected 251 morphospecies with Malaise traps in a pine forest, and Whitfield and Lewis (2001) reported 184 morphospecies collected with the same method in a meadow habitat. These differences are attributed mainly to differences in the size of the study area. Therefore, it would be expected that an increase in the area of the fragments studied and the presence of corridors to increase connectivity between these areas and other places of refuge could lead to an increase in the amount of morphospecies (Connor and McCoy 1979).

4.2 Discussion: plants

Tree cover plots (both native and non-native) were characterized by closed-canopy, multiple vegetation strata and small to medium-sized trees (average DBH ~13 cm for both native and non-native). This indicates that active restoration efforts made in Laguna Cartagena over the past 17 years ago (Weaver and Schwagerl 2008) plus natural secondary succession process have

been successful in the generation of small fragments of secondary forest. In the areas sampled within these forest fragments, I found that 22% of the species recorded in the plots were cultivated and the other 78% corresponds to several native and non-native species that probably colonized these areas from surrounding lands by wind, floods or because they have been dispersed by animals such as birds and bats (Weaver and Schwagerl 2008).

Of the species recorded, the most common among all types of coverage was the native tree *B. buceras*. This result was mainly because nearly two decades ago many seedlings of this species were planted at the refuge (Weaver and Schwagerl 2008), and in addition *Bucida buceras* has been spreading naturally in areas adjacent to the plots where it was initially planted. It is important to note that this species occupies a considerable area within the native tree cover plots and contributes significantly to the vegetation structure in these areas. Their trees have crowns with dense foliage that reach considerable heights, in some cases exceeding 12 m. Indeed *B. buceras* is a canopy dominant in most dry forests in Puerto Rico (Gould et al. 2006, Molina and Lugo 2006).

The second higher cover percent corresponds to *Prosopis juliflora*. This species was only found in plots with non-native tree cover, and the reason for its abundance can be attributed to the previous use of land for cattle grazing, since this type of practice facilitates seed dispersal and further establishment of seedlings (Pasiecznik 2001). Despite being considered a problem as an invasive plant in its non-native range due to its negative effects on soil nutrients, soil phenolics, and the reduction of native species (Kaur et al. 2012), the presence of this species in its native range can be beneficial to other species, because once *P. juliflora* has established in grasslands, it starts a chain of events that can lead to the succession from grassland to forest. This

species, particularly can lead to changes in microclimate; these changes in turn interact with soil changes enhancing plant establishment. Additionally, *P. juliflora* may provide shelter from the wind and shade, creating a positive effect on the water balance by increasing humidity and decreasing evapotranspiration (Archer 1995).

In general, the presence of tree species (like *B. buceras* and *P. juliflora*) generates a positive effect on the area because they provide shade and shelter from the wind, generating a positive effect in water balance by increasing the relative humidity and decreasing evapotranspiration. Additionally, the limited availability of light generated by the crowns reduces grass cover and the consequent threat of fire. Also, these trees provide nesting sites and perches for birds and bats, and sometimes facilitate germination and early growth of species native tree species (Pasiiecznik 2001, Weaver and Schwagerl 2008).

Two climbing vines, *Trichostigma octandrum* and *J. fluminense*, were also frequent in the study area. In particular *T. octandrum* can be established in early and late successional stages in secondary forest; it is localized especially in clearings and at the edges of natural openings (Francis 2004). In the study areas, the coverage of this species was over 60% in plots with non-native tree cover but only ~10% in plots with native tree cover coverage. These differences are probably correlated with the differences in light availability among the two types of canopy cover. Because this species is moderately intolerant of shade, it tends to settle in forest clearings and was largely excluded from native tree plots due to the dense native canopy.

Like the other vine species, *J. fluminense* was highly frequent in the plots with non-native tree cover, covering about 50% of the plots. Its abundance in these plots can be the result of higher light availability in these areas, compared with the native tree cover plots (Hammer 2000).

This species is one of the most problematic in the genus, because it is capable of spreading in a wide variety of habitats, particularly hardwood forests and cultivated grounds. Particularly in Puerto Rico, *J. fluminense* is considered as non-native, as well as in other areas in United States and the Caribbean. Due to its impact on the community it is categorized as Class I in the list of invasive species generated by Florida's Exotic Pest Plant Council's in 2009 (FLEPPC 2009).

Plant species richness was no different among the three types of coverage, mainly because I analyzed together the vegetation above and below 2m. On the other hand, the composition of plant species varied among vegetation types. These differences may be mainly the result of the history of land use (China and Helmer 2003). Land use history could also be the main modulator of vegetation structure, particularly of variables such as stem density and distribution of diameter classes for a forest such as this that is undergoing restoration (Thompson et al. 2002). In particular, the management of lands in the refuge began with the establishment of areas for the cultivation of sugar cane and livestock, resulting in extensive areas dominated by monocots contributing to soil erosion and siltation. However, these conditions were modified more than a decade ago due to grass removal, fire control and reforestation in some areas, which led to the emergence of tree cover patches with native and nonnative vegetation (Weaver & Shchwagerl 2008).

Despite the emergence of these patches of forest, some grassland areas without trees remain. This condition might be explained by the lower elevation in grasslands, which are more susceptible to flooding and less suitable habitats for tree establishment. On the other hand, these areas generally are fairly homogeneous habitats dominated by a single grass species, such as the exotic species *Urochloa mutica*, which can produce phytotoxic compounds that inhibit seed

germination (Chou and Young 1975) and may also be hindering the establishment of shrubs and trees. In contrast, in native canopy cover plots, the reforestation process has produced a closed canopy dominated by crowns of *B. buceras*. In these areas, ranges of height and DBH species are generally higher than those reported by Weaver and Schwagerl (2008), indicating that these fragments continue to gain complexity in terms of structure as time passes. In contrast, the understory is less covered by plants due to the low light availability generated by the dense canopy and the occasional flooding that may be affecting seed germination and promoting mortality in flooding intolerant plants (Kozlowski 1997).

In non-native trees canopy cover plots, the factor which probably had a greater influence on vegetation structure was the development of tree canopy cover. Perhaps *Prosopis* individuals were successfully established in these areas due to soil disturbance by livestock. As these individuals grew they started to establish appropriate conditions for the arriving of other herbaceous and woody species (Archer 1995). When the grass was removed and the recurrent fires controlled, these colonizing species started to develop and begun to create a more complex habitat characteristic of later stages of secondary succession. This, in addition to the planting of some native species, make that currently this habitat is characterized by the presence of large *Prosopis* and *Guazuma* trees in the canopy, but also by areas with light availability in the understory adequate for the establishment of a large amount of herbs and grasses.

4.3 Discussion: multivariate analysis

The ordination results indicate that the parasitoid wasp community is correlated with habitat characteristics, in particular with the structural complexity generated by the presence of

trees. Variables such as tree cover, the amount of tree strata and tree species richness are those that best explain this pattern.

Overall, the most important determinant for the community of wasps is the presence of tree cover. The presence of trees, more than the presence of herbs, is generating structural complexity in the habitat, which can be associated with a greater diversity of hosts and shelters available to parasitoids (Martorell 1975, Hochberg and Hawkins 1992, Sperber et al. 2004). The fact that there is coverage of trees and many vertical layers modifies the conditions of humidity (Power 1992), allowing the generation of suitable microclimatic conditions for the establishment of host and hence of parasitoids (Hochberg 1994, van Nouhuys and Hanski 1999, Sperber et al. 2004). On the other hand, tree species richness can be directly affecting food availability for herbivores, which can modulate the variety and abundance of potential hosts available in the habitat, and consequently the parasitoid richness (Craig 1994, van Nouhuys and Hanski 1999, Sperber et al., 2004, Shaw 2006)

In conclusion, reforestation to recover forest cover is one of the main goals in the restoration process, and it is one of the most significant ones because it generates considerable effects on the animal community. Particularly, with the restoration of canopy cover, suitable microclimatic conditions are generated for parasitoids, as well as a better supply of resources and shelters. With the results I found, I highlight the relevance of small fragments for maintaining insect communities, and the importance of developing future strategies to allow the establishment of connectivity between these fragments to ameliorate the insulation effect of grasslands on the community of wasps. Therefore, I recommend the resumption of reforestation,

or the establishment of appropriate conditions to enhance tree species colonization (whether native or non-native).

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Appendix 1. Species registered in the >2m stratum and number of times they were registered on the different canopy cover types.

Species	NaTr1	NatTr2	NatTr3	NonNatTr1	NonNatTr 2	NonNatTr 3	Grass1	Grass2	Grass3
<i>Andira inermis</i> (W. Wright)		2							
Kunth ex DC.									
<i>Bucida buceras</i> L.	19	21	22						1
<i>Cordia obliqua</i> Willd.					1				
<i>Crescentia cujete</i> L.	1								
<i>Guapira fragrans</i> (Dum. Cours.)	1								
Little									
<i>Guazuma ulmifolia</i> Lam.	1	1	6		4	3			
<i>Jasminum fluminense</i> Vell.		1	1	6	12	11			

Appendix 1 (continuation)

<i>Leucaena leucocephala</i> (Lam.) de Wit	8	4		4	4	
<i>Prosopis juliflora</i> (Sw.) DC.				18	17	11
<i>Sideroxylon foetidissimum</i> Jacq.		1				
<i>Stahlia monosperma</i> (Tul.) Urb.	4	3	3			
<i>Thespesia grandiflora</i> DC.						1
<i>Trichostigma octandrum</i> L.) H. Walt.				15	11	15
Unidentified tree	1					
unidentified tree 1		1				
Unidentified tree 2			1			

Appendix 2. List of plant species recorded in the <2m stratum registered on the sampling plots and number of times they were registered on the different canopy cover types.

Species	NaTr1	NatTr2	NatTr3	NonNat Tr1	NonNat Tr 2	NonNat Tr 3	Grass1	Grass2	Grass3
<i>Achyranthes aspera</i> L.	2			2	2	1			
<i>Blechum pyramidatum</i> (Lam.) Urb.	1			1					
<i>Bucida buceras</i> L.	3	5	5			1			
<i>Cissus verticillata</i> (L.) Nicolson & Jarvis		1	2	2		3		2	
<i>Commelina diffusa</i> Burm. f.	1	1	1		1	1	6		
<i>Convolvulaceae</i>	1								
<i>Crescentia cujete</i> L.		1							

Appendix 2 (continuation)

<i>Guapira fragrans</i> (Dum. Cours.) Little	1						
<i>Guazuma ulmifolia</i> Lam.	2						
<i>Ipomoea batatas</i> (L.) Lam.				5		1	
<i>Ipomoea triloba</i> L.				2			
<i>Jasminum fluminense</i> Vell.		1	5	11	4		
<i>Leucaena leucocephala</i> (Lam.) de Wit	2		2	3			
<i>Ludwigia erecta</i> (L.) H. Hara						2	1
<i>Megathyrsus maximus</i> (Jacq.) B.K. Simon & S.W.L. Jacobs	3		7	9	7		

Appendix 2 (continuation)

<i>Melothria pendula</i> L.		2		
<i>Merremia aegyptia</i> (L.)			1	1
Urb.				
<i>Merremia umbellate</i> (L.)			7	
Urb.				
<i>Paspalum</i> sp.			8	1
Poaceae Msp 4			12	
Poaceae Msp1	6			
Poaceae Msp2	2			
Poaceae Msp3		6		
<i>Rivina humilis</i> L.	4	9		

Appendix 2 (continuation)

<i>Stahlia monosperma</i> (Tul.)	1	1					
Urb.							
<i>Trichostigma octandrum</i>		1	1	7	14	3	
(L.) H. Walt.							
Unidentified seedling	1						
Unidentified seedling 1	1						
Unidentified specimen						2	
Unidentified specimen 1						1	
<i>Urochloa mutica</i> (Forssk.)						6	25
T.Q. Nguyen							25
<i>Vigna luteola</i> (Jacq.)							1
Benth.							

Appendix 3. Species recorded (and identified until the level of species) in the sampled plots in the lagoon tract at Laguna Cartagena NWR: classification according to their status as Native (N), Endemic (E), possibly native (PN), Introduced (I) (classification according to Axelrod 2011). This table also includes what plants were cultivated for the active restoration process in the lagoon tract (Weaver & Schwagerl 2008).

Species	Endemic, Native or introduced	Employed for active restoration in the Lagoon tract
<i>Achyranthes aspera</i>	N	
<i>Andira inermis</i>	N	x
<i>Blechnum pyramidatum</i>	N	
<i>Bucida buceras</i>	N	x
<i>Cissus verticillata</i>	N	
<i>Commelina diffusa</i>	N	
<i>Cordia obliqua</i>	I	
<i>Crescentia cujete</i>	N	x

Appendix 3 (continuation)

<i>Guapira fragrans</i>	N	
<i>Guazuma ulmifolia</i>	N	x
<i>Ipomoea batatas</i>	PN	
<i>Ipomoea triloba</i>	PN	
<i>Jasminum fluminense</i>	I	
<i>Leucaena leucocephala</i>	I	
<i>Ludwigia erecta</i>	N	
<i>Megathyrsus maxima</i>	I	
<i>Melothria pendula</i>	N	
<i>Merremia aegyptia</i>	N	
<i>Merremia umbellata</i>	N	

Appendix 3 (continuation)

<i>Prosopis juliflora</i>	PN	
<i>Rivina humilis</i>	N	
<i>Sideroxylon foetidissimum</i>	N	x
<i>Stahlia monosperma</i>	N	x
<i>Thespesia grandiflora</i>	E	
<i>Trichostigma octandrum</i>	N	
<i>Urochloa mutica</i>	I	
<i>Vigna luteola</i>	I	