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SCHOOL OF NATURAL SCIENCES AND TECHNOLOGY

DIVERSITY AND DYNAMICS OF ARTHROPODS AND MICROBES, AND NUTRIENT RELEASE DURING GREEN LITTER DECOMPOSITION IN A SIMULATED HURRICANE EXPERIMENT

by

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CERTIFICATE OF APPROVAL OF DISSERTATION

The dissertation presented by Ivia I. Moreno Rosado was revised and approved by the members of the Dissertation Committee. The form certifying Fulfillment of Academic Requirements for the Doctorate, signed by the members of the committee, has been filed with the Registrar and with the Center for Graduate Studies and Research of the *Universidad Ana G. Méndez, Recinto de Gurabo.*

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Summary

Hurricanes generate disturbances in forests such as canopy opening, fallen trees and leaves which alter physicochemical characteristics of the habitat. Litter decomposition depends primarily on the interaction among climate, litter quality and biota, as a consequence any change in habitats will result in changes in these factors. Our objective is to evaluate the effects of hurricane driven changes to forests on green litter decomposition, decomposer communities and nutrient mineralization. For this study, three blocks were selected, each with two plots of 20m x 20m, one plot was used for control and the other Canopy opening (Trim). In each subplot, litterbags with different mesh sizes were placed. Each of these litterbags were used as the sampling unit. In each one, decomposer fauna and nutrients were measured, and the weight of green litter from the litterbags was used for measure mass loss through time. Results showed arthropod abundance was significant different through time and the number of taxonomic groups was significantly different between control and trim+detritus. In addition, significant differences in available nutrient between control and trim+detritus, and among litterbags mesh sizes and the number of phylotypes was significant different among litterbag mesh size. For example nitrogen and phosphorous were significantly higher in trim+detritus in large mesh size and

decomposer arthropod abundance was higher in large mesh size. Furthermore mass remaining (%) was significant different through time but not between treatments. Therefore our result showed that abundance of arthropods does not affect the loss of mass, because the effect of arthropods on leaf litter decomposition occurs on a smaller scale, not on a meso-scale, as a result of the interaction between arthropods and microbes. Our result showed that abundance was higher in 84 days in both treatments, while the abundance of fungi was lower in 84 days. Consequently, there was a trend for higher arthropod abundance, low fungi abundance, and nutrient availability, suggesting trophic dynamics mediated by decomposer microbes.

DEDICATION

I want to dedicate this work to my parents Efraín Moreno and Teresa Rosado, for educating me and taking me every day to school and even in my last years of high school. My parents, who did everything in their power to support me in one way or another to complete this goal. Thank you for showing me the way, instilling values and giving me the basic tools to follow me later. I am especially grateful to my family for always support me and believed in me.

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Chapter 1

Introduction

Hurricane Disturbance

Tropical forests are susceptible to a variety of natural disturbances such as hurricanes, landslides and forest fires (Walker 1991, Weaver 1989). In the Caribbean, hurricanes are less severe and frequent (Richardson et al. 2010) and are major disturbances that can have lasting effects on ecological processes and forest structure (Sanford and others 1991, Van Bloem and others 2005). Hurricane disturbance in forests results in canopy opening, fallen trees, and leaves that accumulate on the ground and alter physicochemical characteristics of this habitat, as well as decomposer activity and nutrient fluxes (Walker et al. 1991; González et al. 2014). Hurricanes defoliate and deposit large amounts of biomass (debris) in the forest floor (Walker et al. 1991). In Puerto Rico, Hurricane Hugo caused massive defoliation, loss of branches, and the falling of trees, resulting in 56% of tree defoliation, 9% uprooted trees and 11% logs split (Walker, 1991). Moreover, during a hurricane simulation experiment in Luquillo Experimental Forest (LEF), it was found that an increase in precipitation, soil moisture, increase in solar radiation, and decrease in litter humidity, lead to a decrease in the abundance and diversity of various groups of soil animals (Shiels et al. 2010). The coarse-woody debris added after the passage of a hurricane reduces plant growth due to immobilization of nutrients (Zimmerman et al.1995) brought about by the significant reduction of the functional diversity of microbial communities (Willing et al. 1996). Furthermore, canopy opening also changed the microclimate on the forest soil, drying the surface litter due to increased light (Miller and Lodge, 1997). The increased light reduced litter moisture and inhibited litter fungi (Basidiomycota) simultaneously, which degraded lignin and was responsible for the

translocation of the limiting nutrients, thus causing delay in decomposition (Shiels et al. 2015).

Litter Decomposition

The regulation of the decomposition is given by the three factors that influence this process; the quality of the resource, the physical-chemical conditions, and the decomposer organisms. As a consequence, any change in habitat may produce micro variations in these factors, which in turn affect decomposition (Swift et al. 1979). The quality of the resource is defined by the chemistry of plant residues; the C:N ratio, lignin content, and polyphenols (González et al. 2002). The content of lignin and polyphenols influences leaf litter quality, since the high lignin residues are slowly decomposed, and the polyphenol compounds found in leaf litter also delay decomposition (Brady and Weil 2014). Unlike regular leaf litter, hurricane leaf litter is dominated by green leaves, which have nutrients that have not yet been translocated (Richardson 2010) and have been shown to contain up to two times more nitrogen (N) and phosphorus (P) compared to senescent leaves (Lodge et al. 1991). This alters the quality of litter with nutrients that are retained in green leaves, since they are not subject to nutrient remobilization that occurs during senescence (Marschner 1995). Physicochemical conditions include both climate and soil parent material, and help determine abiotic soil characteristics that influence the litter quality, activity, and composition of the microbial and invertebrate communities (González 2002). The climate modifies the nature and litter decomposition rate on the surface of the soil, which influences the type and abundance of organic matter (Sánchez et al. 2008). In addition, decomposition is mainly the result of microbial activities, while the soil fauna is important to condition the litter and stimulate the actions of the microbial community (Coleman et al. 2004). The rate of decomposition is influenced partially by the combined activities of the soil biota. However, it is important to mention that the rate of

decomposition, together with nutrient dynamics, soil respiration, and soil structure formation, are also associated with other variables (Coleman et al. 2004).

Soil Fauna

The soil fauna is numerous and diverse, with a great variety of species including representatives of all terrestrial phyla. In addition, soil fauna can affect litter decomposition and the rates of mineralization directly (physically modifying the substrate and soil environments) or indirectly (through interactions with the microbial community) (González et al. 2002). Some are herbivores, which only feed directly on the roots of living plants, but mostly subsist on dead organic matter. Others are carnivores, parasites or higher predators (Coleman et al. 2004). In soil systems there is a large group of heterotrophs, these are found at various functional groups within the food web. There are three resolution levels; The micro food web, which include microbes and microfauna predators (nematodes and protozoans) are those animals that directly feed upon them and each other; Litter transformers (microarthropods) are including saprophagous mites and springtails and some macrofauna, wich convert organic matter into organic structure (fecal pellets) and ecosystem engineers (earthworms, termites and ants) are the ones that alter the physical structure of the soil, whose activities create structures that become habitat for other organisms (Wardle, 2002). The litter transformers are an example of direct modulation, they feed on large pieces of litter, transforming them into smaller pieces with a larger surface area stimulating the activity of decomposing microorganisms. On the other hand, there are microfauna predators (collembola and acari) that selectively feed on decomposing fungi reducing their biomass and alter the composition of the community of fungi (Barberena-Arias et al. 2012). Litter arthropods represent an important component in ecosystems, since they modulate the decomposition of organic matter, thus influencing the rate of decomposition and the release of nutrients (Swift et al. 1979).

Microbial Communities

Microbial communities may be affected by hurricane disturbance and forest management practices that remove canopy and debris from forest land. For example, the soil moisture is a key factor controlling the biovolume of fungi (Lodge et al.1994) and microbial biomass in the soil and leaf litter of tropical forests. In tropical forests, soil and litter microbes are composed of a diverse group of microorganisms such as eubacteria, archaea, fungi, actinomycetes, algae, protozoa and nematodes (Ortíz 2008). While the decomposition is progressing, the process is slower and specialized fungi participate, such as Ascomycetes, Basidiomycetes, and Actinomycetes. These organisms participate in the degradation of recalcitrant components (cellulose, lignin, and more complex proteins) (Crespo 2015). Microbial communities in forest soils are involved in important ecosystem processes such as the decomposition of organic matter and the recycling of carbon and inorganic nutrients such as nitrogen (Cantrell et al. 2014). Microbial biomass reflects the state of accumulation and conservation of nutrients in soil, microorganisms make nutrients available for plant growth (González et al. 2002). Microbes colonize and chemically process organic matter through their enzymatic activities that release nutrients from complex compounds and minerals from the soil (Sharma et al. 1995). The release of nutrients generated during litter decomposition is an important nutrient flow pathway in forest ecosystems (González 2014). The rate of nutrients released will depend on several factors, including the initial amount of nutrients in the litter, the structural (molecular) nature of the nutrients in the litter matrix, the microbial demand for nutrients and availability of exogenous sources of the nutrient and composition of the faunistic community (Seastedt, 1984). Therefore short term changes in microbial communities, due to forest alterations may also have significant implications for litter decomposition, soil organic

matter accumulation, nutrient cycling, and the food web dynamics in tropical forests (Cantrell et al. 2014).

Microbial and Soil Fauna Interactions

Litter decomposition involves the activities of soil biota, where interactions occur between microbes and soil fauna, which cause effects in the process. Bacteria and fungi are principal decomposers which participate in the early stages of decomposition and consume mainly sugars and amino acids (Cardona et al. 2009). They are capable of degrading complex carbohydrates in detritus (Sánchez et al. 2008). In the microfood web, the grazing of bacteria by nematodes and protozoa (these feed on bacteria) stimulates microbial productivity. Grazing of fungi may stimulate or reduce the rate of decomposition, which may lead to immobilization of nutrients (Darbyshire et al. 1994). On the other hand microfauna herbivores excrete large amounts of leftover nutrients (nitrogen, phosphorus and sulfur) and these elements are converted from organic to inorganic (mineralized) form, which will then be available to plants. In this process the bulk of net primary production, waste and animal excretions are returned to the soil as dead organic matter. While the soil fauna are secondary consumers, they feed on microbes and others, therefore they have a great importance in the domain of these microbial processes (Wardle 2002). Other organisms that also affect the process of decomposition are the predators of higher level, these alter the microbial activity which causes effects on the mineralization. This has been shown for a wide variety of predators such as; nematodes, mites, spiders and salamanders (Zheng et al. 1993). Although total biomass of biota constitutes a relatively small fraction (1-8%) of total organic matter in the soil, several authors state that the functional importance of these organisms in ecosystems is not directly proportional to their existing biomass (Sánchez et al. 2008).

Previous Studies

Most studies focus on the quality of litter and climatic effects on decomposition, leaving out the soil animals that are part of the process of decomposition. However the decomposition of the litter depends on several soil animal interactions in order to carry out the entire process. Furthermore the interactions of the soil fauna and microbes on nutrient mineralization would depend on the feeding behavior of the soil fauna on the microbial community (González et al. 2002). Similarly, ecological studies in tropical areas most focus on organisms that participate in grazing food webs but not in diversity of invertebrates in soil, leaf litter or dead wood that participate in detrital food webs (González and Barberena-Arias, 2017). Usually decomposition studies focus on mass loss, and few have measured actual nutrient mineralization. While the mineralization of nutrients is regulated by the resolution level of the basal consumers (bacteria and fungi) in the soil food web and this process is affected by soil animals of higher levels (protozoa, nematodes, mites, springtails, millipedes and earthworms) (Wardle 1999). In a recent study carried out to test whether the soil fauna produces different effects in the litter decomposition with different climates or substrates in tropical and temperate ecosystems, the decomposition rates and the nitrogen flow were measured in the control treatments and excluded from the fauna with litterbags (González and Seastedt, 2001). The study have examined two different tree species; *Cecropia scherberiana* (high quality leaf litter) and *Quercus gambeii* (low quality leaf litter) in places with different climates (wet and dry forest and two temperate and subalpine forests). According to the results of González and Seastedt, 2001, soil fauna has a greater effect on litter decomposition in a tropical wet forest than in tropical dry forest or subalpine forests, and soil fauna influenced the percentage of litter mass loss and nitrogen mineralization rates at any given time. It was

shown that all factors (climate, litter quality and soil fauna) independently influence the litter decomposition rate in tropical and subalpine forests.

Previous studies in El Verde (Luquillo Experimental Forest), indicate that the effect of tree falls due to hurricanes and the variations in rainfall that occurs after the opening in the canopy alters the composition of microbial communities and reduce leaf litter species richness (Lodge and Cantrell 1995). In another study of Richardson et al. 2010, they focused on the effects of a hurricane on invertebrate communities in the forest floor used litterbags, to investigate the separate biotic and abiotic consequences of damage caused the hurricanes. In order to carry out the study, the canopy was opened causing a greater runoff, soil moisture and light levels, nonetheless the humidity of the litter decreased. Furthermore, the plots that represented the greatest disturbance, with open canopy and added remains, resulted in a lower abundance and biomass of invertebrates. Therefore, increasing nutritional or habitat complexity may have beneficial effects only if abiotic conditions are adequate (Richardson et al. 2010). However, the effects of hurricanes on decomposition and nutrient recycling, microorganisms and litter fauna, have been less investigated (Richardson et al. 2010, González et al. 2014).

Litter decomposition studies of forest that have been conducted are focused on the effects of disturbances on populations and communities of plants and animals, giving little attention or leaving out microbes and soil animal communities (Willig et al. 1996). Moreover most decomposition studies exclude the explicit importance of the decomposer's biota in the litter decomposition process (González and Seastedt, 2001). Therefore it is important to explore and expand litter decomposition studies, giving importance to the role played by faunistic communities in the decomposition process. To study of interaction between litter invertebrates was using three different mesh sizes of litterbags, using soil animal size as a substitute of the three functional group (micro-food

web, litter transformers and ecosystem engineers) and litter quality by quantifying litter decomposition, available nutrients and invertebrates and microorganisms diversity with the specific objectives: (1) Study the effect of the canopy opening and debris deposition on litter invertebrates, microorganisms and litter decomposition. (2) Evaluate nutrient release, arthropod and microorganisms diversity at three different mesh sizes of litterbags. (3) Determine the rate of litter decomposition at three different mesh sizes of litterbags. This research will contribute to understand the diversity and trophic dynamics of litter invertebrates and microorganisms and their combined contribution to nutrient mineralization. This study will provide information that will be useful to distinguish the contribution of the soil fauna and microorganisms in the process of decomposition of leaf litter. This information will offer us with tools to understand the dynamics in the food web that occur in response to the effect of hurricanes. In addition, the results may contribute to the conservation of soil biodiversity and fertility and create useful tools to suggest new strategies for soil management.

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Chapter 2

Change in arthropod composition in green leaf litter over a six-month litterbag decomposition experiment using different mesh size in a simulated hurricane experiment.

Introduction

Litter decomposition is mainly the result of microbial activities, while the soil fauna is important to condition the litter and stimulate the actions of the microbial community (Coleman et al. 2004). The rate of decomposition is influenced partially by the combined activities of the soil biota. However, it is important to mention that the rate of decomposition, together with nutrient dynamics, soil respiration and soil structure formation, is also associated with other variables (Coleman et al. 2004). In most studies that exclude soil fauna, they use the litterbag decomposition technique in different mesh sizes to exclude specific groups (microfauna, mesofauna and macrofauna) from the soil fauna (Bradfort et al. 2002). The mesofauna has received a lot of attention, since it contains two groups of abundant and diverse arthropods, the *Collembola* (springtails) and *Acari* (mites), together with smaller groups such as *Protura*, *Diplura, Pseudoscorpions, Symphyla* and *Pauropoda*. In addition *Collembola* and the most important group, the Oribatida, are important members of the fauna that have colonized virtually all terrestrial habitats around the world (Kampichler and Brucknuer, 2009). To demonstrate the important role of the composition of the soil fauna in the decomposition, litterbags of different mesh sizes have been used to control the entry of soil animals with different body sizes (Bradford et al. 2002) and determine the impact of different soil animals on the litter decomposition rate (Bokhorst and Wardle, 2013).

The soil biota are numerous and diverse, with a great variety of species including representatives of all terrestrial phyla. Some are herbivores, which only feed directly on

the roots of living plants, but most subsist on dead organic matter. Others are carnivores, parasites or higher predators (Coleman et al. 2004). In soil systems there is a large group of heterotrophs, these are found at various resolution levels within the food web. There are three functional groups (Wardle, 2002); Ecosystem engineers (earthworms, termites and ants) are the ones that alter the physical structure of the soil, the nutrient rate and the energy flow; Litter transformers (microarthropods); The micro food web, which include microbes and microfauna predators (nematodes and protozoans) (Wardle 2002). Ecosystem engineers include larger organisms, such as earthworms and ants, whose activities create structures that become habitat for other organisms, and that have symbioses with microorganisms in their gut (Wardle 1999, 2002). In addition the animals of the soil that are at higher levels (protozoa, nematodes, mites, collembola, millipedes and earthworms) also influence the decomposition process (Wardle 1999). When general categories such as microarthropod, beetles or Acari are used, they not have any functional meaning. However, the precise characterization of the soil community is important to identify interactions with other components of the soil fauna. The importance of an organism in a system is related to the function it plays and not to its size or biomass per se (Moore, Walter, and Hunt, 1988). Therefore there are six general trophic categories of below-ground arthropods: predators, fungivores, bacteriovores, detritivores, herbivores and omnivores (consumers of animal prey and other resources). These are used to demonstrate the regulation of the micro-food and mesobiota and the regulation of the decomposition (Walter 1987, 1988).

Litter arthropods represent an important component in ecosystems, since they modulate the decomposition process of organic matter, thus influencing the rate of decomposition and the release of nutrients (Swift et al. 1979). The fragmenting arthropods are an example of direct modulation, they feed on large pieces of litter transforming them

into smaller pieces with a larger surface area stimulating the activity of decomposing microorganisms. On the other hand, there are the pasture arthropods that selectively feed on decomposing fungi that reduce their biomass and alter the composition of the community of fungi (Barberena-Arias et al. 2012). Furthermore arthropods communities response to disturbance (e.g. Hurricanes) is highly variable because the changes in a habitat (landslides, floods, large amounts of fallen wood and canopy opening) (Walker et al. 1996) may increase resources for some species or reduce it for others (Barberena, 2002). These changes on disturbed habitat can do disappear some species as a result of local extinction of resources (Willig and Camilo 1991). However most studies concentrate on how the quality of litter and climatic variates affect decomposition, leaving out the soil animals that are part of the process (Gonzalez 2002). The effects of soil organisms on decomposition rates are considered inferior to other factors and are often not taken into account in decomposition studies (Wall et al. 2008).

To study the effect of the canopy opening and debris deposition on litter invertebrates, I focused on was in the interaction between litter invertebrates using three different mesh sizes of litterbags, using soil animal size as a substitute of the three functional group (micro-food web, litter transformers and ecosystem engineers). We predict that arthropod diversity of green leaf litter varies among the three litterbag mesh sizes in the control and treatment. To test this hypothesis, we studied the effect of the canopy opening and debris deposition on litter invertebrates and evaluate arthropod diversity in green leaf litter over a six-month litterbag decomposition experiment.

Materials and Methods

Study Site

This study was performed in the Luquillo Experimental Forest (LEF) (Fig. 1), located in northeastern (18.33080, -65.82320, WGS 84) Puerto Rico. The LEF is composed of four life zones that result from changes in elevation, climate and soil characteristics (Willig 1996; (García-Martinó et al. 1996). Specifically, the Tabonuco forest (*Dacryodes excelsa*) is classified as subtropical lower montane wet forest with average monthly of temperature of 21°C in January and 25 °C in September (Brown et al. 1983). Total annual precipitation is approximately 3.5 m (Gracía-Martinó et al. 1996), with approximately 97 rainless days per year. In this forest rainfall is weakly seasonal and has a dry season between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). Litterfall is seasonal, with a main peak from March to June, a secondary peak in September, and minima from December to February (Zou et al. 1995; Lawrence 1996; Zalamea and González 2008; Richardson et al. 2010).

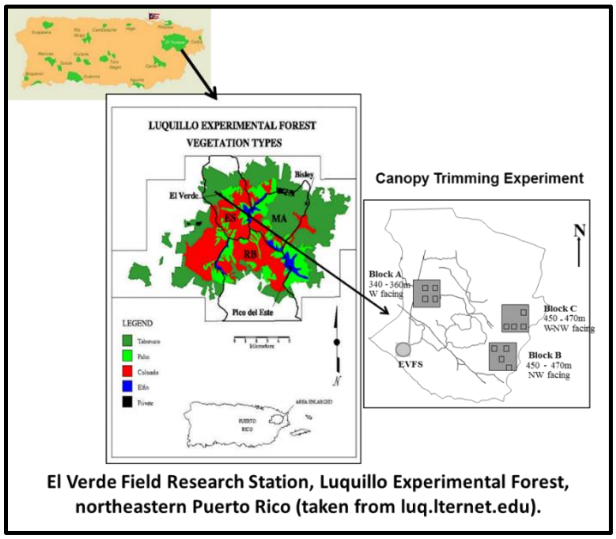

Figure 1. El Verde Field Research Station, Luquillo Experimental Forest, northeastern Puerto Rico.

Field Study Design

This study is part of the Canopy Trimming Experiment 2 performed by the Luquillo Long-Term Ecological Research (LTER) at El Verde Field Station. For this in the Tabonuco forest, three blocks (A, B and C) were selected, in each block two 20m x 20m plots were selected, one plot was control and the other was experimental (Trim+Detritus) (Shiels et al. 2010, Richardson et al. 2010, Shiels and González 2014) (Fig. 2). In the experimental plot, canopy was trimmed and debris was deposited on the ground simulating the impact that a hurricane has in this forest, from now on this is called Trim+Detritus. Each plot was subdivided into 16 sub-plots, and three sub-plots (5m x 5m) were randomly selected. In each subplot, litterbags with different mesh sizes were placed to be collected at four specific times. This experimental design represents 3 blocks x 2 plots/block (1 trim+detritus/ 1 control) x 3 subplots x 3 litterbag mesh sizes x 4 collecting times, for a total of 216 litterbags.

Figure 2. Control Plot **(a)** and Canopy opening (Trim+Detritus) Plot **(b)** showing canopy openness.

Litterbags

The three more common plant species (Zimmerman et al. 2014; Richardson et al. 2010) in the Tabonuco forest, were selected to control for the effect of litter quality, *Manilkara bidentata* (ausubo), *Dacryodes excelsa* (tabonuco) and *Prestoea acuminata var montana* (palma de sierra). Litterbags (Fig. 3a, 3b and 3c) were filled with a combination of green leaves from these 3 species, in equal amounts up to 15g. Litterbags mesh size were selected to represent three functional group (Wardle 2002) using animal body width (Swift et al. 1979) as a proxy. Small mesh (Figure 3a) had a pore size of 0.3 mm^2 mesh – allowing only micro-food web organisms to enter, which include microbes and micro predators (e.g., nematodes and protozoans) that feed upon the microbes (bacteria and fungi that break molecule); Medium mesh (Fig. 3b) had a pore size of 0.5 mm^2– that allowed micro-food web organisms, and mesopredators and small litter transformers them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; that feed on large pieces of litter, transforming them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; Large mesh (Fig. 3c) had a pore size of 3 mm^2 – that allowed all components of the decomposition food web to enter the bag except for large animals such as earthworms or large diplopoda (Wardle 2002). From now on, litterbags with small mesh size will be called small, medium mesh size will be called medium and large mesh size will be called large.

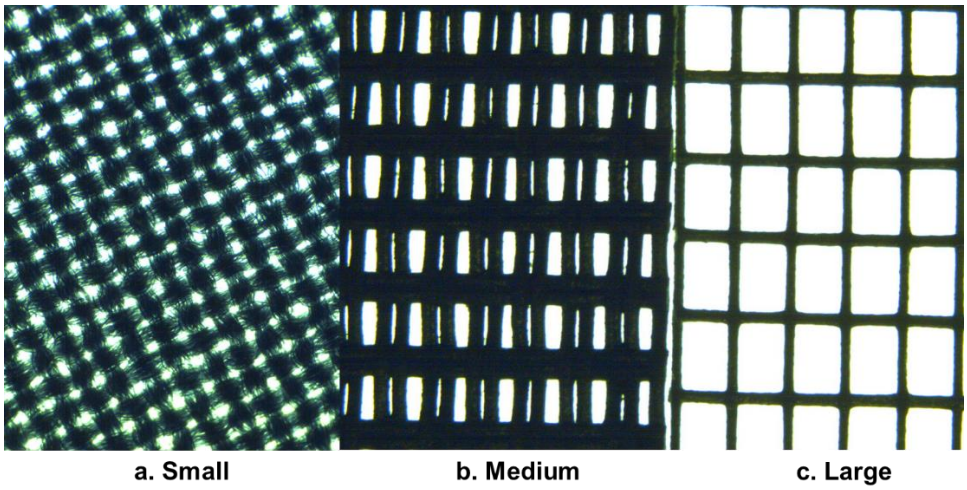

Figure 3. Photo of Litterbags showing differences among mesh sizes: a) small, b) medium and c) large. All photos were taken at the same magnification (40x) under a dissecting microscope. Photo resolution is 1400 x 1000).

Data Collection

Litterbags were retrieved at 21 days, 35 days, 84 days and 168 days after trimming in the laboratory, each sample was placed in Berlese Funnels for one week (Fig. 4a) for arthropod extraction (Walter et al. 1987; Barberena, 2012). The Berlese-Tullgren funnel uses (Walter et al. 1987) a light source to force organisms out of the sample (Sandler et al. 2010) as the sample dries.

Collected arthropods were counted and identified to order and family assigned to a trophic category based on their life stage and feeding habits (Fig. 4b) (Triplehorn and Johnson, 2005) and the abundance was standardized to individuals per gram of dry litter (Ind g^{-1} dry litter). For identification I used dichotomous keys such as Mites and others Microarthropod developed by the University of British Columbia, Biodiversity Research Center (http://www.zoology.ubc.ca/~srivast/mites/index.html), Araneae spiders of Europe

(https://araneae.nmbe.ch/key) and Insect Identification Key a key to Identify Insects in Michigan and beyond (http://www.knowyourinsects.org/index.html) using as reference the classification group, for of *Acari* (*Oribatida*, *Mesostigmata* and *Prostigmata*) in the key. Feeding habits were assessed using Borror and delong's introduction to the study of insects book (Triplehorn and Johnson, 2005).

Figure 4. Sample retrieved from the litterbags will be placed for Berlese-Tullgren funnels for arthropod extraction **(a)** and Arthropods sorted by family and order **(b)**.

A total of eight trophic categories were created: Detritivore-(e.g. *Acari, Coleoptera, Collembola, Diptera, Diplopoda, Thysanoptera, Plecoptera),* feed on decomposing dead organic matter of plants and animals; Omnivore-(e.g. *Blattodea, Hymenoptera),* feed on a mixture of plant and animal matter; leaves, fungus, small insects and dead animals; Predator-(e.g. *Acari, Araneae, Pseudoscorpions, Chilopoda*), feed on other animals (arthropods); Chewing herbivore-(e.g *Coleoptera*, *Lepidoptera)*, chew live plant materials; Animal exudate-(e.g. *Coleoptera* of Family*: Ptiliidae*), they feed on exudations (fluid emitted by an organism) from the bodies of animal (arthropods); Plant exudate- e.g. *Diptera* (Family: *Psychodidae* in adult stage), feed on plants generally exudation (fluid emitted by an organism) from the plant (e.g. flower nectar), Sucking herbivore-(e.g. Homoptera), feed on plant sap; Wood boring- e.g. *Coleoptera* (Family: Scolytinae in adult

stage), feed on decomposing wood and associate microbes (Triplehorn and Johnson, 2005).

Data Analysis

For data analysis we, used IBM Statistical Package for the Social Sciences (SPSS 20) and Principal Component Ordination (PC-ORD 4.0). A General Lineal Model (GLM) was used to determine the effects of treatment, mesh size and time on arthropod abundance and richness. Non metric multidimensional scaling (NMDS) and Multi Response Permutation (MRPP; PC-ORD 1999) were used to compare the species composition of arthropods. NMDS was executed with Sorensen's Coefficient dissimilarity index ($CCs=2 \cdot c/S1+S2$), where: CCs = Sorensen Coefficient, c- the number of species common to both communities, S1 - the number of species in community 1, S2- the number of species in community 2. Sorensen's Coefficient is based on the presence and absence, NMS was set to maximum of three axes and MRPP was used to determine differences in species composition between treatments, mesh sizes and through time.

Results

Arthropod Abundance and Richness

Although abundance in both treatment varied between 2 and 1 Ind g^{-1} dry litter (Fig. 5a), and between 2 Ind g^{-1} dry litter in medium mesh size and 1 Ind g^{-1} dry litter among mesh, there was no significant difference between treatments or among mesh size (Fig 5c).

Arthropod abundance was significant different through time and the number of taxonomic groups was significantly different between control and trim+detritus, among mesh sizes and through time (Table 1). In addition there was a minimum of 1 Ind g^{-1} dry litter in 21 days and a maximum 3 Ind g^{-1} dry litter in in 84 days (Fig. 5e). The number of taxonomic groups was higher in control (Fig. 5b), higher in large mesh size with 3 Ind g^{-1} dry litter (Fig. 5d) and higher in 21 days with a minima of 2 Ind g^{-1} dry litter in 21 days and higher 3 Ind g^{-1} dry litter in in 84 day (Fig. 5d).

Table 1. General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on the Arthropod Abundance and Richness. Showing *df*, *F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05

Effect		Abundance		Richness	
	df	*F*	*P*	*F*	*P*
Treatment	1	1.39	0.240	10.286	**< 0.002**
Size	2	1.425	0.243	29.696	**< 0.000**
Time	3	7.401	**< 0.000**	26.083	**< 0.000**
Treatment x Size	2	1.361	0.259	4.071	0.019
Treatment x Time	3	1.059	0.368	0.655	0.581
Size x Time	6	0.45	0.844	1.798	0.101
Treatment x Size x Time	6	0.581	0.745	0.601	0.729

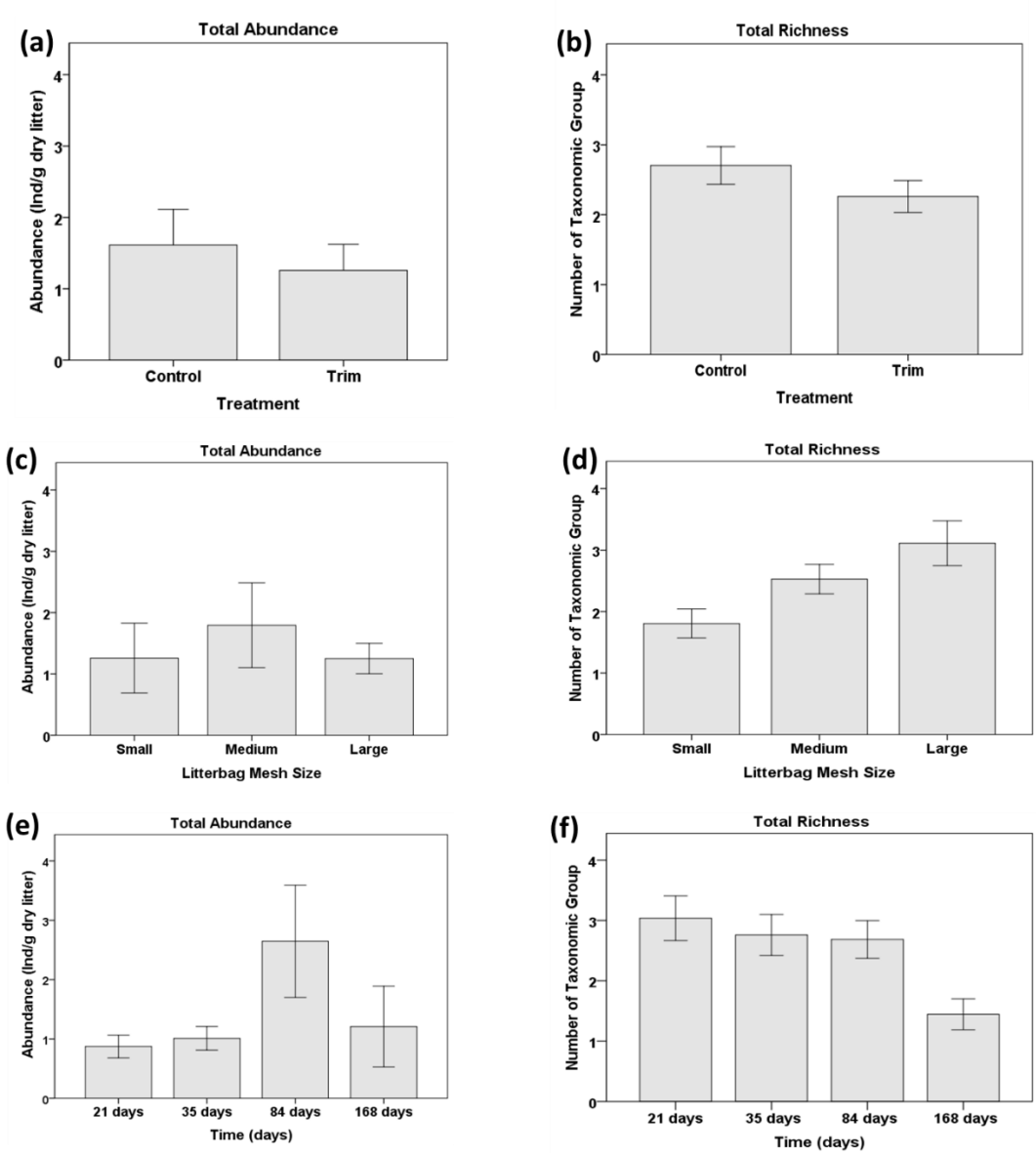

Figure 5. Arthropod abundance and richness (number of taxonomic group): **(a)** Abundance in treatments (Control and Trim+detritus); **(b)** Richness in treatment (Control and Trim+detritus); **(c)** abundant in mesh sizes (small, medium and large); **(d)** Richness in mesh size (small, medium and large); **(e)** Abundance trough the time (21 days, 35 days, 84 days, 168 days); **(f)** Richness trough the time 21 days, 35 days, 84 days,168 days). Bars represent standard error.

A total of 3347 individuals were collected that were distributed in 15 taxonomic groups and *Acari* was the most abundant order. *Acari, Coleoptera, Diptera, Hymenoptera* and *Collembola*, were the most common orders in both treatments. Also, *Blattodea, Lepidoptera*, *Pseudoscorpions, Thysanura, Coleoptera* were common to both treatments. On the other hand, *Plecoptera, Isopoda, Aranae,* and *Chilopoda*, *Diplopoda* and *Homoptera* were unique to control while no group was unique to trim+detritus (Table 2).

The number of orders in small, medium and large mesh sizes was higher in control than trim+detritus. Also, large mesh size was the one that had higher number of order for both treatments and small mesh size in trim+detritus was the one that had fewer order groups (Table 2). These results show a pattern for a higher number of orders in control than in trim+detritus regardless the mesh size. These results suggest that the biodiversity of trim+detritus is a subgroup of control, and that hurricanes affect the number of orders in trim+detritus in all the mesh sizes.

Table 2. Abundance of arthropods per taxonomic group across treatments and mesh size (small, medium and large) of each treatments. (±Standard Error). The letter *O* represent Order and the letter *C* represent Class.

Taxonomic Group	Control			Trim		
	Small	Medium	Large	Small	Medium	Large
O. Acari	58(±3)	108(±2)	170(±3)	31(±1)	98(±3)	115(±3)
O. Coleoptera	1(±0)	1(±0)	6(±0)	1(±1)	16(±1)	9(±1)
O. Diptera	11(±0)	14(±1)	17(±0)	4(±1)	21(±1)	35(±1)
O. Collembola	50(±13)	14(±1)	11(±0)	40(±7)	20(±3)	4(±1)
O. Blattodea	1(±0)	--	2(±0)	--	--	1(±0)
O. Thysanura	1(±0)	--	--	--	--	1(±0)
O. Homoptera	--	--	1(±0)	--	--	--
O. Hymenoptera	5(±1)	65(±9)	12(±2)	--	8(±4)	1(±0)
O. Lepidoptera	--	--	1(±0)	--	2(±0)	10(±1)
O. Pseudoscorpions	--	1(±0)	3(±0)	--	--	1(±0)
O. Isopoda	--	--	1(±0)	--	--	--
O. Aranae	--	--	2(±0)	--	--	--
C. Diplopoda	1(±0)	--	4(±1)	--	--	1(±0)
C. Chilopoda	1(±0)	--	1(±0)	--	--	- -
O. Plecoptera	--	1(±0)	--	--	--	- -

Through time, abundance showed a similar pattern in both control and trim+detritus except at 84 days where it was a peak in both treatments with 10 Ind g^{-1} dry litter in control and 8 Ind g^{-1} dry litter trim+detritus (Fig. 6a and 6b) and between mesh sizes, large mesh size was higher with 4 Ind g^{-1} dry to 10 Ind g^{-1} dry litter than small and medium with 1 to 10 Ind g^{-1} dry litter and regardless at time. This pattern is also found for richness in both treatments with 1 to 5 Ind g^{-1} dry litter in control and 1 to 3 Ind g-1 dry litter in trim+detritus (Fig. 6c and 6d).

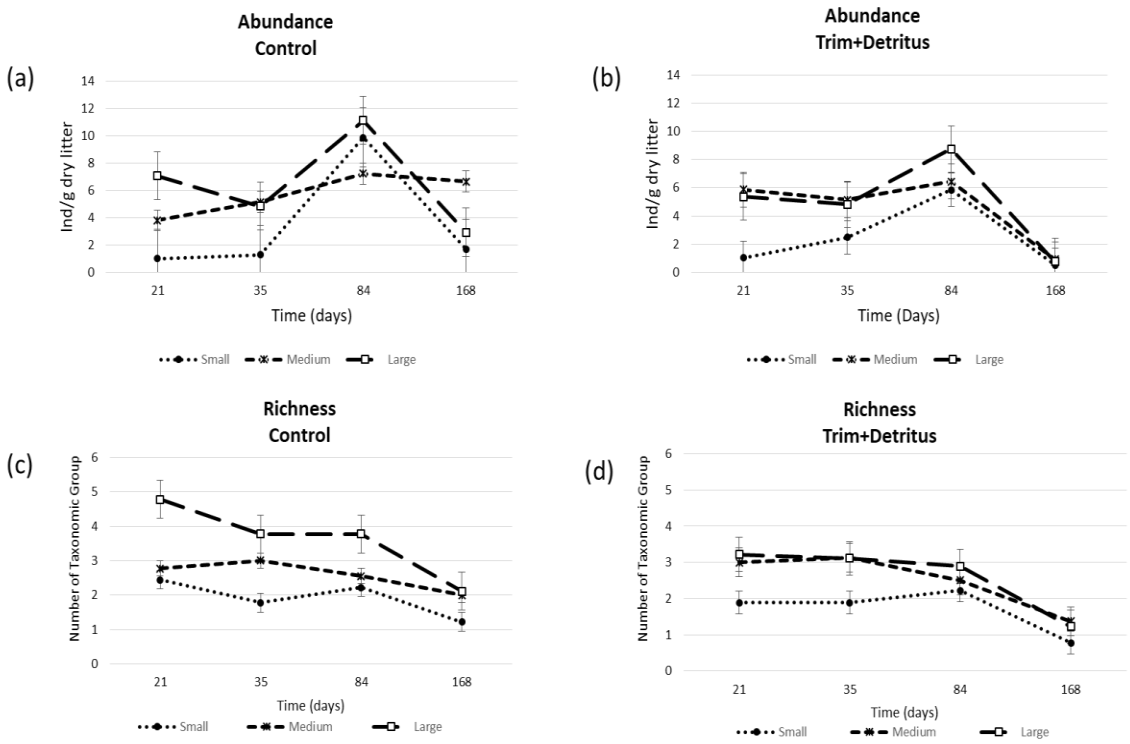

Figure 6. Average arthropods abundance (Ind g^{-1} dry litter) and richness (number of taxonomic group) of arthropods in each mesh size (small, medium and large): **(a)** Total abundance through time for Control and **(b)** Trim+Detritus treatment; **(c)** Richness through time for Control and **(d)** Trim+Detritus treatment. Bars represent standard error.

Arthropod Community Composition

Sorensen distance varies between 0 and 1 with values closer to 1 indicating high within group variability (McCune and Grace 2002). Table 3 shows within group Sorensen distance for treatment, mesh sizes and time, and the distance varied between 0.077 in large mesh size with and 0.650 at 168 days (Table 3). These data suggest that arthropod composition is highly variable across treatments, mesh sizes and time (McCune and Grace 2002).

Table 3. Arthropod community composition group average within Sorensen distance calculated from two different distance matrices for Treatment (control and trim+detritus) three different distance matrices for Litterbag size (small, medium and large) and four different distance matrices for Time (21 days, 35 days, 84 day, and 168 days).

Sorensen distance					
Treatment		**Litterbag size**		**Time (days)**	
Control	0.739	Small	0.733	21 days	0.726
Trim+Detritus	0.732	Medium	0.680	35 days	0.742
		Large	0.777	84 days	0.674
				168 days	0.650

Arthropod community composition was significantly different (MRPP, *P* = 0.0084) between treatments, mesh sizes and time. The test statistic (*T*) show separation between treatments (control and trim+detritus) (*T* = -3.328), between litterbag size (*T* = -5.185) (small, medium and large) and with the higher separation (*T* = -16.141) between times (21 days, 35 days, 84 days, 168 days). Furthermore the agreement statistic (*A*) show heterogeneity equals within groups in litterbag size (*A* = 0.015), treatments (A = 0.007) and time (*A* = 0. 056). These results suggest that identities of arthropod group associate with decomposing litter varies between environmental condition (control and control+detritus), among decomposing functional groups (mesh sizes) and through time (Table 4).

Table 4. Summary statistics for MRPP to compare diversity patterns and species composition of arthropods. Results of Taxonomic Group Sorensen distance, comparing three different factors: Treatment, Litterbag size and Time (days). *T*= test statistic, *P*= P-value and *A*= agreement statistic.

	Observed	Expected	Variance	Skewness	*T*	*P*	*A*
Treatment	0.735	0.740	0.000	-1.276	-3.328	**0.0084**	0.007
Litterbag Mesh Size	0.729	0.740	0.000	-0.903	-5.185	**0.0003**	0.015
Time (days)	0.699	0.740	0.000	-0.737	-16.141	0	0.056

Pairwise comparisons (Table 5) show in litterbag mesh size that large vs small was significant different (MRPP, *P* = 0.000) and the test statistic (*T*) shows high separation between group (*T* = -5.596) also in medium compared to small (MRPP, *P* = 0.001) and (*T* = -5.100). While for large compared to medium there was no significant difference (MRPP, *P* = 0.325) however show heterogeneity equals (A = 0.001) within groups. Moreover for Time (days), 21 days compared to 84 days and 168 days was significant different (MRPP, *P* = 0.000). While 35 days compared to 84 days and 168 days was significant different (MRPP, *P* = 0.000). And 84 days compared to 168 days was significantly different (MRPP, *P* = 0.000). The test statistic (*T*) show higher separation between 84 days compared to 168 days (*T* = -14.280) while 21 days compared to 35 days show heterogeneity equals (A = 0.000) within groups. These data suggest that the groups of arthropods in small mesh size are different than in medium and large. In addition, the data for 21 to 35 days suggest that, in early decomposition, during the first month, arthropods were homogeneous, but in the following times the associated arthropods are highly variable.

Table 5. A posteriori pairwise comparisons for Sorensen distance among mesh sizes (small, medium and large) and across collection days and four different distance matrices for Time (21 days, 35 days, 84 days 168 days). *T*= test statistic, *P*= P-value and *A*= agreement statistic.

Multiple comparisons (Sorensen)			
Litterbag Mesh Size	***T***	***P***	***A***
Large vs. Medium	-0.242	0.325	0.001
Large vs. Small	-5.596	0.000	0.016
Medium vs. Small	-5.100	0.001	0.015
Time (days)			
21 days vs. 35 days	0.043	0.435	0.000
21 days vs. 35 days	-12.153	0.000	0.046
21 days vs. 168 days	-9.904	0.000	0.044
35 days vs. 84 days	-6.447	0.000	0.024
35 days vs. 168 days	-12.434	0.000	0.054
84 days vs. 168 days	-14.280	0.000	0.065

Arthropod Trophic Composition

The number of trophic groups was significantly different among mesh sizes and through time but there was no difference between control and trim+detritus treatment (Table 6). Also, the number of trophic group was higher in small with (2 Ind g^{-1} dry litter) than in large and medium litterbags mesh size (Fig. 7b) and the number of trophic group was similar across time with a minima in 34 days (Fig. 7c).

Table 6. General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on the Number of Trophic Group. Showing *df*, *F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05.

Effect	*df*	*F*	*P*
Treatment	1	3.389482	0.067
Size	2	15.15213	**< 0.000**
Time	3	16.41326	**< 0.000**
Treatment x Size	2	3.067431	0.049
Treatment x Time	3	0.661494	0.577
Size x Time	6	8.709083	0.000
Treatment x Size x Time	6	0.68987	0.658

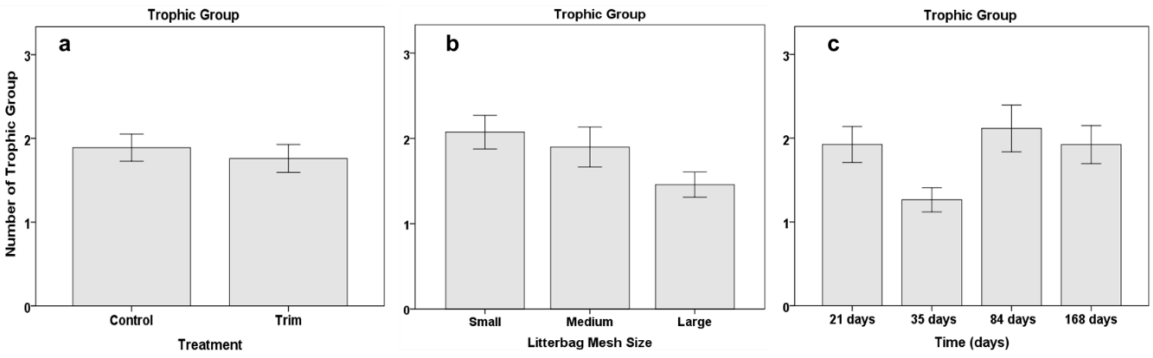

Figure 7. Number of trophic group: 7a) Number of trophic group between treatment (Control and Trim+Detritus); 7b) Number of trophic group between different litterbag size (small, medium and large); 7c) Number of trophic group trough the time (21 days, 35 days, 84 days 168 days). Bars represent standard error.

Abundance and Richness within Trophic Groups

Abundance within trophic group was significantly different through time (Table 7) for detritivore, chewing herbivores and animal exudate. However only chewing herbivore, animal exudate (feed on exudations from the bodies of arthropods), plant exudate (feed on plants generally exudation from plant e.g. flower nectar) and wood boring was significantly different in treatment. Among mesh sizes there was significant difference for predators, chewing herbivore and wood boring.

Table 7. General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on the Trophic Group. Showing *df, F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05.

Effect	Detritivore			Predator		Omnivore		Sucking herbivore		Chewing herbivore		Animal exudate		Plant exudate		Wood boring	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	0.999	0.319	0.329	0.567	3.637	0.058	2.725	0.100	12.338	**< 0.001**	13.368	**< 0.000**	2.939	**< 0.088**	9.139	**< 0.003**
Size	2	0.159	0.853	4.805	**< 0.009**	2.617	0.076	1.415	0.246	12.506	**< 0.000**	1.259	0.286	1.950	0.145	7.045	**< 0.001**
Time	3	9.072	**< 0.000**	1.798	0.149	0.788	0.502	0.920	0.432	6.690	**< 0.000**	5.434	**< 0.001**	1.486	0.220	2.115	0.100
Treatment x Size	2	0.370	0.691	0.586	0.557	1.627	0.199	1.415	0.246	7.297	**< 0.001**	1.043	0.354	1.950	0.145	7.045	**< 0.001**
Treatment x Time	3	0.769	0.513	2.931	**< 0.035**	0.784	0.504	0.920	0.432	7.012	**< 0.000**	5.612	**< 0.001**	1.486	0.220	2.115	0.100
Size x Time	6	0.971	0.446	1.486	0.185	0.861	0.525	0.614	0.719	3.386	**< 0.003**	2.495	**< 0.024**	0.869	0.519	1.737	0.114
Treatment x Size x Time	6	0.441	0.851	1.159	0.330	0.946	0.463	0.614	0.719	3.648	**< 0.002**	2.589	**< 0.020**	0.869	0.519	1.737	0.114

Detritivores, predators and omnivores were the more abundant trophic groups in both treatments (Control and Trim+detritus). Also, detritivores, predators, omnivores, chewing herbivores, sucking herbivores and animal exudate trophic groups were common to both treatment. However Wood boring were present only in control treatment and plant exudate only in trim+detritus treatment (Table 8). The number trophic group in small, medium and large mesh sizes was higher in trim+detritus than in control. Also, large mesh size was the one that had higher number of trophic groups for both treatments and small mesh size in control was the one that had fewer order groups (Table 8). These results

show a pattern for a higher number of trophic groups in trim+detritus than in control regardless the mesh size.

Table 8. Total average trophic group abundance between the different litterbag size (small, medium and large of each treatment. Lowercase letters indicate significant differences among litterbags size. (±Standard Error).

Trophic Group	Control			Trim + Detritus		
	Small	Medium	Large	Small	Medium	Large
Detritivore	110 (±6)	108 (±3)	190 (±3)	79 (±3)	125 (±2)	138 (±2)
Predator	10 (±2)	30 (±1)	25 (±1)	5 (±1)	21 (±1)	20 (±1)
Omnivore	5 (±1)	66 (±9)	14 (±2)	0 (±0)	3 (±4)	- -
Sucking herbivore	- -	0 (±0)	1 (±0)	- -	8 (±0)	2 (±0)
Chewing herbivore	- -	- -	1 (±0)	1 (±0)	2 (±0)	10 (±1)
Animal exudate	- -	- -	- -	3 (±1)	9 (±1)	6 (±1)
Plant exudate	- -	- -	- -	- -	- -	2 (±0)
Wood boring	- -	- -	2 (±0)	- -	- -	- -

Within trophic group abundance varied in each mesh size (small, medium and large) through time for Control and Trim+Detritus. Detritivore, Predator and Omnivore were the more abundant trophic group in both treatments (Control and Trim+Detritus) (Table 8) and (Fig. 8Aa, 8Bb and 8Cc). Detritivore was trend higher in control treatment (Fig. 8A) and in small litterbag mesh size than in medium and large litterbag mesh size, these last both was a similar abundance. Moreover the abundance was higher in 84 days through time; Predator: was trend higher in trim+detritus treatment (Fig. 8b) and higher medium litterbag litter mesh size than in small and large litterbag mesh size and the abundance was trend higher in 84 days through time; Omnivore: was trend higher in control treatment (Fig. 8C) and in medium litterbag mesh size than in small and large

litterbag mesh size. While the abundance was trend higher in 168 days through time in control treatment.

While sucking herbivore: was trend higher in control treatment (Fig. 8D) and in large litterbag mesh size than in small and medium litterbag mesh size. Moreover the abundance was higher in 35 days through time; Chewing herbivore: was higher in trim+detritus treatment (Fig. 8e) and was significant different among litterbag size, in large than in small and medium litterbag. In addition the abundance was higher in 21 days through time; Animal exudate: was higher in trim+detritus treatment (Fig. 8f) and trend higher in medium litterbag than in small and large litterbag. Moreover the abundance was higher in 35 days through time; Plant exudate: was only in trim+detritus treatment (Fig. 8e) and was trend higher in large litterbag mesh size than in medium litterbag mesh size. Furthermore the abundance was trend higher in 21 days through time; Wood boring: was only in control treatment (Fig. 8H) and was trend higher in large litterbag than in small litterbag mesh size. Moreover the abundance was trend higher in 84 days through time.

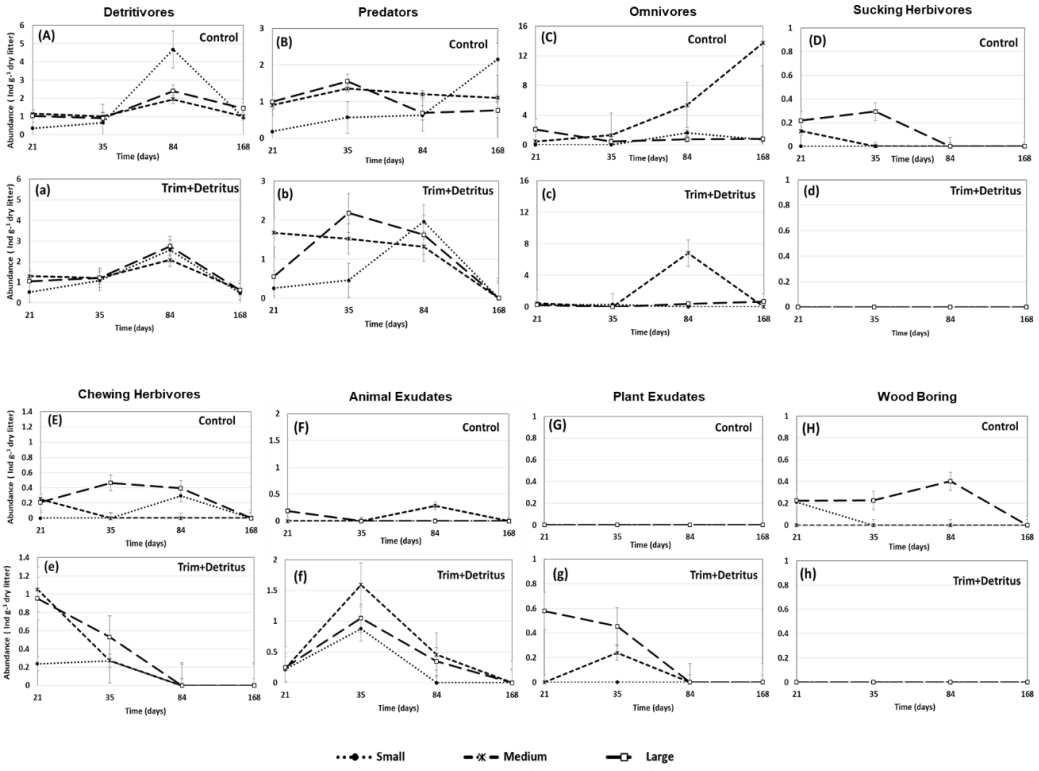

Figure 8. Average trophic group abundance (Ind g^{-1} dry litter) in each mesh size (small, medium and large) through time for Control and Trim+Detritus: (Aa) Detritivore (feed on decomposing dead organic matter of plants and animals), (Bb) Predator (feed on other animals), (Cc) Omnivore (feed on a mixture of plant and animal matter), (Dd) Sucking Herbivore (feed on shrubs, trees and herbaceous and extract plant sap), (Ee) Chewing Herbivore (feed on plant material, (Ff) Animal Exudate (feed exudation from the animal body), (Gg) Plant Exudate (feed exudation from plant) and (Hh) Wood boring (feed mainly on fungi in the wood). Bars represent standard error.

Discussion

Arthropod abundance and richness

In this study, abundance was significant different through time but not between treatment and among mesh size. In addition, abundance was higher in 84 days in both treatments. Canopy trimming resulted in abiotic changes: increase throughfall, higher soil moisture, higher solar radiation and decrease litter moisture (Shiels et al. 2015). Furthermore canopy opening is the major determinant of community change by reducing arthropod diversity and biomass (decrease detritivores; e.g. *Coleoptera* and *Diplopoda*) and changes in fungivores (e.g. *Collembola* and *Mites*) (Richardson et al. 2010). We predicted that arthropod diversity of green leaf litter varies among the three mesh sizes in the control and trim+detritus treatment. However abundance was not affected with mesh size or treatments.

Our results showed that richness was significantly different between control and trim+detritus treatment, among mesh sizes and through time. The changes in habitat caused by disturbances (e.g. hurricane) affect the composition of arthropod communities since changes in abundance and distribution of resources can modify the microhabitat used by arthropods (Barberena, 2012). In addition, canopy opening and debris (trim+detritus treatment) provides more organic matter for arthropods, this stimulates the activity of soil animals in the early time (days). In this study, *Acari* was the most abundant order and common in both treatments and the number of taxonomic group was higher in control than trim+detritus and in time 21 days (early time) in large mesh size. These results showed a patter for a higher number of orders in control, suggest that the biodiversity of trim+detritus is a subgroup of control, and that hurricanes affect the number of orders in trim+detritus in all mesh sizes.

Abundance of arthropods and richness showed a similar pattern among sizes in both treatments. Through time, the small mesh size was always the lowest and large mesh size was always the highest, while medium mesh size was intermediate for both. Furthermore, abundance results showed a peak in 84 days, while in richness there was a decrease for the same time. These data suggest that abundance and richness of arthropods are not affect similarly, it also suggests that the biodiversity of trim+detritus is a subgroup of control and the effects of the hurricane affect the number of order in trim+ detritus in all sizes of mesh.

Arthropod community composition

Our results about Sorensen distance suggest that arthropod composition is highly variable across treatments, mesh sizes and time, showed a high dispersion of species between treatments, litterbag mesh size and through time. Also, arthropod community results suggest that identities of arthropod group associate with decomposing litter varies between environmental condition (control and trim+detritus). For example, Barberena (2002) found that although the structure of the habitat is affected (e.g. hurricane disturbance), arthropods with high dispersal capacity (e.g. Diptera, Acari, Collembola) can contribute to great changes in insect communities because they move across wide spatial ranges following their resources. Our results suggest that identities of arthropod group associate with decomposing litter varies between environmental condition (control and trim+detritus) and for 21 and 35 days the data suggest that arthropods in early decomposition during the first month were more homogeneous than the following times were arthropods highly variable.

The species composition was significantly different across mesh sizes and through time. There were 15 orders, small mesh did not show any unique order, while large mesh size had four unique orders. While through time the highest number of orders was in 21 days and the lowest number was at 168 days. In addition, in small mesh size only microfood web arthropods entered, in medium mesh size mesoarthropods entered and in large all size arthropods were allowed . These data suggest a great variability between the functional groups (mesh size) and through time and also that the effect of trim+detritus affects the composition of species and taxonomic groups. Also, given that species composition was significantly different across mesh sizes, then arthropods associated to decomposing matter vary in relation to decomposer functional groups. Therefore, even though we can assume that large mesh size to include microfoodweb and mesoarthropods (represented in small and medium respectively), the fauna representative of different functional groups is associated to decomposing organic matter resulting in different food webs that differentially affect decomposition.

Arthropod trophic composition

In this study the number trophic group in small, medium and large mesh sizes was higher in trim+detritus than control and *Acari* was a most abundant taxonomic group for both treatments. Also, Detritivore, Predator and Omnivore were more abundant trophic groups in both treatments (Control and Trim+Detritus). Detritivores (e.g. *Acari, Coleoptera*, *Collembola*) and Predators (e.g. *Ac*ari, *Araneae)* they are important role in the litter decomposition. In Puerto Rico studies (Petersen 1982) numerically, mites usually comprise the most abundant taxon in forest litter. Also, our results show a pattern for a higher number of trophic groups in trim+detritus than control regardless the mesh size.

These results suggest that the trophic group varies little in time and space, but the species composition of the trophic group can be very variable.

The results of trophic groups showed that detritivores, predators and omnivores were the most common in all sizes and through time for both treatments. Detritivore was the most abundant for both treatments and was highest in small mesh size, where most of the organisms that feed on decomposing organic matter are found. In addition, our results show that in control the detritivores had a peak in 84 days while the predators had a peak in 168 days, suggesting trophic dynamics where detritivores are prey for predators (Wardle 2002). These data suggest that while the decomposition proceeds, resource quality changes producing variations in detritivores, for example early decomposition might represent a high quality resource that promotes detritivore abundance, while later on resource quality decreases and detritivore abundance also decrease. In the mesh sizes it was observed that small and medium only had specific trophic groups, this based on the functional groups that could enter each of them, while in large mesh size it could entered those of small and large, however if in large they entered other organisms, then the composition of those groups changed.

Conclusion

In this study, we showed mesh size not affect total abundance but can have a significant effect on taxonomic groups and trophic groups. Thus, we conclude that through time arthropod abundance, taxonomic groups and trophic groups were significantly different with a peak in 84 days. While *Acari* (detritivores) was the most abundant and common for both treatments (control and trim+detritus). Furthermore the taxonomic groups of trim+detritus were a subgroup of Control, however the number of trophic group

in control were a subgroup of trim+detritus. These data suggest that the composition of the trophic groups does not vary according to the composition of the taxonomic groups. Because there was always some species that represented the trophic group, although not all the taxonomic groups were present in both treatments and in all sizes. Consequently, in this research we could saw that litterbag decomposition technique in different mesh sizes to exclude specific groups is useful for that exclude soil fauna and see the different role played of soil fauna in litter decomposition. In addition, this research gives data about the importance to know the role played by animal soil communities in the litter decomposition process.

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Chapter 3

Effect of litterbag mesh size on Fungi during green litter decomposition in a simulated hurricane experiment.

Introduction

Canopy defoliation and the deposition of large amounts of biomass, after a hurricane, generate disturbances that can increase the loss of nutrients in the forest, this will depend on the management of the residues, the response of the microbiota to the disturbance, and the chemical and physical characteristics of the soil (Miller and Lodge 1997). The coarse-woody debris added after the passage of a hurricane reduces plant growth due to immobilization of nutrients (Zimmerman et al.1995) by significantly reducing the functional diversity of microbial communities (Willig et al. 1996). White-rot basidiomycetes are the most efficient biodegraders of lignin, breaking down bonds to expose the assimilable cellulose and hemicelluloses surrounding lignin. This assimilable cellulose and hemicellulose is available for the bacterial community to degrade it. While litter with more lignocellulose promotes the fungal decomposition pathway, which in turn favors soil and litter food webs dominated by arthropods. In addition, the reduction of moisture in litter inhibits fungi (Basidiomycota), which degrade lignin and are responsible for the translocation of the limiting nutrients, thus causing delay in decomposition (Shiels et al. 2015).

Litter decomposition depends primarily on the interaction among climate, litter quality and biota, as a consequence any change in habitats will result in changes in these factors. Also litter decomposition by micro-organisms is highly dependent on microclimate including in the litterbag environment (Aerts, 2006). Soil organisms have direct and indirect effects on litter decomposition (Petersen and Luxton, 1982) in litterbag the direct effects

include the consumption of litter and elimination of litter from litterbag, while indirect effects, include mesofauna imply modification of direct effect of one group of organisms (Bradfort et al. 2002) through the action of another (e.g. mesofauna that increasing surface for microbial colonization or collembolan overgrazing of fungi, reducing fungal biomass) (Petersen and Luxton, 1982). Consequently, any effects of litterbag mesh size on microclimate are therefore likely to affect the enzymatic processes and animal activity on the litter within the bags. For example, the mesh size could create a barrier of water entry into the bag affect the moisture in the bag (Bokhorst and Wardle, 2013). Therefore understanding the mechanistic processes of litter decomposition is essential for predicting nutrient cycling dynamics in tropical forests. While models of litter decay mostly rely on climate and litter chemistry, it is increasingly apparent that the decomposer communities (fungi, bacteria, and arthropods) interact during leaf litter decomposition significantly influencing decay rates and mineralization of nutrients.

Furthermore, microbial community structures may be affected by hurricane disturbance and forest management practices that remove canopy and debris from forest land (Cantrell et al. 2014). Canopy opening also change the microclimate in forest soil, while increasing soil moisture due to reduced evapotranspiration (Richardson et al. 2010). Short term changes in microbial communities due forest alterations may also have significant implications for litter decomposition, soil organic matter accumulation, nutrient cycling, and the food web dynamics in tropical forests (Cantrell et al. 2014). For example, moisture is a key factor controlling the biovolume of fungi (Lodge et al.1995) and microbial biomass in the soil and leaf litter of tropical forests. Microbial communities in forest soils are involved in important ecosystem processes such as the decomposition of organic matter and the recycling of carbon and inorganic nutrients such as nitrogen (Cantrell et al. 2014). Microbes colonize and chemically process organic matter through their enzymatic

activities that release nutrients from complex compounds and minerals from the soil (Sharma et al. 1995). Microbial biomass reflects the state of accumulation and conservation of nutrients in soil and microorganisms make nutrients available for plant growth (González et al. 2002).

Even though Lodge et al. (1994) and Miller and Lodge (1997) document the effect of disturbances in soil and leaf litter microbial communities, most of the studies that have been conducted are focused on the effects of disturbances on populations and communities of plants and animals, giving little attention or leaving out microbial communities (Willing et al. 1996). For this reason, I focused in study the effect of a hurricane in the food web dynamics specifically in microbial communities (fungi). Previous studies has demonstrated that the primary effect of hurricanes on detrital communities is through changes in the microclimate associated with canopy opening (increased solar radiation and decrease in leaf litter moisture) (Miller and Lodge, 1997). While Lodge and Cantrell (1995) found that canopy opening caused by Hurricane Hugo resulted in basidiomycete colony extinctions on ridges. Consequently, we predict that microbial diversity of green leaf litter varies among the three litter bag mesh sizes in the control and treatment, expecting that the decrease in green leaf litter moisture, associated with the opening canopy affects the diversity of fungi. To test this hypothesis, we studied the effect of the canopy opening and debris deposition on microbial communities (fungi) diversity green leaf litter over a six-month litterbag decomposition experiment.

Materials and Methods

Study Site

This study was performed in the Luquillo Experimental Forest (LEF) (Fig. 1), located in northeastern (18.33080, -65.82320, WGS 84) Puerto Rico. The LEF is composed of four life zones that result from changes in elevation, climate and soil characteristics (Willig 1996; (García-Martinó et al. 1996). Specifically, the Tabonuco forest (*Dacryodes excelsa*) is classified as subtropical lower montane wet forest with average monthly of temperature of 21°C in January and 25 °C in September (Brown et al. 1983). Total annual precipitation is approximately 3.5 m (Gracía-Martinó et al. 1996), with approximately 97 rainless days per year. In this forest rainfall is weakly seasonal and has a dry season between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). Litterfall is seasonal, with a main peak from March to June, a secondary peak in September, and minima from December to February (Zou et al. 1995; Lawrence 1996; Zalamea and González 2008; Richardson et al. 2010).

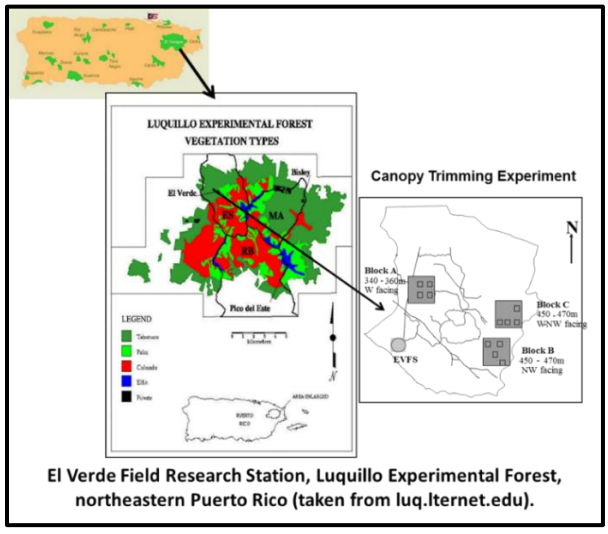

Figure 1. El Verde Field Research Station, Luquillo Experimental Forest, northeastern Puerto Rico.

Field Study Design

This study is part of the Canopy Trimming Experiment 2 performed by the Luquillo Long-Term Ecological Research (LTER) at El Verde Field Station. For this, three blocks (A, B and C) were selected, in each block two 20m x 20m plots two were selected, one plot was control and the other was experiment (Trim+Detritus) (Shiels et al. 2010, Richardson et al. 2010, Shiels and González 2014) (Fig. 2). In the experimental plot, canopy was trimmed and debris was deposited on the ground simulating the impact that a hurricane has in this forest, for now on this is called Trim+Detritus. Each plot was subdivided into 16 sub-plots, and three sub-plots (5m x 5m) were randomly selected. In each subplot, litterbags with different mesh sizes were placed to be collected at four specific times. This experimental design represents 3 blocks x 2 plots/block (1 trim+detritus/ 1 control) x 3 subplots x 3 litterbag mesh sizes x 4 collecting times, for a total of 216 litterbags.

Figure 2. Control Plot **(a)** and Canopy opening (Trim+Detritus) Plot **(b)** showing canopy openness.

Litterbags

The three more common plant species (Zimmerman et al. 2014; Richardson et al. 2010) in the Tabonuco forest, were selected to control for the effect of litter quality, *Manilkara bidentata* (ausubo), *Dacryodes excelsa* (tabonuco) and *Prestoea acuminata var montana* (palma de sierra). Litterbags (Fig. 3a, 3b and 3c) were filled with a combination of green leaves from these 3 species, in equal amounts up to 15g. Litterbags mesh size were selected to representing three functional group (Wardle 2002) using animal body width (Swift et al. 1979) as a proxy. Small mesh (Figure 3a) had a pore size of 0.3 mm^2 mesh) – allowing only micro-food web organisms to enter, which include microbes and micro predators (e.g., nematodes and protozoans) that feed upon the microbes (bacteria and fungi that break molecule); Medium mesh (Fig. 3b) had a pore size of 0.5 mm^2– that allowed micro-food web organisms and mesopredators and small litter transformers that feed on large pieces of litter, transforming them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; Large mesh (Fig. 3c) had a pore size of 3 mm^2 – that allowed all component of the decomposition food web to enter the bag expected for large animals such as earthworm or large diplopoda (Wardle 2002). From now on, litterbags with small mesh size will be called small, medium mesh size will be called medium and large mesh size will be called large.

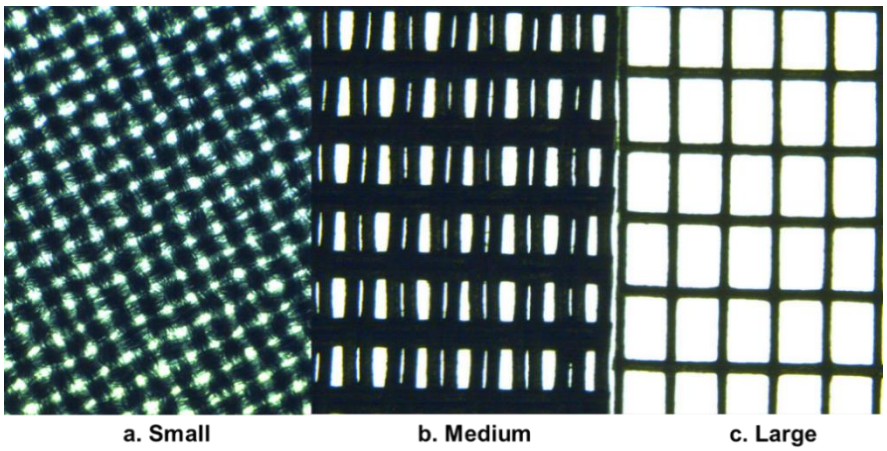

Figure 3. Image of Litterbags mesh sizes: a) small, b) medium and c) large. All photos were taken at the same magnification (40x) under dissecting microscope. Photo resolution is 1400 x 1000).

Study of Litter Microbial Communities

DNA Extraction

DNA was extracted from 0.3g of green leaf litter sample, using "UtraClean™ Soil DNA Isolation Kit" (MoBio Laboratory, Solana Beach, CA). DNA quality was assessed by electrophoresis with 1% agarose gel and DNA concentration was calculated with a biophotometer.

DNA Amplification

To amplify specific segments of DNA, the "Polymerase Chain Reaction" (PCR) was used. Amplification it was done using the enzyme "Red Taq DNA Polymerase" (Sigma Aldrich, St. Louis, Mo). The fungal ITS region of the rDNA were PCR amplified using forward primer fluorescently labeled. The molecular markers that were used are the

internal transcribed region (ITS) for fungi. The fungal ITS region was amplified using primers ITS1F-FAM and ITS4.

Metagenomics Community Analysis

To carry description and estimate the diversity of microbial communities present in green leaf litter, the TRFLP technique was used. PCR amplicons were enzymatically digested with *HaeIII* for ITS (Cantrell et al. 2013), following the manufacturer's protocols (Invitrogen^{TM}, United Kingdom). TRFLP community analysis has been used to determine the microbial community structure and distribution in different ecosystems and disturbance regimes (Cantrell et al. 2013). This technique will allow us to compare changes in community structure. TRFLP requires specific restriction enzymes to cut the DNA.

Data analysis

Two-Ways Permanova with Bray-Curtis similarity index were used to determine significant differences between treatments, litterbags mesh sizes, blocks and through time using the relative abundance of each TRFLP Phylotype present in each sample. PCoA cluster analysis using the Bray–Curtis similarity index with PAST Version 2.03 was used to see how samples cluster together.

Results

The Two-Way Permanova analysis showed that the number of phylotypes was significant different among litterbag mesh size (P= 0.00) (Table 1A) and blocks (P=0.00) (Table 1A) and treatment (Table1A).

Table 1. Two-Way Permanova using Bray Curtis test for the effect of treatment, size and time on the diversity of fungi. Showing *df*, *F* and *P* values for number of phylotypes. *P* values in bold represent a *P* values < 0.05.

A

Source	*df*	*F*	*P*
Block	2	8.41	**0.00**
Treatment	1	2.11	**0.03**
Interaction	2	-1.17	0.192

C

Source	*df*	*F*	*P*
Treatment	1	1.71	0.08
Time	3	0.79	0.51
Interaction	3	-0.31	0.26

B

Source	*df*	*F*	*P*
Mesh	2	3.92	**0.00**
Time	3	0.75	0.37
Interaction	6	-0.78	0.71

D

Source	*df*	*F*	*P*
Treatment	1	1.66	0.07
Mesh	2	3.75	**0.00**
Interaction	2	-1.68	0.97

Number of phylotypes was higher for both treatments in large litterbags mesh size than small and medium for 21 days, 35 days and 84 days (Fig. 4A). While number of phylotypes was higher in small litterbag for 168 days in control treatment compared with trim+detritus treatment (Fig.4B). However the results don't show number of phylotypes for large litterbag mesh size in 168 days, because we did not get the amplifications for ITS. (Fig. 4A and 4B).

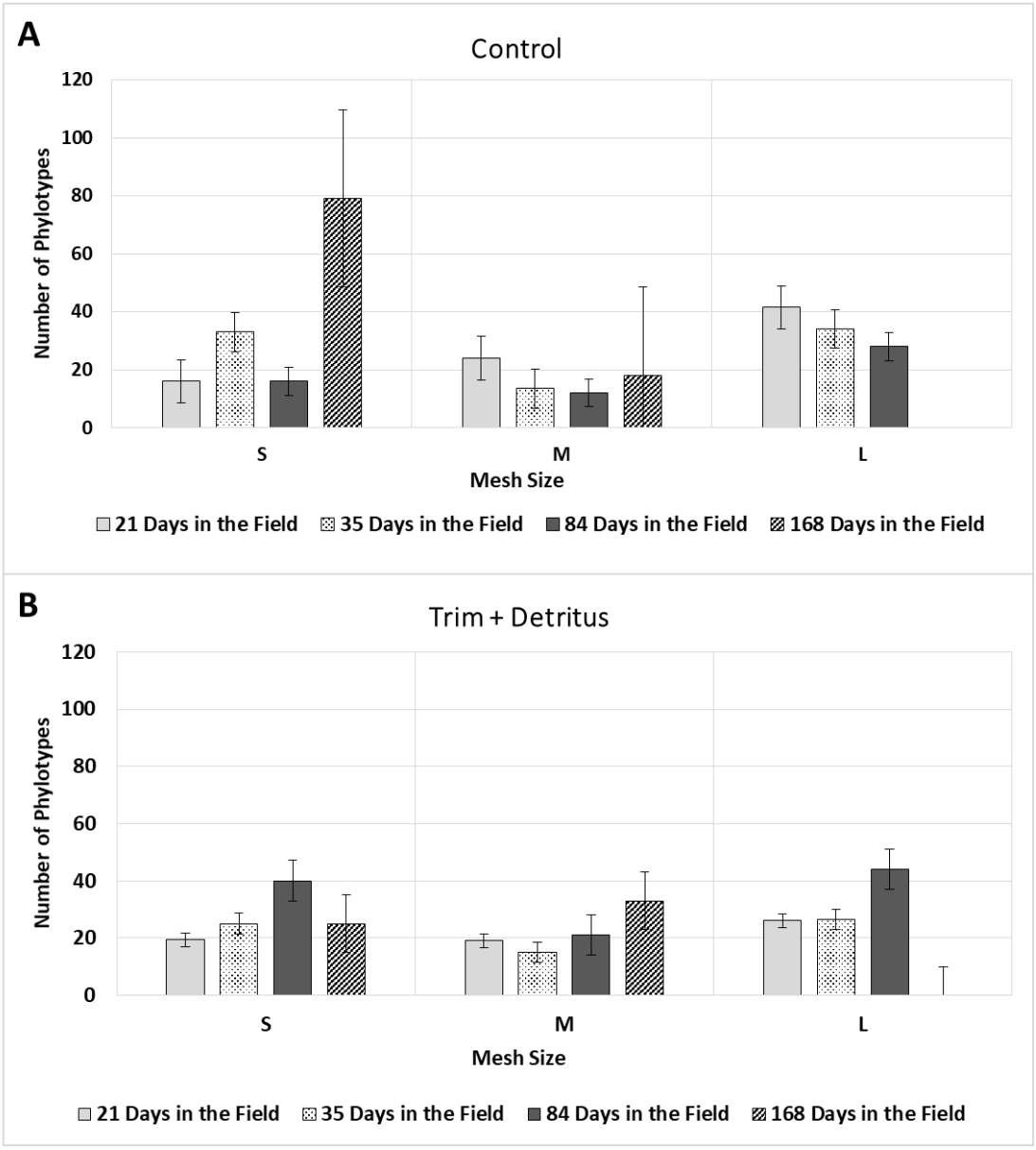

Figure 4. Number of phylotypes of fungi in each time (21 days, 35 days, 84 days and 168 days) for mesh size (small, medium and large) in Control (A) and Trim+Detritus (B) treatment.

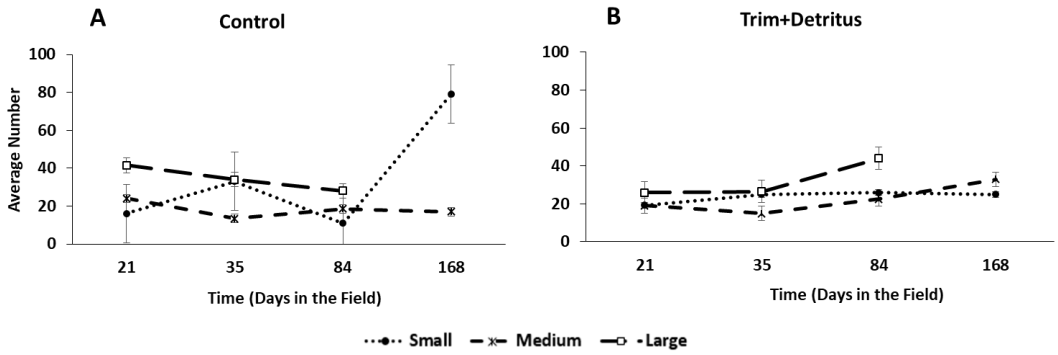

Figure 5. Average number of phylotypes in each mesh size (small, medium and large) through time for Control (A) and Trim+Detritus (B) treatment**.**

In addition average number was higher in small, medium and large litterbag for 21 days, 35 days and 84 days in control treatment (Fig. 5A), also during the six months (2014-2015) less precipitation was observed in 168 days after canopy trimming (Fig.6).

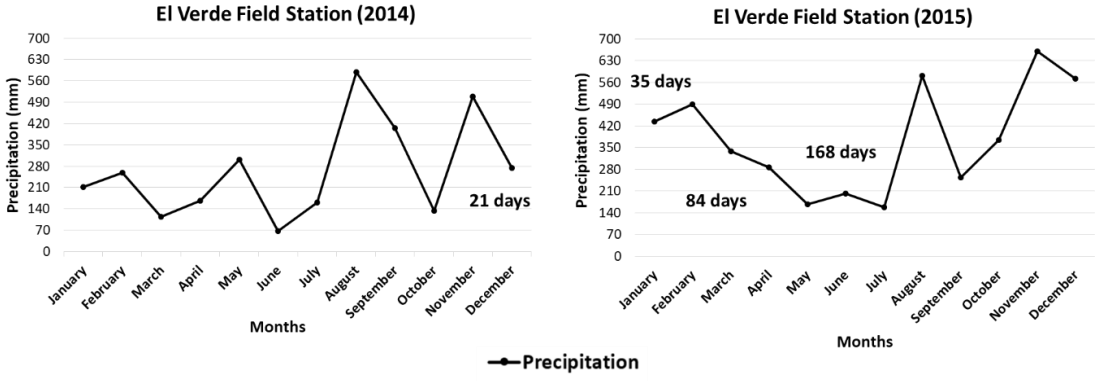

Figure 6. Diagram of General Precipitation (mm) for one year (2014-2015) to four times: 21 days, 35 days, 84 days and 168 days during six months (168 days) after canopy trimming in Luquillo Experimental Forest in El Verde Field Station located in the northeastern part (18.33080, -65.82320, WGS 84) of Puerto Rico

While the PCoA cluster analysis of green leaf litter microbial community with the Bray-Curtis similarity index showed clustering in the samples of small and medium litterbag mesh size with 13% of variability, also showed clustering in the treatments (control and trim+detritus) although treatment factor was not significantly different in the two-way Permanova analysis. In addition showed greater percentage (38%) of variability for large litterbag size (Fig.7)

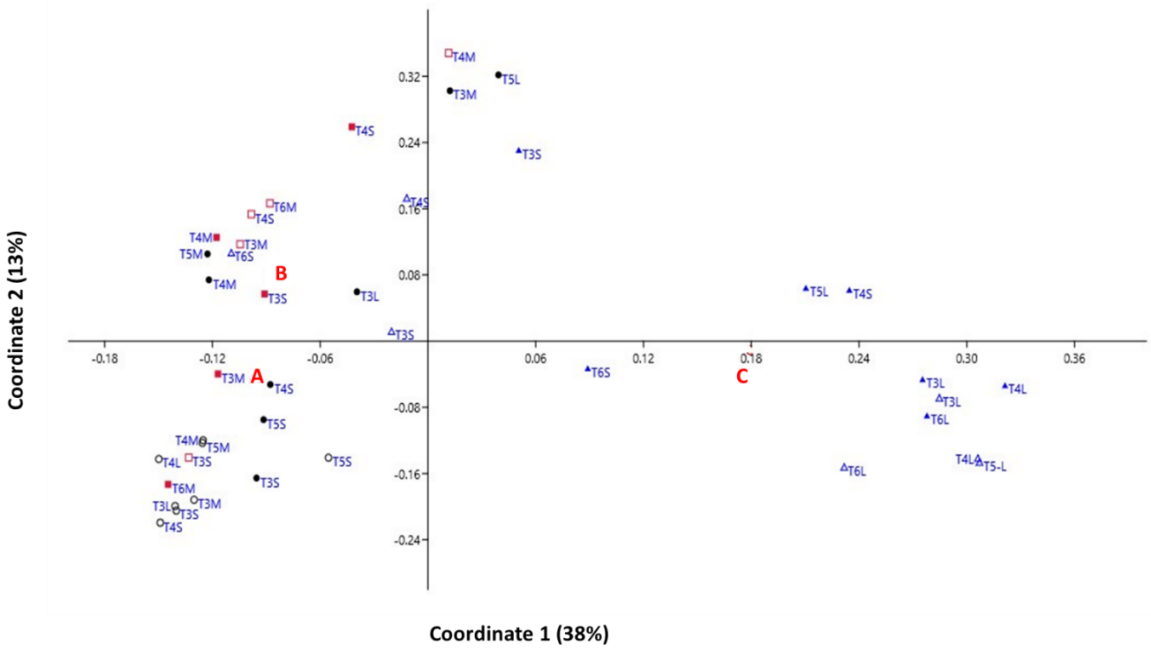

Figure 7. PCoA cluster analysis using the Bray–Curtis similarity index of the green leaf litter microbial community (fungi). Coordinate 1 with 38% of variability and coordinate 2 with 13% of variability. The filled symbol = Control treatment and open symbol = Trim+Detritus treatment.

Discussion

Litter decomposition by micro-organisms is highly dependent on microclimate including in the litterbag environment (Aerts, 2006). Therefore differences in abiotic factors such as moisture, light, nutrients, and temperature between treatments (Bokhorst & Wardle, 2013) may partly explain the differences observed in a number of phylotypes among treatments (control and trim+detritus). Our results showed that the average number of phylotypes was higher in small, medium and large litterbag size in control treatment (canopy is closed) for 21 days, 35 days and 84 days. Also, results showed a relation with the results of general precipitation in the Luquillo Experimental Forest for six months (2014-2015), with more precipitation and higher average number of phylotypes in 21 days and 84 days after canopy trimming.

Previous studies from the first simulated hurricane experiment in the Luquillo Experimental Forest (LEF) found that soil and leaf litter microbial community composition responded to canopy opening and debris deposition (Cantrell et al. 2014). Our results showed a higher number of phylotypes in control treatment (canopy closed) where the entry of light was less, this caused the litterbags to stay with higher humidity for a longer time preserving connectivity of fungi.

Conclusion

In this study the difference in abiotic factors such as humidity, solar radiation, temperature between treatments can explain the differences observed in the number of phylotypes. For example in previous study in the Luquillo Experimental Forest (LEF) litter moisture was lower in trimmed plots due to increased solar radiation and wind (Richardson et al. 2010; Lodge et al. 2014).This observation reflects the susceptibility of microbial communities to effect of canopy opening. In addition, any effects of litterbag mesh size on

microclimate are therefore likely to affect the enzymatic processes and animal activity on the litter within the bags (Bokhorst and Wardle, 2013). Our results showed that a number of phylotypes were higher for both treatments (control and trim+detritus) in large litterbags size than small and medium for 21 days, 35 days and 84 days. While our results showed that a number of phylotypes were higher in small litterbag in 168 days (when the precipitation was less), where the size would prevent more moisture loss in control treatment compared with trim+detritus treatment. Therefore results support our hypothesis in which we established that microbial diversity of green leaf litter varies among the three litterbag mesh sizes in the control and treatment (trim+detritus). These results will be further analyzed and interpreted in the context of food web dynamics.

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Chapter 4

Effect of litterbag mesh size on decomposition and nutrient mineralization from green leaves in a simulated hurricane experiment.

Introduction

Hurricanes generate disturbance in forests, altering biotic and abiotic conditions affecting is on microbial activity and the flow of nutrients in the soil. Hurricanes defoliate and deposit large amounts of biomass (debris) in the forest floor (Walker et al. 1991). Overall, a hurricane could drastically alter patterns in litter and nutrient cycling (Gonzalez et al. 2014). Hurricanes alter the physical structure of the forests by creating canopy opening which increase understory light (Shiels et al. 2015). An opening in the canopy, can increase the amount of solar radiation entering the forest floor and alters the temperature and moisture conditions on the forest floor (Richardson et al. 2010).

A previous study Shiels et al. 2010 showed that canopy openness and deposition of detritus are important determinants of the response of woody plants. They found that an increased opening in the canopy allows the greater availability of light, and this is the dominant factor that affects the regeneration of forests after a hurricane (Shiels et al. 2010). However soil nutrients limit plant growth in the humid tropical forests therefore, the recently fallen litter can increase nutrients and soil organic matter, and thus benefit the plants (Lodge et al. 1991, Denslow; Ellison and Sanford 1998). Therefore, the combination of debris and opening in the canopy, increasing light levels, humidity, nutrient inputs and recycling of nitrogen and phosphorus in the soil are factors that influence the trophic dynamics in soil and forest litter (Rivera 2008).

The forest floor is composed largely of soil biomass and litter and the soil biomass is composed largely of microorganisms, which are critical for fertility. The litter that falls to

the forest floor (canopy leaves, branches and, fruit and seeds) provides a habitat for major detritivores and fungivore invertebrates that aid in decomposition (Richardson et al. 2005). Litter decomposition results in nutrient mineralization that enters the soil solution and becomes available for plants (Wardle 1999). Soil fauna plays an important role in the biological turnover and release of nutrients from plant residues by the arthropod fragmenting plant residues, generating greater activity of microorganisms and grazing of the microflora by the soil fauna (Anderson et al. 1983).

The availability of nitrogen (N) and phosphorus (P) will depend on the decomposition of the organic matter and the release of these in the mineralization process. After carbon and oxygen, nitrogen is the next most abundant element in the dry matter of the plant. The cycle of N begins through the decomposition of organic matter in the soil, which provides the N available for plant and microbial absorption returning to the ground through dead organic matter or grazing animal wastes (McNeill and Unkovich 2007). While for the phosphorus (P) cycle, the microbial biomass is the force that drives the soil organic P dynamics (Bünemann and Condron 2007). The rate of nutrients released will depend on several factors, including the initial amount of nutrients in the litter, the structural (molecular) nature of the nutrients in the litter matrix, the microbial demand for nutrients and availability of exogenous sources of the nutrient and composition of the faunistic community (Seastedt, 1984). The release of nutrients generated during litter decomposition is an important nutrient flow pathway in forest ecosystems (González 2014).

Previous studies in El Verde (inside the Luquillo Experimental Forest (LEF) in Puerto Rico), indicate that opening in the canopy dries the litter, alters the composition of microbial communities and reduce leaf litter species richness (Lodge et al. 2014). However, most of the studies that have been conducted in the forest are focused on the

effects of disturbances on populations and communities of plants and animals, giving little attention or leaving out microbial communities and litter invertebrates (Willig et al. 1996). For this reason, I focused on the interaction between litter invertebrates was using three different mesh sizes of litterbags, using soil animal size as a substitute of the three functional groups (micro-food web, litter transformers, and ecosystem engineers) by quantifying litter decomposition, available nutrients and invertebrates and microorganisms diversity. We predict that arthropod abundance, the rate of decomposition (mass remaining %) of green leaf litter and the available nutrient concentration varies among the three litterbag mesh sizes in the control and trim+detritus treatments. To test this hypothesis, we studied the effect of the canopy opening and debris deposition on litter invertebrates and evaluate arthropod abundance in green leaf litter, mass remaining (%) and available nutrient concentration over a six-month litterbag decomposition experiment in the Luquillo Experimental Forest in Puerto Rico.

Materials and Methods

Study Site

This study was performed in the Luquillo Experimental Forest (LEF) (Fig. 1), located in northeastern (18.33080, -65.82320, WGS 84) Puerto Rico. The LEF is composed of four life zones that result from changes in elevation, climate and soil characteristics (Willig 1996; (García-Martinó et al. 1996). Specifically, the Tabonuco forest (*Dacryodes excelsa*) is classified as subtropical lower montane wet forest with average monthly of temperature of 21°C in January and 25 °C in September (Brown et al. 1983). Total annual precipitation is approximately 3.5 m (Gracía-Martinó et al. 1996), with approximately 97 rainless days per year. In this forest rainfall is weakly seasonal and has a dry season between December and March (most commonly March)

(http://lternet.edu/data/ lterdb14/data/). Litterfall is seasonal, with a main peak from March to June, a secondary peak in September, and minima from December to February (Zou et al. 1995; Lawrence 1996; Zalamea and González 2008; Richardson et al. 2010).

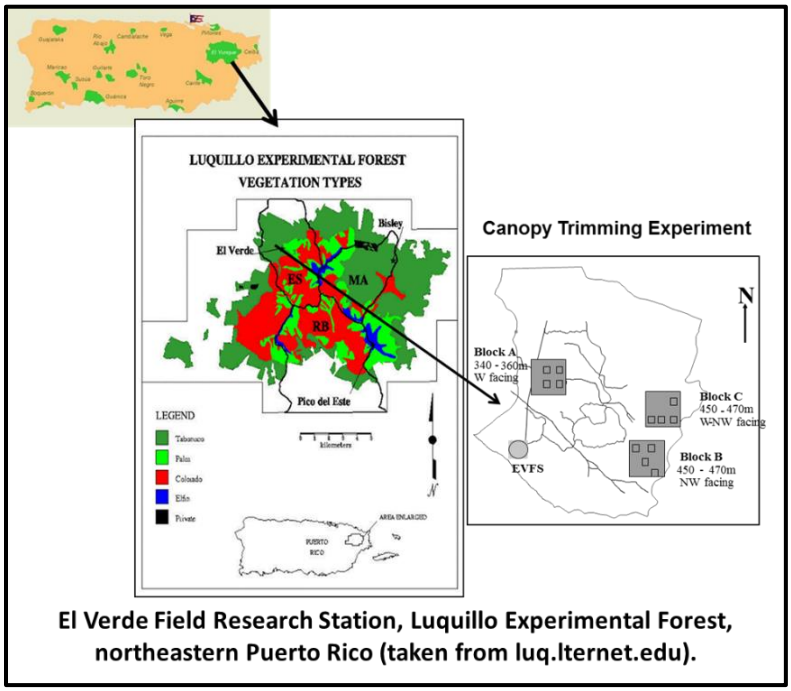

Figure 1. El Verde Field Research Station, Luquillo Experimental Forest, northeastern Puerto Rico.

Field Study Design

This study is part of the Canopy Trimming Experiment 2 performed by the Luquillo Long-Term Ecological Research (LTER) at El Verde Field Station. For this in the Tabonuco forest, three blocks (A, B and C) were selected, in each block two 20m x 20m plots were selected, one plot was control and the other was experimental (Trim+Detritus) (Shiels et al. 2010, Richardson et al. 2010, Shiels and González 2014) (Fig. 2). In the experimental plot, canopy was trimmed and debris was deposited on the ground

simulating the impact that a hurricane has in this forest, from now on this is called Trim+Detritus. Each plot was subdivided into 16 sub-plots, and three sub-plots (5m x 5m) were randomly selected. In each subplot, litterbags with different mesh sizes were placed to be collected at four specific times. This experimental design represents 3 blocks x 2 plots/block (1 trim+detritus/ 1 control) x 3 subplots x 3 litterbag mesh sizes x 4 collecting times, for a total of 216 litterbags.

Figure 2. Control Plot **(a)** and Canopy opening (Trim+Detritus) Plot **(b)** showing canopy openness.

Litterbags

The three more common plant species (Zimmerman et al. 2014; Richardson et al. 2010) in the Tabonuco forest, were selected to control for the effect of litter quality, *Manilkara bidentata* (ausubo), *Dacryodes excelsa* (tabonuco) and *Prestoea acuminata var montana* (palma de sierra). Litterbags (Fig. 3a, 3b and 3c) were filled with a combination of green leaves from these 3 species, in equal amounts up to 15g. Litterbags mesh size were selected to represent three functional group (Wardle 2002) using animal body width (Swift et al. 1979) as a proxy. Small mesh (Figure 3a) had a pore size of 0.3 mm^2 mesh – allowing only micro-food web organisms to enter, which include microbes and micro

predators (e.g., nematodes and protozoans) that feed upon the microbes (bacteria and fungi that break molecule); Medium mesh (Fig. 3b) had a pore size of 0.5 mm^2– that allowed micro-food web organisms, and mesopredators and small litter transformers them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; that feed on large pieces of litter, transforming them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; Large mesh (Fig. 3c) had a pore size of 3 mm^2 – that allowed all components of the decomposition food web to enter the bag except for large animals such as earthworms or large diplopoda (Wardle 2002). From now on, litterbags with small mesh size will be called small, medium mesh size will be called medium and large mesh size will be called large. Allowing 3 different of decomposer functional groups to enter the bags (See Chapter 2) that differentially affect decomposition therefore nutrient mineralization.

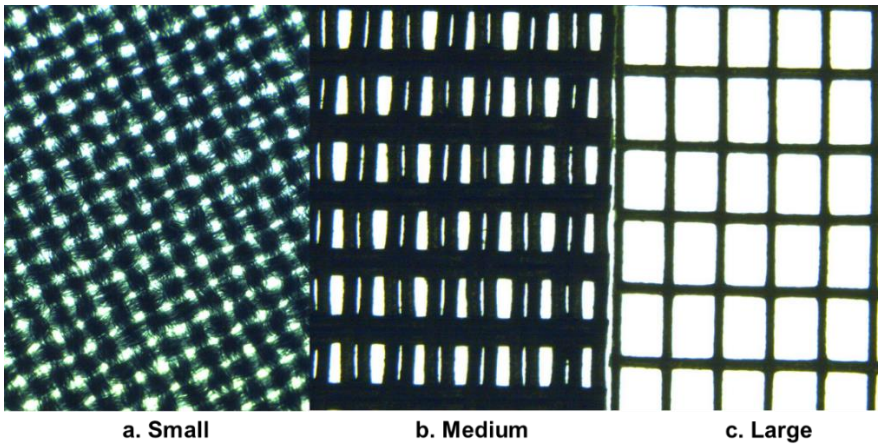

Figure 3. Photo of Litterbags showing differences among mesh sizes: a) small, b) medium and c) large. All photos were taken at the same magnification (40x) under a dissecting microscope. Photo resolution is 1400 x 1000).

Data Collection

Mass Loss

The litter sample for mass loss study was the same litter sample we used to funnel extraction, therefore before arthropod extraction, litter sample were weighed (wet weight) and later after extraction is put in the oven-dried at 60˚C for one week, the sample were weighed again (dry weight). To calculate mass loss, a simple exponential decay function model was used as proposed by Jenny et al. (1949) and discussed by Olson in (1963). This model proposes that the decomposition follows a unique pattern: $Xt = X_o e^{-kt}$. Where X_t is the fraction of leaf litter remaining at time t (days), X_o is the initial amount of litter, and k is the decomposition constant.

Nutrients

Nutrients were quantified using PRS ™-probes which adsorb nutrients that enter the soil solution. One week prior to sampling, two pairs of each membrane (2 anionic and 2 cationic) were placed inside the litterbags were placed under the litter (Fig. 4). PRS ™-probes was sent to Western Ag Innovations for nutrient analyses. We used two different membrane one anion probes (orange) that have a positively-charged membrane to adsorb all negatively-charged anions (nitrate, phosphate and sulphate) and one cation probes (purple) that have negatively-charged membrane to adsorb all positively-charged cations (ammonium, potassium, calcium and magnesium). This probes are used for quantifying spatial and temporal variations in nutrient rates for all ions (Western Ag 2019) (http://www.westernag.ca/innovations/technology/basics).

Figure 4. Two pairs of each PRS Probes (2 anionic and 2 cationic) were buried inside litterbags one week prior of sampling.

Data Analysis

We performed General lineal model: Univariate Analysis of Variance using IBM Statistical Package for the Social Sciences (SPSS 20) and Principal Component Ordination (PC-ORD 4.0) were used to determine the significant difference of available nutrient concentration (ug/10cm^2/1week) and mass remaining (%) between treatments, litterbags mesh sizes and through time.

In addition, we used Principal Components Analysis (PCA) with randomization test, the cross-products matrix contains correlation coefficients among groups and the eigenvectors, scaled to unit length, and these can be used as coordinates in a distance. PCA was used to visualize the data in a dimension that projects high dimension data in two axes. This sorting technique is used to analyze the relation of the data.

Results

Available Nutrient Concentration

Available nitrogen (NH4-N) and potassium (K) were significantly different between control and trim+detritus, among mesh sizes and through time (Table 1). While phosphorus (P) was significantly different among mesh sizes and through time (Table 1).

Nitrogen was higher in large mesh size in trim+detritus with 20 to 100 ug/10cm^2/one-week than in control with 20 to 60 ug/10cm^2/1week (Fig. 5A and 5a), also phosphorus with 10 ug/10cm^2/one-week in trim+detritus and 2 to 10 ug/10cm^2/one-week (Fig. 8B and 5b). Potassium showed a similar pattern in both treatments with a higher nutrient available in large mesh size with 50 to 300 ug/10cm^2/one-week in control and 100 to 400 in trim+detritus (Fig. 5C and 5c). The three nutrients showed a similar pattern with a minimum at 84 days (Fig. 5). Also, nutrients was always higher in large mesh size than small and medium for both treatments. For example nitrogen was higher in large mesh size with a peak more than 80 ug/10cm2/one-week in 35 days.

Table 1. General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on the Available Nutrient Concentration, Total Arthropod Abundance and Mass Remaining %. Showing *df*, *F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05.

Effect		NH4-N		P		K	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	8.120	**< 0.005**	0.045	0.832	6.618	**< 0.011**
Size	2	11.354	**< 0.000**	5.806	**< 0.004**	4.382	**< 0.014**
Time	3	17.627	**< 0.000**	4.062	**< 0.008**	53.379	**< 0.000**
Treatment x Size	2	5.792	0.004	0.004	0.996	1.564	0.212
Treatment x Time	3	2.561	0.056	0.276	0.842	2.141	0.096
Size x Time	6	3.258	0.005	4.426	0.000	0.432	0.857
Treatment x Size x Time	6	1.912	0.081	0.152	0.989	1.321	0.250

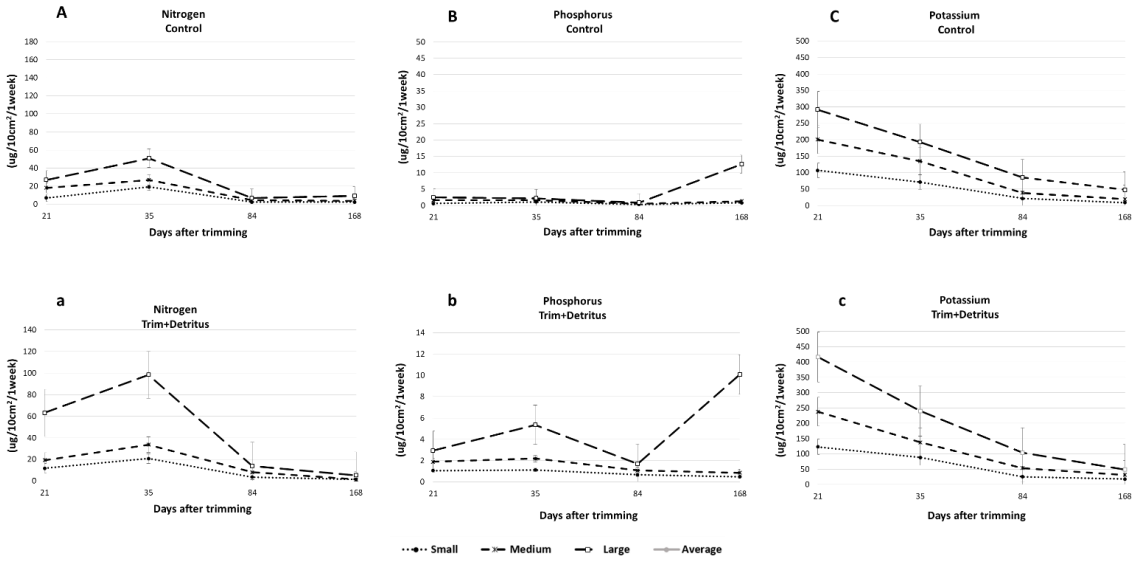

Figure 5. Average (±s.e.) available nutrient concentration, i.e. nitrogen, phosphorus, and potassium, in each litterbag mesh size and for Control (A, B and C) and Trim+Detritus (a, b and c) plots. Bars represent standard error.

Also during the six months (2014-2015) less precipitation was observed in 168 days after canopy trimming (Fig.6). This event of drought is not normal for the forest, because the dry season at Luquillo Experimental Forest (LEF) is between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). During the study a severe drought was observed starting in December 2014 and lasted till August 2015. Drought affect microbes and arthropods activity (Bouskill et al. 2016; Liu et al. 2013; Schowalter, 2017) and therefore affect available nutrients in the soil.

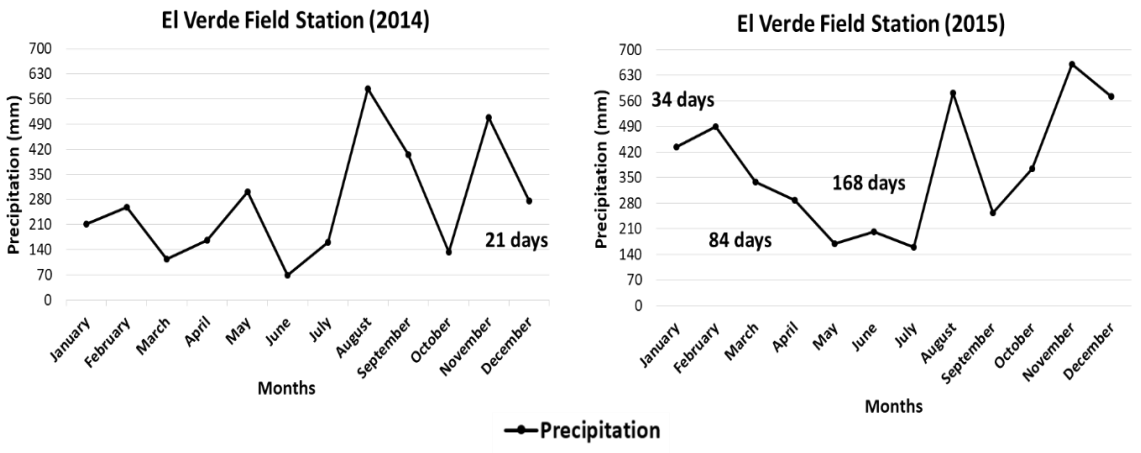

Figure 6. Diagram of General Precipitation (mm) for one year (2014-2015) to four times: 21 days, 35 days, 84 days and 168 days during six months (168 days) after canopy trimming in Luquillo Experimental Forest in El Verde Field Station located in the northeastern part (18.33080, -65.82320, WGS 84) of Puerto Rico.

Principal components analysis (PCA) with randomization test (Table 2) showed that only Axis 1 was significant was correlated with nutrients (P = 0.008) (Table 2A) for which the Eigenvalue was 1.36 explained 46.213 % (Table 2B). Axis 1 showed a significant correlation, in addition nitrogen had an eigenvector of 0.07054, and this suggests that nitrogen is correlated to the axis 1. While phosphorus showed an eigenvector of -0.0168, which suggests that this is further from the axis 1 (Table 2). These results suggest that sampling units are discriminated along axis 1 with samples with high nitrogen towards positive axis values and sample with low nitrogen towards negative axis values.

While the left values show low concentration of nutrients available than right values show higher concentration (Fig. 7). For example through time we found that in 21 days some sampling units had low nitrogen concentration, while at 35 days these sampling

units increased their nitrogen concentrations (Fig 7). Which suggests a higher concentration of nutrients in those mesh sizes and that period of time.

Table 2. Principal components analysis (PCA). Randomization results test of Correlation among groups variance (A) extracted (3 axes) and eigenvalues from real data and randomization (B).

A

Axis	Eigenvalue	Eigenvector	% of Variance	Cum. % of Var.	Broken-Stick Eigenvalue
1	1.386	0.7054	46.213	46.213	1.833
2	1.025	-0.0168	34.163	80.377	0.833
3	0.589	0.7086	19.623	100	0.333

B

		Eigenvalues from randomizations			
Axis	Eigenvalue from real data	Minimum	Average	Maximum	P
1	**1.3864**	**1.005**	**1.1115**	**1.5338**	**0.008**
2	1.0249	0.89292	0.99745	1.1018	0.128
3	0.58869	0.46587	0.891	0.99194	0.995

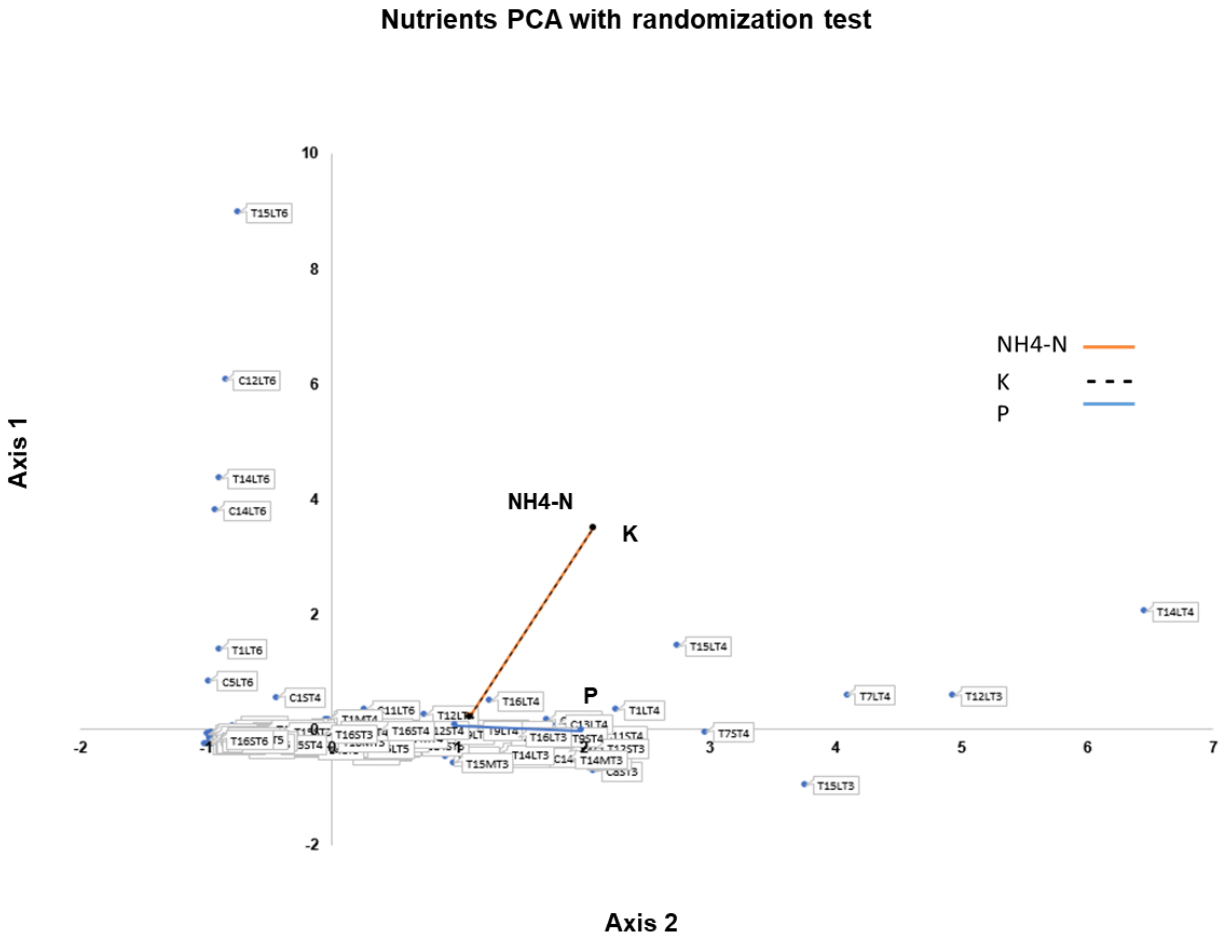

Figure 7. Principal component analysis (PCA) with randomization test for available nutrient concentration; nitrogen (NH4-N), Phosphorus (K) and Potassium (P).

Mass Remaining (%)

Mass remaining (%) was significant different (P < 0.05) through time (Table 3). Furthermore, mass remaining (%) in control treatment (Fig. 8a) and trim+detritus treatment (Fig. 8b) followed a similar pattern.

Mass remaining, showed a pattern for both treatments (control and trim+detritus) in the first phase of decomposition, with an changed from 100 to 60%, resulting at 60% of mass remaining and 40% of mass loss in Control and trim+detritus, during the first 21 days after trimming (Fig. 8a and 8b). While, it showed a pattern much slower pace during the last 168 days. However was not significantly different in weight loss between treatments.

Table 3. General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on Mass Remaining %. Showing *df*, *F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05.

Effect	Mass remaining (%)		
	df	*F*	*P*
Treatment	1	1.911	0.168
Size	2	1.021	0.362
Time	3	83.078	**0.000**
Treatment x Size	2	0.84	0.433
Treatment x Time	3	1.126	0.340
Size x Time	6	0.385	0.888
Treatment x Size x Time	6	0.655	0.686

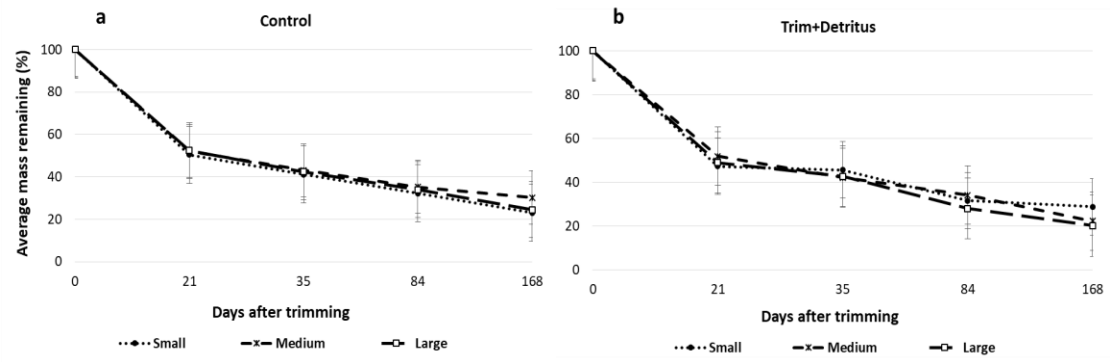

Figure 8. Average (±s.e.) mass remaining (%) in each mesh size of litterbag and for Control (a) and Trim+Detritus (b) plots and through time. Bars represent standard error.

Discussion

Total abundance: We found that total abundance was significant different through time but not between treatment, we know the changes in habitat caused by disturbances (e.g. hurricane) affect the composition of arthropod communities since changes in abundance and distribution of resources can modify the microhabitat used by arthropods (Barberena 2002). Furthermore, the effect of canopy opening and debris (trim+detritus treatment) provides more organic matter for arthropods, this stimulates the activity of soil animals in the early time (days). For example, our results showed a total abundance trend for all litterbags mesh sizes in 84 days in both treatments.

Available Nutrient Concentration: The Canopy opening effect increase solar radiation, throughfall, and soil moisture (Shiels et al. 2015) with increased throughfall and surface organic matter, excess N was converted by soil bacteria to nitrate, which leached into groundwater (Shiels et al. 2015). In our study, nitrogen, phosphorus, and potassium were significantly different among litterbag mesh sizes and through time, in addition were significantly higher in large litterbag. This result suggests more loss of nutrients in large

litterbag by throughfall. In addition, increased solar radiation by canopy opening effect increase a pioneer plants, while debris (trim+detritus treatment) provides more organic matter for arthropods, stimulating the activity of soil animals in the early time (days) and increased herbivory resulting in an increase of leaf nitrogen and phosphorus concentration (Shiels et al. 2015). Our study showed a trend higher nitrogen release in 21 to 35 days that suggest that result was for by the high activity of soil animals in early days. In addition, in our study potassium (K) trend were a fast and higher release in large litterbag, which suggests an important role for leaching. This is consistent with previous reports that K is the most mobile element during decomposition (Swift et al. 1979, Anderson et al. 1983). While phosphorus (P) in comparison with other nutrients (nitrogen and potassium); the concentration of phosphorus in the soil solution is generally low (Stewart et al. 1990). It has been suggested that the P in a decomposing litter is subject to the same pattern of immobilization and uptake by micro-organisms (Peterson & Rolfe 1982). Our results showed low P release which suggests an immobilization of P uptake by microbe.

Mass Remaining **(%):** In this study, the mass remaining were significant different through time but not between treatments. Even though followed a similar pattern in between treatments (Control and Trim+Detritus). In our experiment at the meso-scale level, the physical-chemical factors (temperature and humidity) and the quality of the resource (litter) are equal in both treatments. While the abundance and variety of taxonomic and trophic groups vary for both treatments. However, the results of the mass remaining does not show a significant difference between the treatments. Therefore our result showed that abundance of arthropods does not affect the loss of mass, because the effect of arthropods on leaf litter decomposition occurs on a smaller scale, not on a meso-scale.

Conclusion

Therefore we found that litterbag mesh size significantly affected available nutrients concentration and arthropods abundance, while the effect of canopy opening and debris (trim+detritus treatment) provides more organic matter for arthropods, this stimulates the activity of soil animals in the early time (days) showing a total abundance higher for all litterbag size in 35 to 84 days in both treatments. In addition, our results showed mass remaining was not significantly different between treatments, suggest that abundance of arthropods does not affect the loss of mass. The effect of arthropods on leaf litter decomposition occurs on a smaller scale, not on a meso-scale. Therefore, our results are aligned and support what we all know from the literature previous studies it has been found that at the meso-scale level, the predominant factors in litter decomposition are: resource quality and environmental conditions. Based on evidence from multisite experiments at regional and global scales, while soil animals are considered key regulators of decomposition at local scales (Wall et al. 2008). In addition, there was a trend for higher arthropod abundance, higher nutrient availability in large litterbag mesh size suggesting trophic dynamics mediated by decomposer microbes.

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Chapter 5

Integration of Arthropod and Fungi Abundance in a simulated hurricane experiment

Introduction

Hurricanes generate disturbances in forests such as canopy opening, fallen trees, and leaves which alter physicochemical characteristics of the habitat, as well as, decomposer activity and nutrient fluxes (Walker et al. 1991; González et al. 2014). Litter decomposition depends primarily on the interaction among climate, litter quality, and biota, as a consequence any change in habitats will result in changes in these factors (Swift et al. 1979). Furthermore, canopy opening also change the microclimate on the forest soil, drying surface litter due to increased light (Miller and Lodge, 1997), therefore the increased light reduced litter moisture at the same time inhibits the litter fungi (Basidiomycota), which degrade lignin and are responsible for the translocation of the limiting nutrients, thus causing delay in decomposition (Shiels et al. 2015).

The regulation of the decomposition is given by the three factors that influence this process; the quality of the resource, the physical-chemical conditions, and the decomposer organisms, as a consequence any change in habitat may produce micro variations in these factors which in turn affect decomposition (Swift et al. 1979). Therefore, understanding the mechanistic processes of litter decay is essential for predicting nutrient cycling dynamics in tropical forests. Litter decomposition is mainly the result of microbial activities, while the soil fauna is important to condition the litter and stimulate the actions of the microbial community (Coleman et al. 2004). The rate of decomposition is influenced partially by the combined activities of the soil biota. Consequently, quantify the influence of soil fauna on the litter decomposition is more difficult than that of microorganisms because it is largely an indirect effect. Therefore by regulating bacterial and fungal

populations, protozoa and nematodes that make up the microfauna can alter litter decay and nutrient turnover (Hättenschwiler et al. 2005).

Usually, decomposition studies focus on mass loss, and few have measured actual nutrient mineralization. While the mineralization of nutrients is regulated by the resolution level of the basal consumers (bacteria and fungi) in the soil food web and this process is affected by soil animals of higher levels (protozoa, nematodes, mites, springtails, millipedes and earthworms) (Wardle 1999). In addition, most studies concentrate and observe the quality of litter and climatic effects on decomposition, leaving out the soil animals that are part of the process of decomposition. However, the decomposition of the litter depends on several soil animal interactions in order to carry out the entire litter decomposition process. Furthermore, the interactions of the soil fauna and microbes on nutrient mineralization would depend on the feeding behavior of the soil fauna on the microbial community (González and Seastedt, 2001).

Our objective was evaluate the effects of hurricane driven changes to forests on green litter decomposition, decomposer communities and nutrient mineralization. For this, the study focused on the interaction between litter invertebrates using three different mesh sizes of litterbags, using soil animal size as a substitute of the three functional group (micro-food web, litter transformers and ecosystem engineers) and litter quality by quantifying litter decomposition, available nutrients and invertebrates and microorganisms diversity with the specific objectives: (1) Study the effect of the canopy opening and debris deposition on litter invertebrates, microorganisms and litter decomposition. (2) Evaluate nutrient release, arthropod and microorganisms diversity at three different pores sizes of litterbags. (3) Determine the rate of litter decomposition at three different pores sizes of litterbags.

Materials and Methods

Study Site

This study was performed in the Luquillo Experimental Forest (LEF) (Fig. 1), located in northeastern (18.33080, -65.82320, WGS 84) Puerto Rico. The LEF is composed of four life zones that result from changes in elevation, climate and soil characteristics (Willig 1996; (García-Martinó et al. 1996). Specifically, the Tabonuco forest (*Dacryodes excelsa*) is classified as subtropical lower montane wet forest with average monthly of temperature of 21°C in January and 25 °C in September (Brown et al. 1983). Total annual precipitation is approximately 3.5 m (Gracía-Martinó et al. 1996), with approximately 97 rainless days per year. In this forest rainfall is weakly seasonal and has a dry season between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). Litterfall is seasonal, with a main peak from March to June, a secondary peak in September, and minima from December to February (Zou et al. 1995; Lawrence 1996; Zalamea and González 2008; Richardson et al. 2010).

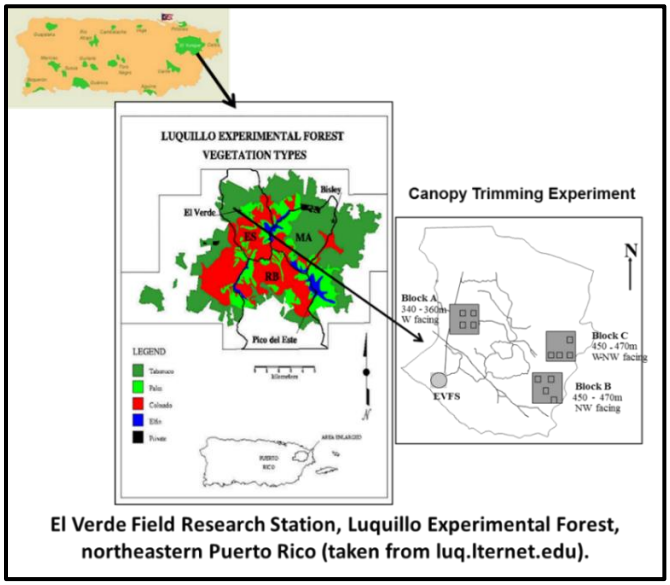

Figure 1. El Verde Field Research Station, Luquillo Experimental Forest, northeastern Puerto Rico.

Field Study Design

This study is part of the Canopy Trimming Experiment 2 performed by the Luquillo Long-Term Ecological Research (LTER) at El Verde Field Station. For this, three blocks (A, B and C) were selected, in each block two 20m x 20m plots two were selected, one plot was control and the other was experiment (Trim+Detritus) (Shiels et al. 2010, Richardson et al. 2010, Shiels and González 2014) (Fig. 2). In the experimental plot, canopy was trimmed and debris was deposited on the ground simulating the impact that a hurricane has in this forest, for now on this is called Trim+Detritus. Each plot was subdivided into 16 sub-plots, and three sub-plots (5m x 5m) were randomly selected. In each subplot, litterbags with different mesh sizes were placed to be collected at four specific times. This experimental design represents 3 blocks x 2 plots/block (1 trim+detritus/ 1 control) x 3 subplots x 3 litterbag mesh sizes x 4 collecting times, for a total of 216 litterbags.

Figure 2. Control Plot **(a)** and Canopy opening (Trim+Detritus) Plot **(b)** showing canopy openness.

Litterbags

The three more common plant species (Zimmerman et al. 2014; Richardson et al. 2010) in the Tabonuco forest, were selected to control for the effect of litter quality,

Manilkara bidentata (ausubo), *Dacryodes excelsa* (tabonuco) and *Prestoea acuminata var montana* (palma de sierra). Litterbags (Fig. 3a, 3b and 3c) were filled with a combination of green leaves from these 3 species, in equal amounts up to 15g. Litterbags mesh size were selected to representing three functional group (Wardle 2002) using animal body width (Swift et al. 1979) as a proxy. Small mesh (Figure 3a) had a pore size of 0.3 mm^2 mesh) – allowing only micro-food web organisms to enter, which include microbes and micro predators (e.g., nematodes and protozoans) that feed upon the microbes (bacteria and fungi that break molecule); Medium mesh (Fig. 3b) had a pore size of 0.5 mm^2– that allowed micro-food web organisms and mesopredators and small litter transformers that feed on large pieces of litter, transforming them into smaller pieces and this highly favorable for microbial growth, stimulated the activity of decomposing microorganisms; Large mesh (Fig. 3c) had a pore size of 3 mm^2 – that allowed all component of the decomposition food web to enter the bag expected for large animals such as earthworm or large diplopoda (Wardle 2002). From now on, litterbags with small mesh size will be called small, medium mesh size will be called medium and large mesh size will be called large.

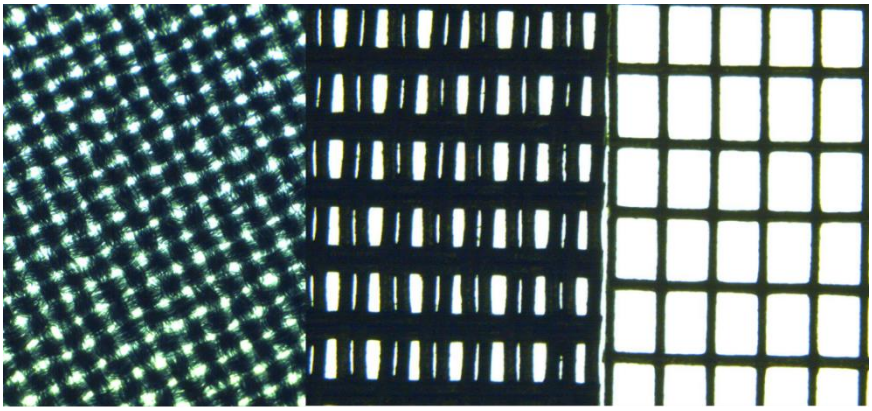

Figure 3. Image of Litterbags mesh sizes: a) small, b) medium and c) large. All photos were taken at the same magnification (40x) under dissecting microscope. Photo resolution is 1400 x 1000).

Data Collection

Nutrients

Nutrients were quantified using PRS ™-probes which adsorb nutrients that enter the soil solution (http://www.westernag.ca/innov/). One week prior to sampling, two pairs of each membrane (2 anionic and 2 cationic) were placed inside the litterbags were placed under the litter (Fig. 4). PRS ™-probes was sent to Westernag Innovations for nutrient analyses (http://www.westernag.ca/innovations/technology/basics).

Figure 4. Two pairs of each PRS Probes (2 anionic and 2 cationic) were buried inside litterbags one week prior of sampling.

Arthropod

Litterbags were retrieved for six months (168 days) after canopy trimming, in four times: 21 days, 35 days, 84 days and 168 days. The litter sample retrieved from inside the litterbags were placed in Berlese Funnels (Fig. 5a) for arthropod extraction (Walter et al. 1987; Barberena, 2002). The Berlese-Tullgren funnels (Walter et al.1987) is used to extract the active invertebrates living the litter. This method consists of a funnel which is placed in a sieve with a diameter of 3 mm mesh and then placing a volume of soil litter, with a light source intensity of 40 W was used at 40 cm for one week. The organisms flee from the light, making it easy and quick to extract those (Sandler et al. 2010). As the sample is dried, the invertebrates are concentrated in the lower part of the same and end up falling into a container with 70% alcohol as preservative, located at the end of the funnel.

Arthropod were sorted by family and order and assigned to a trophic category based on their life stage and feeding habits (Fig. 5b) Among the most abundant groups, *Acari* were sorted by suborder based on the dichotomous key (www.zoology.ubc/ca) using as reference the classification group of *Acari* (*Oribatida*, *Mesostigmata,* and *Prostigmata*) in the key. However, *Collembola, Coleoptera, Diptera,* and *Hymenoptera* were sorted by family because life stages are difficult to distinguish.

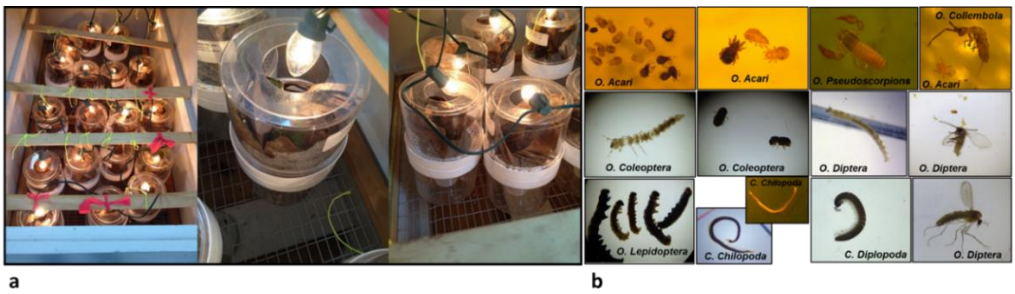

Figure 5. Sample retrieved from the litterbags will be placed for Berlese-Tullgren funnels for arthropod extraction **(a)** and Arthropods sorted by family and order **(b)**.

Study of Litter Microbial Communities

DNA Extraction

DNA was extracted from 0.3g of green leaf litter sample, using "UtraClean™ Soil DNA Isolation Kit" (MoBio Laboratory, Solana Beach, CA). DNA quality was assessed by electrophoresis with 1% agarose gel and DNA concentration was calculated with a biophotometer.

DNA Amplification

To amplify specific segments of DNA, the "Polymerase Chain Reaction" (PCR) was used. Amplification it was done using the enzyme "Red Taq DNA Polymerase" (Sigma Aldrich, St. Louis, Mo). The fungal ITS region of the rDNA were PCR amplified using forward primer fluorescently labeled. The molecular markers that were used is the internal transcribed region (ITS) for fungi. The fungal ITS region was amplified using primers ITS1F-FAM and ITS4.

Metagenomics Community Analysis

To carry a description and estimate the diversity of microbial communities present in green leaf litter, the TRFLP technique was used. PCR amplicons were enzymatically digested with *HaeIII* for ITS (Cantrell et al. 2013), following the manufacturer's protocols (Invitrogen™, United Kingdom). TRFLP community analysis has been used to determine the microbial community structure and distribution in different ecosystems and disturbance regimes (Cantrell et al. 2013). This technique will allow us to compare changes in community structure. TRFLP requires specific restriction enzymes to cut the DNA.

Data analysis

We performed General Lineal Model: Univariate Analysis of Variance using IBM SPSS 20 and PC-ORD 4.0 software and the arthropods abundance was standardized to individuals per gram of dry litter (Ind g^{-1} dry litter). Moreover, Univariate analysis of variance were used to determine the significant difference of available nutrient concentration (ug/10cm2/1week) and mass remaining (%) between treatments, litterbags mesh sizes and through time.

Furthermore, Two-Way Permanova with the index of similarity Bray-Curtis with PAST Version 2.03, were used to determine the significant difference of fungi diversity between treatments, litterbags mesh sizes, blocks and through time.

Results

Available Nutrient Concentration: The total nitrogen (NH4-N) and potassium (K) concentration were significantly different ($P < 0.05$) between control and trim+detritus treatment, among litterbag sizes and through time (Table 1). While phosphorus (P) was significantly different among litterbag mesh sizes and through time (Table 1). In addition nitrogen showed a decreased in 84 days for both treatments (Fig. 6a and 6b).

Total arthropod abundance was significant different ($P < 0.05$) through time (Table 1) and was trend higher in control treatment for large litterbag mesh size (Fig. 8a). Furthermore total arthropod abundance was higher for all litterbag mesh sizes in 84 days in both treatments (Fig 8a and 8b).

Table 1. Result of General lineal model: Univariate Analysis of Variance for the effect of treatment, size and time on the Available Nutrient Concentration, Total Arthropod Abundance. Showing *df*, *F* and *P* values for total abundance. *P* values in bold represent a *P* values < 0.05.

Effect	NH4-N			P		K		Arthropod Abundance	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1	8.120	**< 0.005**	0.045	0.832	6.618	**< 0.011**	1.390	0.240
Size	2	11.354	**< 0.000**	5.806	**< 0.004**	4.382	**< 0.014**	1.425	0.243
Time	3	17.627	**< 0.000**	4.062	**< 0.008**	53.379	**< 0.000**	7.401	**< 0.000**
Treatment x Size	2	5.792	0.004	0.004	0.996	1.564	0.212	1.361	0.259
Treatment x Time	3	2.561	0.056	0.276	0.842	2.141	0.096	1.059	0.368
Size x Time	6	3.258	0.005	4.426	0.000	0.432	0.857	0.450	0.844
Treatment x Size x Time	6	1.912	0.081	0.152	0.989	1.321	0.25	0.581	0.745

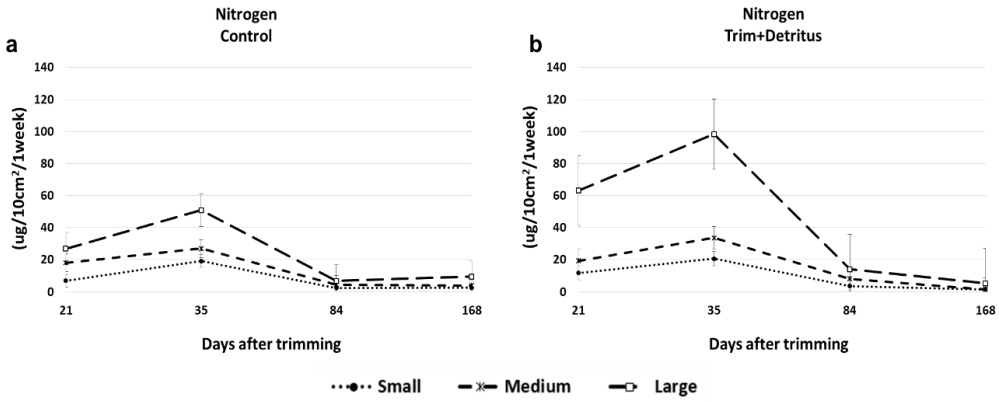

Figure 6. Average (±s.e.) available nutrient concentration of nitrogen, in each mesh size of litterbag and for Control (a) and Trim+Detritus (b). Bars represent standard error.

The Two-Way Permanova analysis showed that the number of phylotypes was significantly different among litterbag mesh size (*P*= 0.00) (Table 2A) and blocks (*P*=0.00) (Table 2A) and treatment (Table2A). While number of phylotypes trend a low for all litterbag mesh size for both treatment in 84 days (Fig 7a and 7b).

Table 2. Result of Two-Way Permanova using Bray Curtis test for the effect of treatment, size and time on the diversity of fungi. Showing *df*, *F* and *P* values for number of phylotypes. *P* values in bold represent a *P* values < 0.05.

A

Source	*df*	*F*	*P*
Block	2	8.41	**0.00**
Treatment	1	2.11	**0.03**
Interaction	2	-1.17	0.192

B

Source	*df*	*F*	*P*
Mesh	2	3.92	**0.00**
Time	3	0.75	0.37
Interaction	6	-0.78	0.71

C

Source	*df*	*F*	*P*
Treatment	1	1.71	0.08
Time	3	0.79	0.51
Interaction	3	-0.31	0.26

D

Source	*df*	*F*	*P*
Treatment	1	1.66	0.07
Mesh	2	3.75	**0.00**
Interaction	2	-1.68	0.97

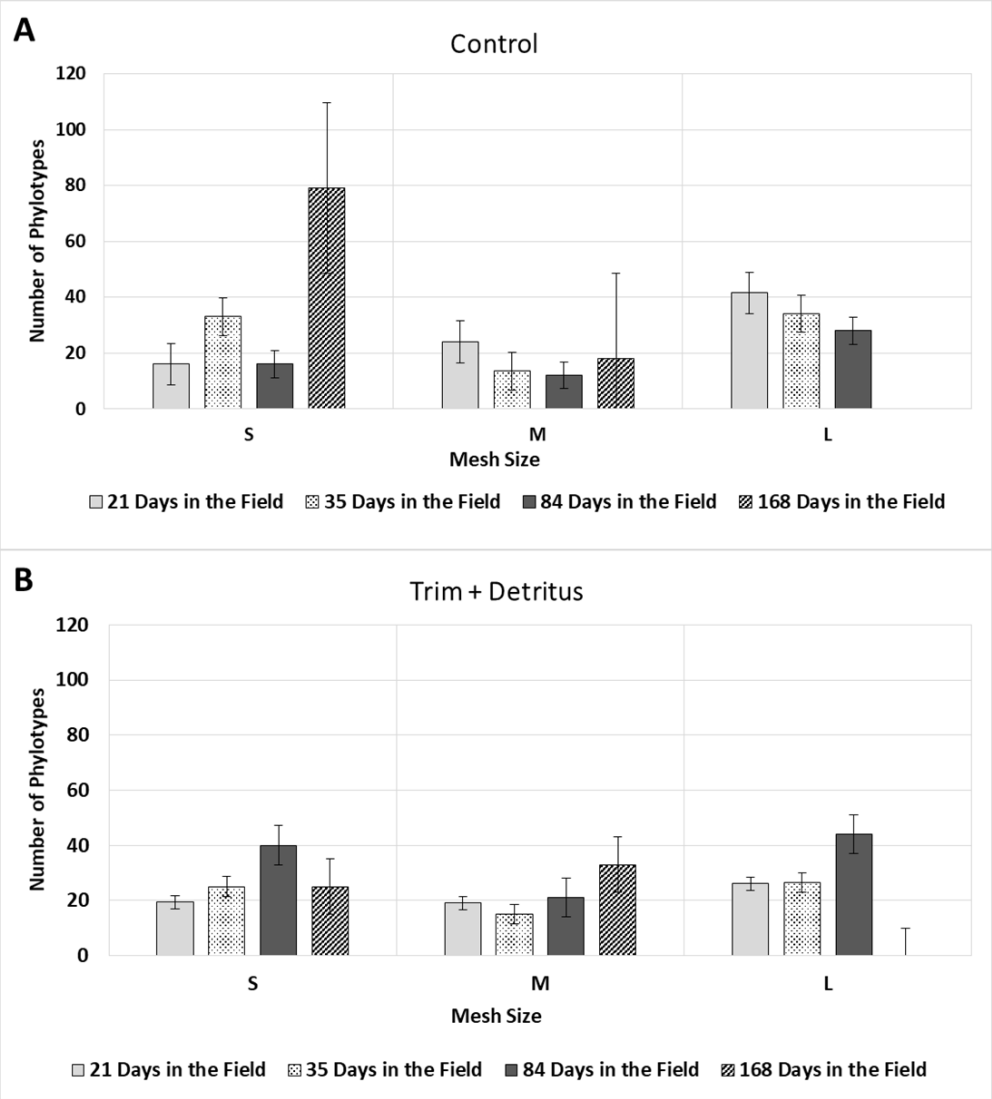

Figure 7. Number of phylotypes of fungi in each time (21 days, 35 days, 84 days and 168 days) for mesh size (small, medium and large) in Control (A) and Trim+Detritus (B) treatment. Bars represent standard error.

Total arthropod abundance was significant different ($P < 0.05$) through time (Table 1) and was trend higher in control treatment for large litterbag mesh size (Fig. 8a). Furthermore total arthropod abundance was higher for all litterbag mesh sizes in 84 days in both treatments (Fig 8a and 8b).

Average abundance was trend higher in large litterbag mesh size (Fig.6a and 6b) for both treatment (control and trim+detritus) and higher in 84 days for both treatments (Fig 6a and 6b). While the number of taxonomic groups was higher in 21 days through time and in the large litterbag mesh size for both treatments (Fig. 6c and 6d).

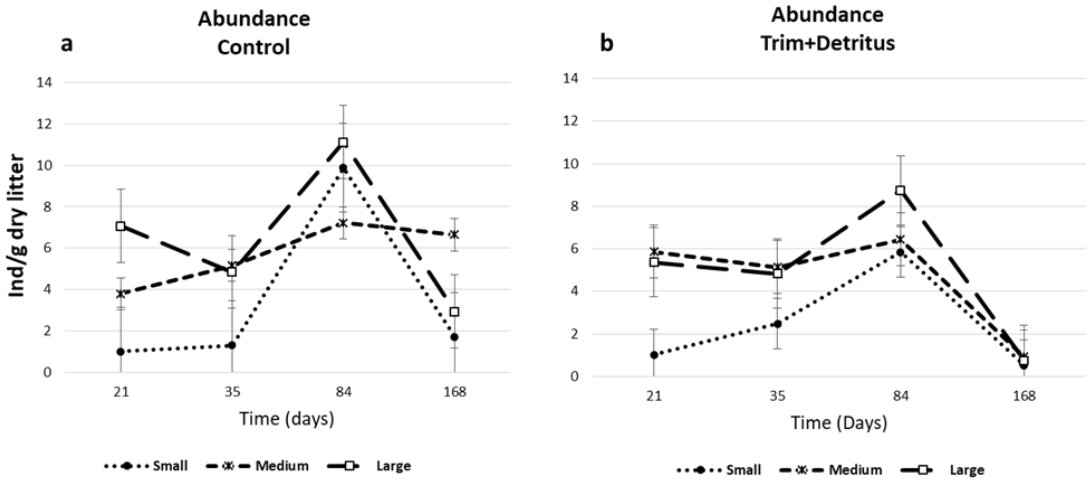

Figure 8. Average arthropods abundance (Ind g^{-1} dry litter) in each mesh size (small, medium and large): **(a)** Total abundance through time for Control and **(b)** Trim+Detritus treatment. Bars represent standard error.

Integration

Abundance of Arthropod and Fungi: Pearson Correlation between abundance of arthropods and fungi were significantly P-value of 0.049 with a correlation index of -.233 (Table 3). These results showed an inversely proportional negative significant correlation, (Fig. 9).

Table 3. Pearson Correlation between abundance of fungi and arthropods

		Abundance (Ind/g dry litter)	Average
Abundance (Ind/g dry litter)	Pearson Correlation	1	**-.233***
	Sig. (2 tailed)		**0.049**
	N	72	72
Average	Pearson Correlation	**-.233***	1
	Sig. (2 tailed)	**0.049**	
	N	72	72

*Correlation is significant at the 0.05 level (2-tailed).

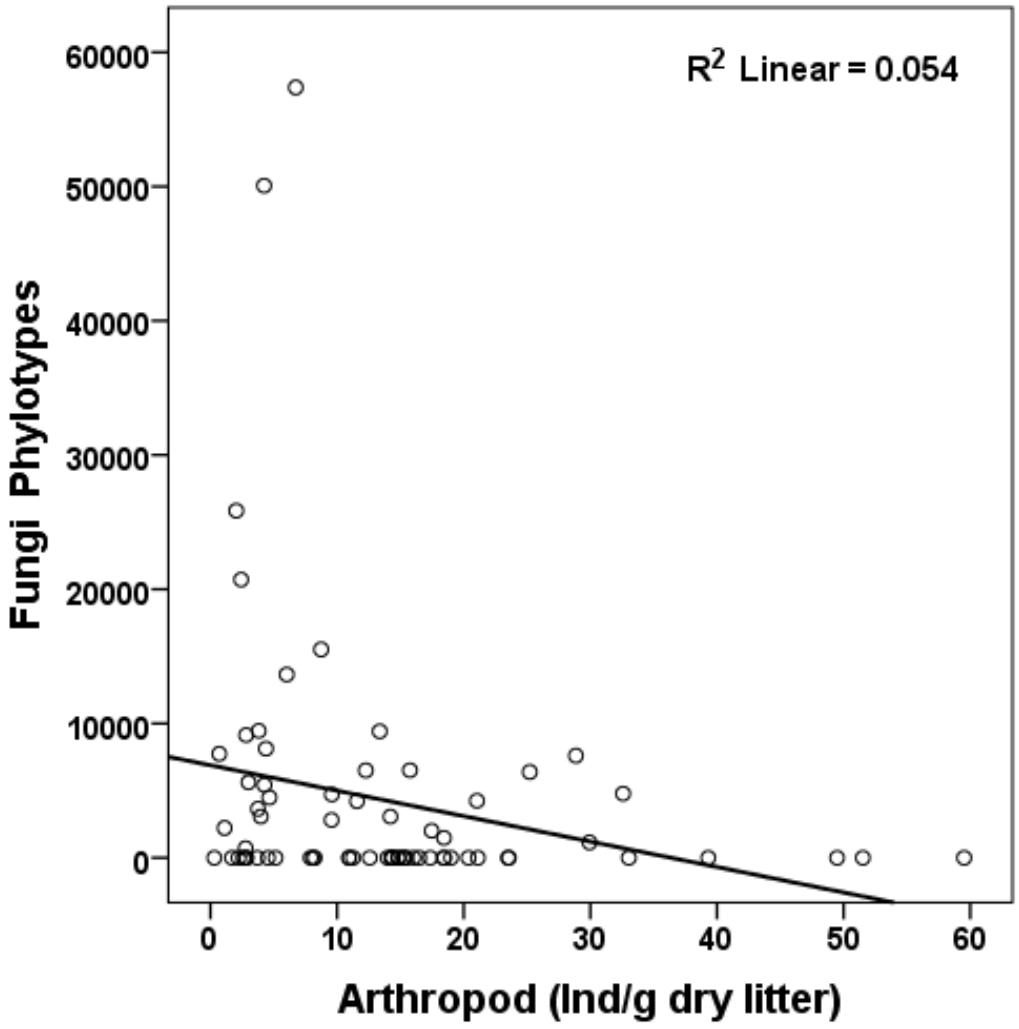

Figure 9. Pearson Correlation between arthropods and fungi abundance. Negative Significant Correlation, inversely proportional.

Top-Down Effects: Negative inversely proportional significant correlation, suggest that increased arthropod abundance decrease fungi abundance and nitrogen concentration.

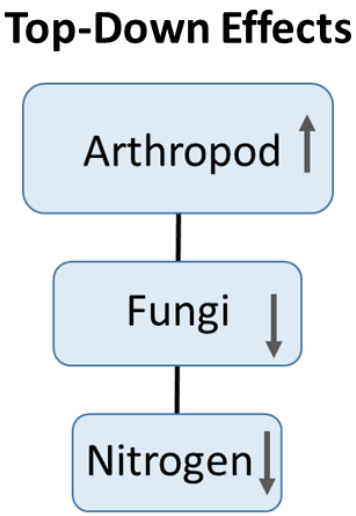

Figure 10. Diagram of Top-Down Effects of Arthropod Abundance in Fungi Abundance and Nitrogen Concentration.

Also during the six months (2014-2015) less precipitation was observed in 168 days after canopy trimming (Fig.6). This event of drought is not normal for the forest, because the dry season at Luquillo Experimental Forest (LEF) is between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). During the study a severe drought was observed starting in December 2014 and lasted till August 2015. . Drought events will affect microbes and arthropods diversity (Bouskill et al. 2016; Liu et al. 2013; Schowalter, 2017). This data coincide with decrease in artrophod abundance, available nutrients and fungi in the 168 days after trimming.

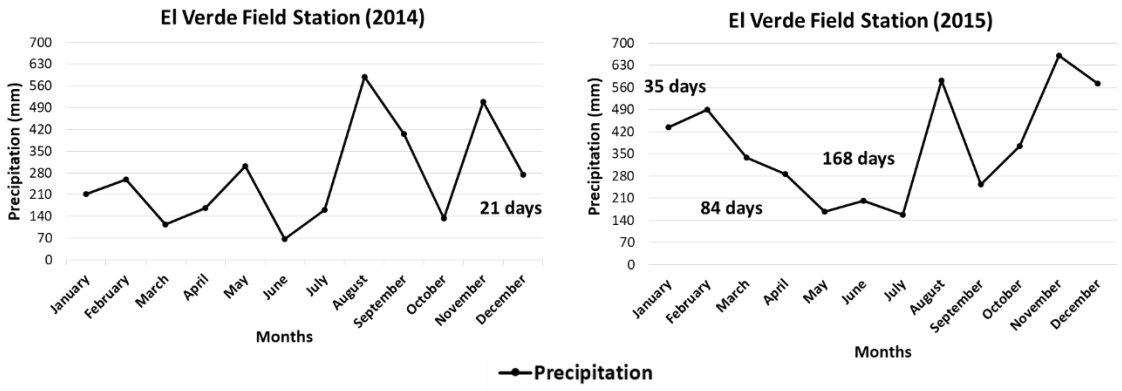

Figure 11. Diagram of General Precipitation (mm) for one year (2014-2015) to four times: 21 days, 35 days, 84 days and 168 days during six months (168 days) after canopy trimming in Luquillo Experimental Forest in El Verde Field Station located in the northeastern part (18.33080, -65.82320, WGS 84) of Puerto Rico**.**

Discussion

Litter decomposition by micro-organisms is highly dependent on microclimate including in the litterbag environment (Aerts, 2006). In addition, any effects of litterbag mesh size on microclimate are therefore likely to affect the enzymatic processes and animal activity on the litter within the bags. While the effect of canopy opening and debris (trim+detritus treatment) provides more organic matter for arthropods, this stimulates the activity of soil animals in the early time (days), for example our results showing a higher total abundance for all litterbags sizes in time 35 days in both treatments. In our study, we found that the total abundance was significantly different through time ($P < 0.05$) but not between treatment and among litterbag mesh size. Furthermore, total abundance was

higher in 84 days in both treatments, while the abundance of fungus was less in 84 days. In our results Acari was the most abundant order and, collembola with acari was the most common order in both treatment. These results are associated with the reduction of macroinvertebrates (consume and fragment litter) and a greater abundance of microbivores (mites, collembola) that feed on microfungi (Richardson et al. 2010). Consequently, there was a trend for higher arthropod abundance, low fungi abundance, and nutrient availability, suggesting trophic dynamics mediated by decomposer microbes.

Trim Detritus Treatment and Litterbag Mesh Size: The response of Canopy opening and deposition detritus response represent the cumulative effects of a hurricane (Shiels et al. 2015) For example, our results suggest a cascading effect (Fig. 12), which including increase solar, which decrease litter moisture. In addition, increased solar radiation by canopy opening effect increase a pioneer plants, while debris (trim detritus treatment) provides more organic matter for arthropods, stimulating the activity of soil animals in the early time (days) and increased herbivory resulting in an increase of leaf nitrogen and phosphorus concentration (Shiels et al. 2015). However increased an abundance of microbivores (mites, collembola) that feed on microfungi (Richardson et al. 2010) decreasing microbial communitie (Fungi) and decrease nitrogen release by inmobilization.

Furthermore we found that abundance of arthropod and fungi has a Pearson Correlation significantly P-value of 0.049 with a correlation index of -.233. In addition, our results suggest a Top-Down Effects (Fig.10), when increase in abundance of arthropods in 84 days (Fig. 8a and 8b), decreased the abundance of fungi (Fig. 7A and 7B) in 84 days and also the concentration of nitrogen in 84 days (Fig.6) This coincides with the Cascading Effects of canopy openness that lead to most of the shifts in the forest biota and biotic processes, which included the decreased abundance and diversity of several animal

groups (Shiels et al. 2015). Also, we had a several drought in June, that not normal for this forest, because the dry season at Luquillo Experimental Forest (LEF) is between December and March (most commonly March) (http://lternet.edu/data/ lterdb14/data/). But for in this study we had a sever dry season on June (168 days after trimming) and this data coincide with decrease artrophod abundance, available nutrients and fungi in the 168 days after trimming.

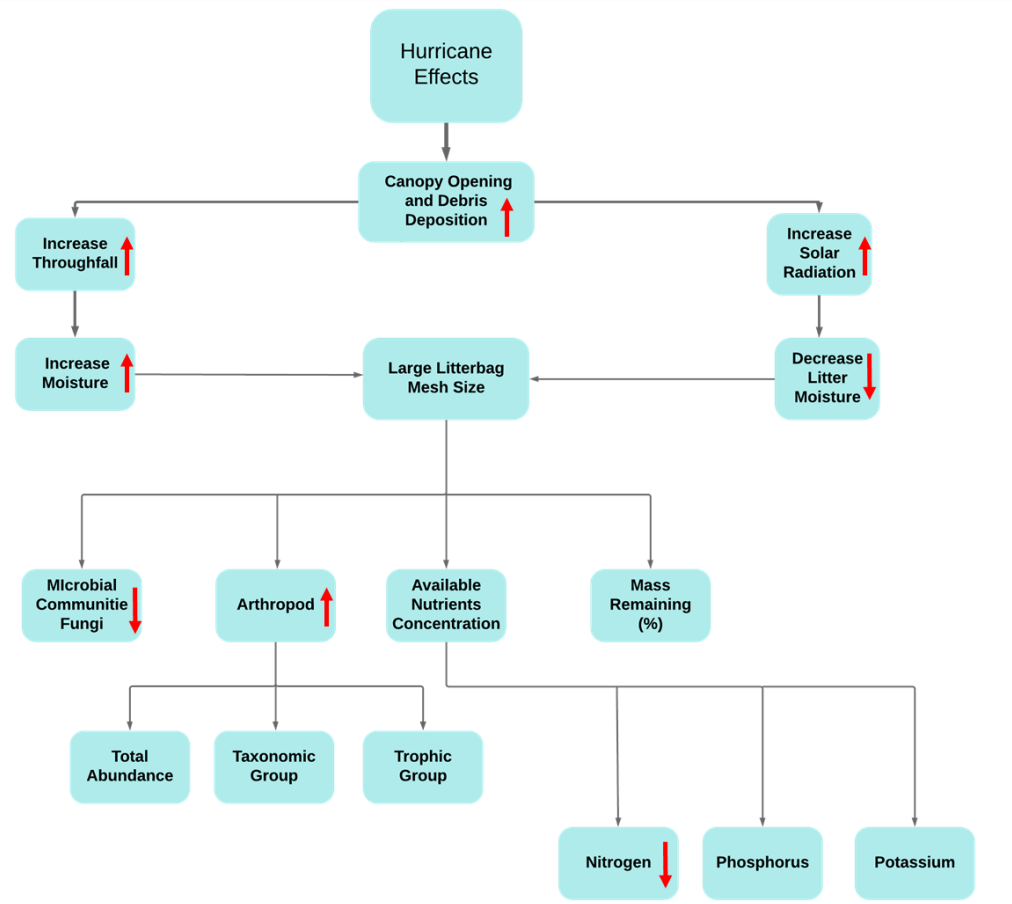

Figure 12. Diagram of integration of Trim Detritus (canopy opening and detritus deposition) treatment and litterbag mesh size.

Conclusion

In our study, the mass remaining was controlled by meso-scale factors: Climate and Resource Quality. Mass remaining were significantly different through time but not between treatments. In our experiment at the meso-scale level, the physical-chemical factors (temperature and humidity) and the quality of the resource (litter) are equal in both treatments. While the abundance significantly different ($P < 0.05$) trough time and variety of taxonomic and trophic groups vary for both treatments. However, the results of the mass remaining does not show a significant difference between the treatments. Furthermore, in our study, the number of taxonomic groups was significantly different ($P < 0.05$) between control and trim+detritus treatment, among litterbag mesh sizes ($P < 0.05$) and through time ($P < 0.05$). Therefore our result showed that abundance of arthropods does not affect the loss of mass, because the effect of arthropods on leaf litter decomposition occurs on a smaller scale, not on a meso-scale, as a result of the interaction between arthropods and microbes.

Furthermore, our results showed, nitrogen, phosphorus, and potassium were significantly different among litterbag mesh sizes and through time ($P <0.05$), and were significantly higher in large litterbag. Consequently our results suggest that available nutrients concentration was affected by microbial biomass (Fungi). While microbial communities (Fungi) was a significant correlation (P-value =0.049) inversely proportional (.-233) between arthropods and fungi abundance. These results suggest a Top-Down Effects when an increase in abundance of arthropods in 84 days decreased the abundance of fungi in 84 days and also the concentration of nitrogen in 84 days.

In our study, we found that litterbag mesh size significantly affected nutrient released, arthropods abundance, and structure of the fungal community. For example, there was a trend for higher arthropod abundance, higher nutrient availability and larger

mass loss in large litterbags mesh size, suggesting trophic dynamics mediated by all decomposer communities. These results approve our hypothesis that arthropod diversity of green leaf litter, nutrient availability and microbe community would vary among the three litterbag mesh sizes in the control and treatment.

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