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Effects of a simulated hurricane disturbance on forest floor microbial communities

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ABSTRACT

Forest floor microbial communities play a critical role in the processes of decomposition and nutrient cycling. The impact of cultivation, contamination, fire, and land management on soil microbial communities have been studied but there are few studies of microbial responses to the effects of tropical storms. The Canopy Trimming Experiment was executed in the Luquillo Experimental Forest of Puerto Rico to decouple two prominent effects of a hurricane—canopy opening and debris deposition on the forest floor—on forest biota and processes. We studied the independent and interactive hurricane effects of canopy openness and debris deposition on the relative abundance and diversity of microorganisms in soil and leaf litter using ester link fatty acids methyl esters (EL-FAME) analysis, and terminal restriction fragment length polymorphism (TRFLP) profile. Non-metric multi-dimensional scaling analysis of soil FAME showed soil microbial community composition was significantly different between pre- and post-hurricane periods including in the unmanipulated plots and among blocks, but there was no significant separation among treatments. This shows that there are strong spatiotemporal dynamics in the structure of soil microbial communities which masked hurricane effects (canopy opening and deposition of green debris). The degree of difference among treatments decreased with time in soil which suggests that our study may have started too late after the manipulations and therefore missed the effects of canopy opening and debris addition. This reflects the resilience of the soil microbial communities. The richness of soil bacterial TRF's however, showed a significant positive response to added debris. Neither fungal nor bacterial NMDS clusters for leaf microbial communities showed significant grouping by treatment, time or litter cohorts. Significant differences were observed through time for fungal diversity in green leaves and for both bacterial and fungal diversity in senesced leaves. Senesced leaves microbial succession apparently stopped when both the canopy and debris were removed, and there was a suggestive trimming by time interaction which reflects the susceptibility of the leaf litter microbial community. Our findings contribute to the understanding of how microbial community structures can be affected by hurricane disturbances and forestry management practices that remove canopy and debris from the forest floor, and shows the need to analyze the microbial community immediately after the disturbance. Short-term changes in microbial communities due to forest disturbances can have significant implications for litter decomposition, soil organic matter accumulation, nutrient cycling, and food web dynamics in tropical forests. All of these factors should be taken into consideration when selecting the appropriate forest management practice.

1. Introduction

Forest floor microbial communities mediate important ecosystem processes such as the decomposition of organic matter and recycling of carbon and inorganic nutrients such as nitrogen. These microbial processes are crucial for maintaining ecosystem function

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and stability as well as providing environmental resistance or resilience to change (de Vries and Shade, 2013). Ecosystem stability is defined as the ability of a community to return to the original equilibrium condition after passing through different intermediate states after a disturbance. The concept of stability includes the terms resistance (community does not change) and resilience (rate of returning to the original equilibrium condition) (de Vries and Shade, 2013). The resistance and resilience of a microbial community will depend on the diversity and function of the community. As explained by de Vries and Shade (2013), a microbial community composed of r-strategists (fast growers) will be highly resilient, thriving in nutrient rich and highly disturbed environments while if it is composed of K-strategists (slow growers) it will be more resistant to disturbances and be characteristic of nutrient poor environments. In microbial communities of environments that are less disturbed, the strongest competitors will survive and excluded other species, making the community less diverse (Lin et al., 2011). Species that show high tolerance to altered conditions (forest thinning, e.g. nutrients, temperature, pH) are characteristic of highly disturbed environments (Lin et al., 2011). Forest management practices can alter the structure and function of microbial communities and understanding how these communities respond to disturbances will facilitate a better understanding of which management practices are best at maintaining ecosystem stability (Lin et al., 2011).

Global climate change is expected to affect patterns of precipitation, temperature and litter inputs and it is likely to increase the frequency of intense tropical cyclones (Michener et al., 1997). The large quantities of biomass transferred by hurricanes (cyclones) from the canopy to the forest floor increase soil nutrient pools (Lodge et al., 1991). Canopy openings also change the microclimate on the forest floor, drying surface litter due to increased solar radiation and wind while increasing soil moisture due to reduced evapotranspiration (Miller and Lodge, 1997; Richardson et al., 2010). The effects of hurricane disturbances are similar to those of forestry operations that open the canopy and leave branches and leafy debris on the forest floor. Moisture is a key factor controlling fungal biovolume (Lodge, 1993; Lodge et al., 1994) and microbial biomass in soil and leaf litter of tropical forests. Soil moisture in wet tropical forests affects oxygen concentrations by causing fluctuating redox potentials that strongly influence the structure of microbial communities and hence the products and fates of carbon in the ecosystem (Pett-Ridge and Firestone, 2005; Pett-Ridge et al., 2006; De Angelis et al., 2010).

Microbial activity is responsible for most litter decay in tropical forests, though leaching (Cleveland et al., 2006) and litter arthropods (Heneghan et al., 1999; Richardson et al., 2010) also contribute significantly to mass loss. Different groups of microbial decomposers are adapted to degrade different types of substrates, and are adapted to different environmental conditions, leading to microbial community succession (Lodge, 1996). Labile compounds are degraded first by fast-growing bacteria and sugar fungi, while recalcitrant resources (e.g., lignin) may be decomposed later primarily by fungi, especially white-rot basidiomycetes that use hyphal strands and cords to colonize new resources and to import nutrients from their previous food base (Miller and Lodge, 1997). In contrast to temperate forests, colonization of leaf litter by white-rot basidiomycete fungi in wet tropical forest commences during the first few days to weeks after litterfall (Lodge et al., 2008; Sinsabaugh et al., 2002). Degradation of lignocellulose by white-rot fungi provides additional types of resources that are decayed by other microbes, leading to further microbial community changes. Disturbances that open the canopy and deposit green debris, whether from a natural disturbance (e.g., hurricane) or anthropogenic activity (e.g., silvicultural thinning) could significantly affect microbial decomposer communities and rates of decomposition through their effects on forest floor environment and litter qualities (Miller and Lodge, 1997; Lin et al., 2011). One important factor to consider when analyzing the effect of disturbances is the knowledge of the seasonal dynamics of microbial communities (de Vries and Shade, 2013). The diversity of soil microbes varies over periods of hours to weeks and these changes can be related to specific microbial group (Bardgett et al., 2005).

Studies of the impact of disturbances on microbial communities have primarily measured biomass, respiration (bulk community), and enzyme responses rather than diversity and community structure which can provide information of which members of the microbial community are actually changing therefore providing a better understanding of ecosystem stability (Griffiths and Philipot, 2013; Griffiths et al., 2004; de Vries and Shade, 2013). The effects of natural disturbances on leaf and soil fungi have been studied in the forest adjacent to El Verde Field Station (EVFS), a tropical wet forest in the Luquillo Experimental Forest (LEF) in Puerto Rico that experiences frequent tropical storms and hurricanes, landslides and tree falls (Cowley, 1970; Lodge and Cantrell, 1995; Lodge, 1996; Stephenson et al., 1999; Brokaw et al., 2012). The earlier studies of disturbance effects on decomposer fungi in Puerto Rico were reviewed by Lodge (1997) and Miller and Lodge (1997). Additionally, Stephenson et al. (1999) found that diversity of amoebae and slime molds (protostelids, dictyostelids and myxomycetes) in the litter layer was highest in the most disturbed part of the EVFS corresponding to areas with higher functional diversity and abundance of bacteria, which are the primary food sources of the amoebae and slime molds (Willig et al., 1996). One of the main environmental factors that negatively affects the diversity and abundance of fungi in wet tropical forests such as the LEF is drying (Lodge, 1993; Lodge et al., 1994; Lodge and Cantrell, 1995). Following Hurricane Hugo's passage over the LEF, some species of agaric leaf decomposer fungi disappeared from sites on ridges that were exposed to greater sun and wind, including *Collybia johnstonii* (Lodge and Cantrell, 1995). Data on changes in microbial biomass and nutrient storage in response to hurricane disturbance and drying in the LEF were used to illustrate fungal importance in controlling nutrient availability to trees in wet tropical forests through pulsed nutrient dynamics (Lodge et al., 1994; Miller and Lodge, 1997).

The objective of our research was to determine the independent and interactive effects of the two main effects of a hurricane – canopy opening and debris addition – on the diversity of microorganisms in soil and leaf litter. We hypothesized that the structure of the microbial community in soil samples would change under canopy opening due to increased soil moisture. We also expected that the structure of the microbial community in leaf litter would change through time due successional processes during decomposition and that canopy opening would have a stronger effect on leaf litter than on soil microbial communities. Changes in the microbial community can have consequences for decomposition of organic matter, the fate of carbon and nutrients and the stability of the ecosystem.

2. Methods

2.1. Experimental design

The Canopy Trimming Experiment (CTE) was executed near EVFS to decouple two prominent effects of a hurricane–canopy opening (trim) and canopy debris deposition onto the forest floor. The CTE experiment followed a 2×2 factorial design that included four treatment combinations: (A) no trim + no debris (NT + ND) – this represents the unmanipulated treatment; (B) no trim + debris (NT + D) – this represents the effect of debris addition under closed

canopy conditions; (C) trim + debris (T + D) – this represents the combined effect of canopy opening and debris deposition after a hurricane, (D) trim + no debris (T + ND) – this represents the effect of canopy opening alone, and no canopy debris was added to the forest floor. Briefly, three replicate blocks (A, B, and C) each with four treatments (30 × 30 m plots) were established in tabonuco (*Dacryodes excelsa*) forest in the LEF near EVFS, Puerto Rico (see map in Shiels and González, 2014, this issue). In Trim plots (6 plots), branches and stems ⩽10 cm diameter were cut at least 3 m height from all trees ⩾10 cm DBH, and palm fronds were removed from palm trees (*Prestoea acuminata* var. *montana*) that were at least 3 m height. In each of the six debris addition plots, an equal mass of canopy debris (5408 ± 143 kg; mean ± SE dry mass) was spread evenly across the ground. For a detailed description of the site, experimental design, and treatment methodology, see Richardson et al. (2010), Shiels et al. (2010), or Shiels and González (2014, this issue).

2.2. Sampling

Soil sampling for microbial analyses was done in five 5 × 5 m subplots established for soil analysis in the interior 20 × 20 m area of each plot. Soil cores were collected using polyvinyl chloride (plastic) tubes 5 cm in diameter and 10 cm in length after removal of surface litter. A pooled sample was prepared combining soil samples from each subplot to obtain one representative sample per plot (3 samples per treatment at each sampling period). The plots were established in October 2002. Soil samples were collected every 3 months from November 2002 to August 2003 and this represents the pre-treatment period. It was expected that the application of the treatments (canopy trimming and addition of debris) would begin in September 2003 but permitting problems delayed the process. It took approximately 8 months (November 2004–June 2005; Shiels et al., 2010) to execute the canopy trimming and the redistribution of canopy debris. After the application of the treatments, soil samples were taken every three months from August 2005 to June 2006, which is considered the post-treatment period. The number of samples per sampling period is 12 for a total of 96 samples for FAME analyses. Samples were stored in sterile plastic bags at −20 °C for fatty acid and DNA extraction.

DNA extraction was also conducted on leaf litter obtained from within open-mesh plastic baskets (35 × 25 cm; see Lodge et al., 2014 this issue, for enclosure design and description). The baskets were modified by replacing the bottom with 2 mm nylon mesh. Litter enclosures (baskets) were placed in five randomly selected subplots per plot 1 month after treatments were completed for the last block. Existing forest floor was transferred to the bottom of the basket, followed by a layer of 1 mm mesh plastic window cap screen, then a monolayer of weighed senesced leaves, followed by another screen. Baskets were placed on the bare soil where the organic layer (detritus layer) had been removed. Senesced (fresh fallen) leaves were of *D. excelsa* (tabonuco) that had been collected within 24 h after they abscised and they were surface air-dried (10 g surface air dried was equivalent to 6.3–6.8 g oven dried at 60 °C). In treatments receiving debris deposition, an additional layer of weighed green leaves was added. Green leaves were comprised of a mixture of 100 g fresh weight of three co-dominant tree species collected from the understory. The green leaf mixture was equal (in g/m^2) to the mean green leaf deposition in tabonuco forest that resulted from Hurricane Hugo (Lodge et al., 1991). DNA of leaf endophytes and epiphylls was not extracted from the leaves placed in the baskets because they were constant among all treatments and because they are quickly replaced by decomposers on the forest floor (Lodge, 1997). Baskets were placed in the subplots the first week of July 2005 after the treatments were applied. One basket per subplot (i.e., 5 per plot) was destructively sampled starting in October 2005, and a new screen was placed in each remaining basket to separate leaf litter cohorts. The number of (basidiomycete) fungal connections between litter cohorts was determined (see Lodge et al., 2014, this issue). Two g fresh weight of senesced and green leaf litter was removed from each of the five baskets and pooled within plots, then stored at −20 °C until DNA extraction. The remaining material from these cohorts was used to determine wet to dry weight ratios, mass loss and phosphorus content (Lodge et al., 2014, this issue).The sampling periods analyzed for leaf litter microbial communities were 14, 28 and 53 weeks. Because these samples represent microbial communities at different stages of succession, detection of treatment effect requires comparison to the unmanipulated treatment at the same time step and not to the initial. The number of samples analyzed for senesced leaves was 36 and for green leaves 18 (total leaf litter samples 54).

2.3. Ester link fatty acid methyl ester (EL-FAME) community structure analysis

Fatty acids were extracted directly from 3 g of soil using the method described by Schutter and Dick (2000) for EL-FAME. This technique has been used to study abundance and composition of microbial communities. Briefly, the method used a mild alkaline methylation followed by pH neutralization with 1 M acetic acid and EL-FAME extraction using hexane. Extracts were cleaned using a NH_2 column to remove humic substances. Samples were stored at −20 °C until analyzed. Samples were analyzed in a gas chromatograph mass spectrometer (Hewlett Packard 6890). For a summary of the fatty acids used to characterize the microbial communities see Table 1.

2.4. TRFLP community structure analysis

A homogenous 0.3–0.5 g sample of each litter or soil sample was extracted for DNA using MoBio Ultraclean Soil DNA extraction kit. DNA quality was assessed by electrophoresis with 1% agarose gel and DNA concentration was calculated with a biophotometer. TRFLP community analysis has been used to determine the microbial community structure and distribution in different ecosystems and disturbance regimes (Lord et al. (2002), Pérez-Jiménez and Kerkhof (2005), Cantrell et al. (2013)). Briefly, the 16S bacterial rDNA and the fungal ITS region of the rDNA were PCR amplified separately from genomic DNA extracts using forward primer fluorescently labeled. The 16S bacterial rDNA was amplified using primers 27F-FAM and 1525R and the fungal ITS region was with primers ITS1-FAM and ITS4. Amplicons were enzymatically digested with specific restriction enzymes, *Mnl*I for 16S rDNA and *Hae*III for ITS (Cantrell et al., 2013), following the manufacturer's protocols (Invitrogen™, United Kingdom). After digestion, samples were ethanol precipitated to eliminate impurities and dried. The samples were re-suspended in formamide with a GenScan 500 Liz size standard (ABI, Warrington, UK). Samples were run on an ABI 3130 Genetic Analyzer (ABI, USA) and TRFLP profiles were generated using GeneMapper Software version 4.0 (ABI, USA).

2.5. Statistical analyses

2.5.1. Soil EL-FAME data

We used non-metric multidimensional scaling (NMDS), available through the VEGAN package in R (Oksanen et al., 2013), to analyze a set of 49 different fatty acid concentrations (indicative of the soil microbial communities) found in soil samples at the experimental plots. NMDS is a data visualization and dimensionality-reduction technique that re-projects high-dimension data onto

Table 1
Groups of fatty acids used in the present study.

Microbial group	Fatty acid	Refs.
Fungi/bacteria ratio	18:2ω6/i15:0 + a15:0 + 15:0 + i16:0 + 16:1ω7t + i17:0 + a17:0 + *cy*17 + 17:0 + 18:1ω7c + *cy*19:0	Frostergård and Bååth (1996)
Gram +	i15:0 + a15:0 + i16:0 + i17:0 + a17:0	White et al. (1996), Zelles (1997)
Gram –	*cy*17:0 + *cy*19:0 + 18:1ω7c + 17:1ω7 + 16:1ω7t	White et al. (1996), Zelles (1997)
Fungi	18:2ω6	Frostegård et al. (1993), Zelles (1997)
Actinomycetes	10Me16:0 + 10Me18:0	Zelles et al. (1994)
Protozoa	20:3ω6	White et al. (1996)

a few (commonly two) new axes. In this sense, it is similar to principal components analysis (PCA). As a second step, the researcher can conduct tests to see if the position of points on the NMDS axes significantly relate to ancillary factors and covariates. We tested three factors (plot, treatment and block), one temporal covariate (time, measured in months since the start of the experiment), and 15 chemical covariates (Cl, NO_3-N, SO_4-S, Na, K, Ca, NH_4-N, PO_4-P, DOC, TDN-N, DON-N, Silica, CO_2 flux, CH_4 Flux, N_2O Flux; unpublished data from W. Silver and W. McDowell available in the Luquillo LTER web site). The chemical covariates were measured at the same plots, during the same sampling periods, as the fatty acids.

Once the iterative NMDS solution is found, the R-VEGAN package uses a random permutation method (“envfit”) for determining whether the factors and covariates are significantly influencing the NMDS scores (i.e., the locations of each observation in ordination space). For each covariate, this test compares the length of the fitted vector on the NMDS axes to a distribution of fitted vector lengths calculated after randomly permuting the dataset a number of times (i.e., randomly assigning a covariate value to each point in ordination space). The *p*-value (significance level) for the covariate is determined as the percent of the randomly-permuted fitted vector lengths that the actual fitted vector length exceeds. For categorical factors, “envfit” calculates the differences between the average NMDS scores at each level of the factor. It then compares these factor-level differences to a distribution of differences calculated over a number of random permutations of the data (i.e., randomly assigning each point in ordination space to a factor level). As with covariates, the *p*-value for the factor is the percent of the randomly-permuted factor-level differences that the actual difference exceeds. If the actual factor-level difference was greater than 93.6% of the randomly-permuted differences, the p-value for that factor would be 0.064. For our significance tests, we ran 10,000 permutations to construct the distributions of fitted vector lengths and factor-level differences. Contour plots (for covariates) and 95% confidence ellipse plots (for factors) were used to interpret the direction of influence on NMDS scores. In addition, we performed a multivariate analysis of covariance (MANCOVA) in *R* to test for differences among factor-level means (block, treatment and plot) and to test the significance of the time covariate. For this analysis, the NMDS scores on axis 1 and axis 2 were the dependent variables.

2.5.2. Soil and leaf litter TRFLP data

To test whether soil or leaf microbial communities using terminal restriction fragments (TRFs) data was affected by the four treatment combinations, a TRFs presence and absence matrix was prepared and a NMDS cluster analysis was performed using the Bray–Curtis similarity index with PAST Version 2.03 (Hammer et al., 2001) and analyzed using ANOSIM (Analysis of similarities) to determine significant differences between treatments ($p \leqslant 0.05$). ANOSIM is a non-parametric analysis to test significant differences between groups based in any similarity index. The use of NMDS with Bray–Curtis similarity index and ANOSIM are recommended for TRFLP data (Rees et al., 2004). Green leaves were only added to the T + D and NT + D treatments while senesced leaves were added to all treatments. Leaf litter TRF data were also used to calculate alpha and beta diversity. Soil TRF data were analyzed with a mixed-effects repeated measure model using the transformed number of different TRFs (double-squared root transformation). The model was run with trim, debris, season and pre-/post-treatment as Model I factors and block as a model II factor and 10 DF. For the leaf litter TRF data, a repeated measures two-way factorial linear mixed-effects model with trim and time as model I factors, and block as a model II factor was used for green leaves; a three-way factorial linear mixed-effects model with trim, debris, and time after manipulation as model I factors, and block as a model II factor (i.e. random effect) was used for senesced leaves. The factorial mixed-effects model analyses were performed using the program R (R Core Team, 2013). A principal component analysis was done by using the number of different TRFs per treatment combination and litter cohort together with the data from Lodge et al. (2014, this issue) on hyphal connectivity, percent of mass remaining and percent of moisture using the program PAST.

3. Results

3.1. Soil microbial communities

Both FAME and 16S rDNA TRFLP results showed that the microbial community composition in soil changed between the pre- and post-treatment samples including the unmanipulated plots. Inspection of climate data from El Verde on the LTER website (http://climhy.lternet.edu/plot.pl) revealed that monthly mean precipitation was somewhat lower during the pre-treatment than in the post-treatment wet and dry season samplings (ca. 200 and 80 mm vs. 215 and 140 mm), but the longer pre-treatment sampling for FAME spanned a very dry year (2002) and two very wet years (2003–2004). The NMDS analysis for the FAME results indicated that there was clear and significant ($p < 0.0001$) separation in microbial communities throughout time between the pre- and post-treatment measurements based on lipid markers, while there were no significant differences in the lipid profile among the various treatment combinations ($p = 0.2677$) (Fig. 1 and Table 2). On the other hand, the three replicate blocks (A, B, C) were significantly different ($p = 0.01438$) (Table 2). The fungal lipid marker 18:2ω6 characterized the pre-treatment soil communities while the bacterial *cy*19:0 (anaerobic, Gram negative) and the i15:0 (Gram positive) lipid markers characterized the post-treatment. The results indicate that Cl, Na, K, DON-N, N_2O and CO_2 fluxes are significantly related to NMDS2 which is what primarily separates pre-treatment from post treatment lipid profiles, while NH4-N is aligned with changes in NMDS1 which is what separates the replicate blocks (A, B, C) (Table 3). The contour plot indicates (results not shown) that NH4-N was higher in Block A relative to Block C and likely explains the variability among replicate blocks. Cl, Na, K, and DON-N concentrations decrease as the lipid profiles change through time (from pre-treatment to post treatment samples) (Table 3). In contrast, N_2O and CO_2 fluxes significantly increase through time (N_2O $p < 0.0001$ and CO_2 $p = 0.07129$). Inputs

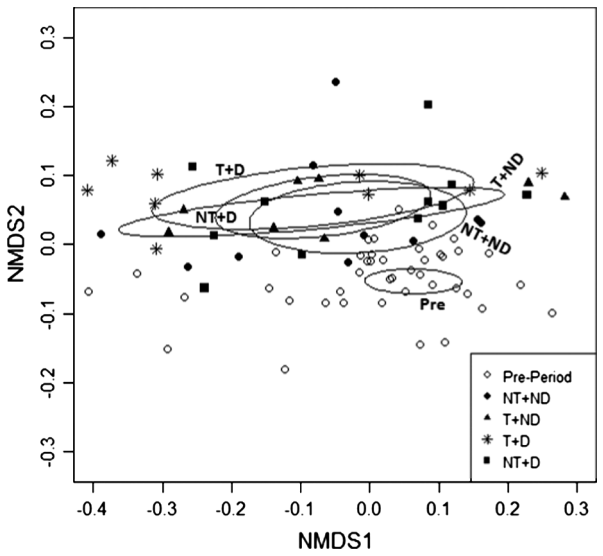

Fig. 1. NMDS cluster of soil EL-FAME with all four treatments combined. The 95% confidence ellipses around the centroid (mean) of each group are plotted. There are significant differences between pre and post treatment samples (Goodness of fit $p = 0.0010$).

of Cl and Na originate from sea salt aerosol in rainwater while higher N_2O fluxes result from stimulation of certain anaerobic bacteria following heavy rainfall as well as when soil is waterlogged because of reduced transpiration associated with defoliation. Consequently, a significant factor affecting soil microbial communities was rainfall 2 and 4 weeks prior to sampling (Goodness of fit: 2 weeks $p = 0.0160$, 4 weeks $p = 0.0288$). Since no significant differences were observed between the treatment combinations, we decided to run separate analyses using sampling date as the independent variable to test whether a significant difference could be observed as a function of the time between treatment application and sampling event. The results showed a trend of declining treatment effect through time (Aug 2005 $p = 0.19$, Dec 2005 $p = 0.55$, Mar 2006 $p = 0.49$, Jun 2006 $p = 0.88$) indicating that treatment effects had declined below significant levels by the first sampling and continued to decline through time. The NMDS cluster analysis of the soil 16S rDNA TRFs data using Bray–Curtis similarity index showed some clustering into pre- and post-treatment but these groupings were not significantly different (One-Way ANOSIM $p = 0.9300$) (Fig. 2).

The richness of bacterial TRFs in soil shows a significant three way interaction between debris, season and pre-/post-treatment ($p = 0.0370$). Debris addition had a strong positive effect on TRF richness in soil in Spring regardless whether the canopy was trimmed (Fig. 3). In Fall, however, debris addition had no effect on TRF richness in untrimmed plots, but had a positive effect in the trimmed plots, which explains the significant three way interaction (Fig. 3).

Table 3
Probability values from permutation test (envfit in R-Vegan package) and concentration trend for environmental parameters. Only parameters that were significant at $\alpha < 0.1$ were included. Values indicate results from 10,000 permutations.

Environmental parameter	Permutation test p-value	Concentration trend through time	Coordinate parallel to trend
Cl	0.0568	Decrease	NMDS2
Na	0.0777	Decrease	NMDS2
K	0.0606	Decrease	NMDS2
NH_4-N	0.0250	Increase	NMDS1
DON-N	0.0665	Decrease	NMDS2
CO_2	0.0713	Increase	NMDS2
N_2O	<0.0001	Increase	NMDS2

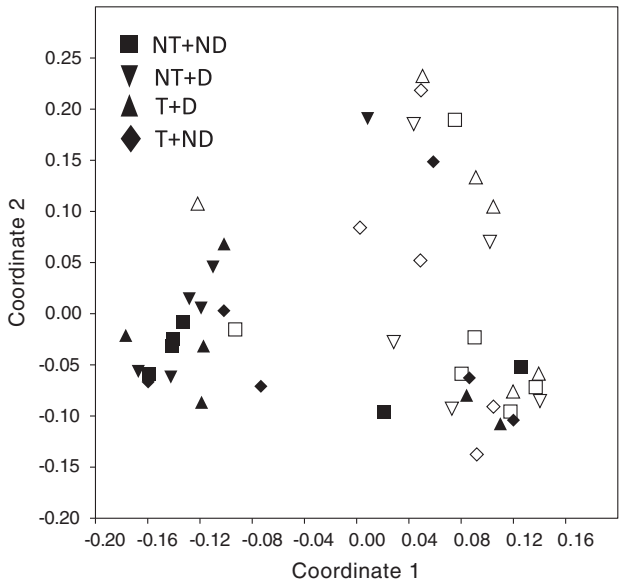

Fig. 2. NMDS cluster using Bray–Curtis similarity coefficient of the soil microbial community based on bacterial 16S rDNA TRFs data. (Open symbols = pre-treatment samples, filled symbols = post-treatment samples). One-way ANOSIM revealed no significant differences between the microbial communities ($p = 0.93$).

3.2. Leaf litter microbial community

The repeated measures analyses of transformed TRF richness from senesced leaves showed significant changes through time for both bacteria and fungi ($p = 0.042$ and 0.046, respectively), and a suggestive trim by time interaction ($p = 0.075$) (Table 4). In senesced leaves, the unmanipulated treatment has a strong peak for fungal TRF richness at 28 weeks whereas the other treatments

Table 2
Probability values from a multivariate analysis of covariance (MANCOVA) and random permutation method (envfit in R-Vegan package) applied to test for differences among factor-level means (block, treatment and plot) and to test the significance of time and environmental covariates. NMDS scores on axis 1 and axis 2 were the dependent variables.

Factor	Description	MANOVA p-value	Permutation p-value
Block	Replicate blocks A, B, and C	0.01438	0.0199
Plot	Each individual plot measured through time	0.2677	0.1157
Time	Months since the first sampling date	<0.0001	<0.0001
Treatment	P: All plots in the period before treatments were applied No Trim, No Debris Trim, No Debris Trim, Debris No Trim, Debris	0.618	0.9336

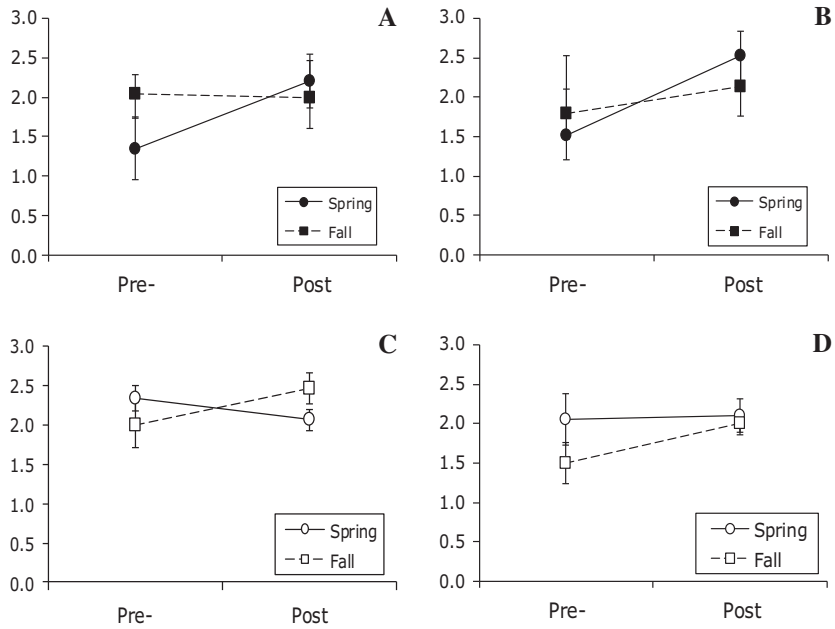

Fig. 3. Interaction graphs of soil bacterial 16S richness based on number of different TRFs in each treatment. A. No trim plus debris. B. Trim plus debris. C. No trim plus no debris. D. Trim plus no debris. A mixed-effect repeated measure analysis was performed on the transformed number of different TRFs (double-squared root transformation). The model was run with trim, debris, season and pre-/post as model I factors and block as a model II factor and 10 DF. The only significant result ($p = 0.0370$) was the three-way interaction of debris by season by pre-/post treatment.

Table 4
Probability values from a two-way factorial linear mixed-effects model with repeated measures analysis of the numbers of different TRFs in senesced and green leaves. Trim, debris, and time were included as model I factors, and block as a model II factor for senesced leaves; trim was not a factor in green leaves as they were only placed in plots with added debris. The number of different TRFs was transformed using the double square root transformation. Samples from the first collection in which no TRFs were detected were assumed to have resulted from PCR failure and were recoded as missing values. Significant values ($p < 0.05$) are in bold while those approaching significance ($0.1 > p > 0.05$) are underlined.

Source	DF	Trim	Debris	Time	Trim:Debris	Trim:Time	Debris:Time	Trim:Debris:Time
Senesced leaves								
Fungi	17	0.124	0.620	**0.046**	0.444	0.075	0.713	0.693
Bacteria	17	0.857	0.591	**0.043**	0.978	0.385	0.944	0.609
Green leaves								
Fungi	9	0.894		**0.022**		0.363		
Bacteria	9	0.348		0.526		0.371		

do not (Fig. 4). Neither fungal nor bacterial NMDS clusters showed significant grouping by treatment combination, time or litter cohorts (green vs senesced leaves (figures not shown) (Bacterial One-Way ANOSIM $p = 0.3195$; Fungal One-Way ANOSIM $p = 0.1834$). For green leaves, there was a significant effect of time using transformed fungal TRF richness ($p = 0.022$), but no effect of trim (Table 4). In green leaves the fungal community declined at 53 weeks (Fig. 4).

The number of different TRFs for both fungi and bacteria changed through time in all treatments with the exception of T + ND suggesting that microbial succession in senesced leaf litter was arrested where the canopy was opened and debris was removed (note that the data points for T + ND remain in the same quadrant through time in Fig 5). The figure also shows shifts in the number of different TRFs between litter cohorts. The factors that contribute more to PC1 are the number of fungal TRFs and fungal connectivity between litter cohorts, and for PC2 are the percent of mass remaining and number of bacterial TRFs. Therefore, the community shifts from fungal dominated during the early stages of decomposition to bacterial dominated during late stages of decomposition.

4. Discussion

Soil microbial communities are important for nutrient cycling in forest ecosystems and mediate processes important for soil fertility and forest productivity. Griffiths and Philipot (2013) reviewed the impact of different types of disturbances such as cultivation, fire, land management and contamination on the stability (resistant and resilience) of soil microbial communities. The reviewed studies used a diversity of techniques including fatty acids, TRFLP clone libraries and next generation sequencing; yet, none of the reviewed papers included natural disturbances such as tropical storms or hurricanes. We hypothesize that the structure of the microbial community in soil samples would change in response to canopy opening, with subsequent reduction in evapotranspiration and increase in soil moisture. Instead, our results show that there are strong spatiotemporal dynamics in the structure of soil microbial communities which masked one of the hurricane effects (canopy opening) as was found for gastropods (Willig et al., 2014). Temporal dynamics were influenced by inter-annual variation in rainfall, whereas spatial dynamics are associated with natural small-scale heterogeneity of tabonuco forest. Our results

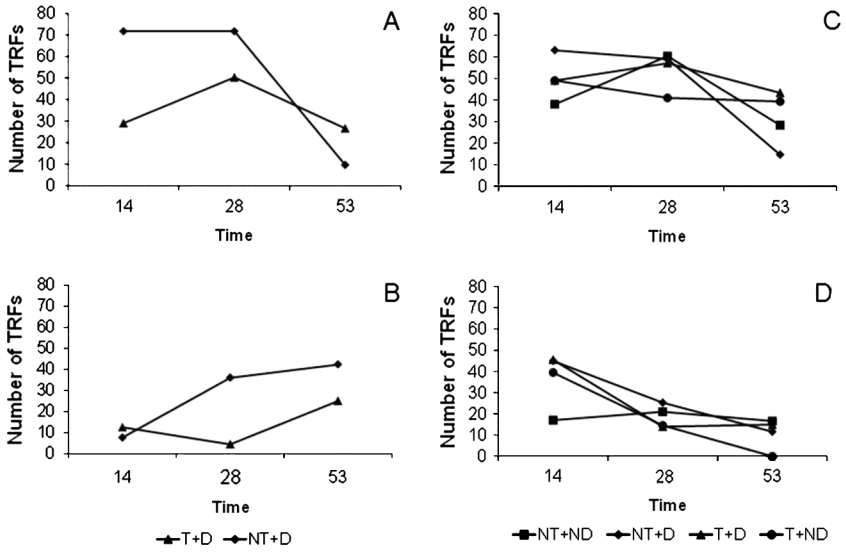

Fig. 4. Comparison of the leaf litter microbial communities in the leaf litter decomposition experiment. (A) and (B). On green leaves, the average number of TRFs for the fungal ITS rDNA (A) and bacterial 16S rDNA (B). The only treatments where green leaf trials were conducted were in the no trim plus debris (NT + D) and trim plus debris (T + D). (C) and (D). Senesced leaves average number of TRFs for the fungal ITS rDNA (C) and bacterial 16S rDNA (D). Senesced leaves were measured for microbial communities in all four treatments. Time represents the number of decomposition weeks. Error bars represent the standard deviation of the mean.

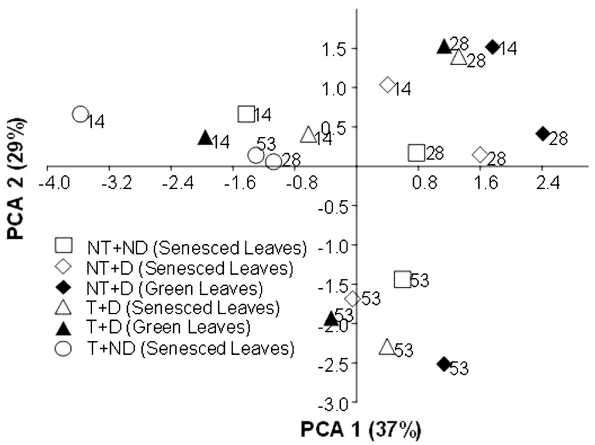

Fig. 5. Principal component analysis of the number of different TRFs, number of fungal connectivity, percent of mass remaining and percent of moisture for in each treatment and litter cohort in the leaf litter decomposition experiment.

support the hypothesis that there are successional changes in the structure of the microbial community in leaf litter through time as decomposition progresses and there is a suggestive interaction between canopy opening and time. The most surprising result was the arrested microbial succession in trimmed plots in which canopy and debris was removed, corresponding to the slowest rates of mass loss among treatment combinations found by Lodge et al. (2014, this issue).

4.1. Soil microbial communities

The only analysis that detected significant treatment effects on the soil microbial community was the two-way repeated measures analysis of soil bacterial TRF richness. Debris deposition had a strong positive effect on soil bacterial TRF richness in Spring, but only in trimmed plots in Fall. Although the NMDS did not detect significant treatment effects, significant relationships in soil microbial community changes were found with variables related to rainfall patterns such as cumulative rainfall prior to sampling as well as soil Cl and Na ion concentrations as observed by others (Walker et al., 2013). Soil lipid markers shifted from fungal dominance in the drier pre-treatment years to bacterial dominance in the wetter post-treatment years. The shift from K-strategists to r-strategists reflects the resilience of the soil microbial community, and could be related to the increase in soil nutrients and moisture. Soil moisture and redox potential are important factors affecting soil microbial communities and the processes they mediate. Higher cumulative rainfall during post treatment sampling was associated with higher flux rates of N_2O and a decrease in DON-N based on NMDS analysis, and corresponded with an increase in anaerobic bacteria in the soil community. High rainfall can affect oxygen concentrations in soils (Lodge et al., 1994; Lodge, 1996; Pett-Ridge and Firestone, 2005), decreasing the redox potential. Increased N mineralization from hurricane debris and possibly reduced N uptake by defoliated trees after Hurricane Hugo resulted in bacteria-mediated denitrification and large losses of N via N_2O efflux from soil (Steudler et al., 1991). Although canopy opening increased soil moisture in trimmed plots (Richardson et al., 2010) as a result of reduced evapotranspiration, we did not find significant treatment effects using fatty acids analysis, despite the significant differences observed in the soil microbial community between pre- and post-treatment samples. In comparison, soil bacterial TRF composition also separated by pre- and post-treatment years in Fig. 2 even though the ANOSIM analysis did not detect significant differences between pre- and post-treatment. Pooling of samples within plots reduced the number of replicates to three per treatment, making it more difficult to detect differences statistically. Pooling within a plot was necessary, however, due to financial and logistical considerations (sample processing and analyses) as well as to avoid pseudoreplication. Additionally, significant inter-annual differences in rainfall and temperature between pre- and post-treatment years may have overwhelmed differences between treatments. Further, the effects of opening the canopy and debris addition may have been missed due to the delay in sampling and the staggered completion dates for each of the blocks (e.g., completion of block A was 6 months after completion of block B). Decreasing differences among treatments with time is concordant with this assumption. These observations suggest that soil microbial communities are highly resilient, and are probably able to return to the original

equilibrium state within 2 months of a disturbance. Unlike observed changes in LEF following hurricanes for plants and animals, any changes in the microbial community were very short-lived (Brokaw et al., 2012). Griffiths and Philipot (2013) emphasized the importance of taking measurements soon after a disturbance in order to understand the stability of the soil microbial community.

The changes from fungi to bacteria may reflect the high soil moisture observed when the canopy was opened, as well as increases in rainfall during the post-treatment year. Our results indicate that microbial communities are correlated more strongly with cumulative rainfall prior to sampling (two to four weeks) than soil moisture at the time of sampling which can be skewed by heavy rains just prior to sampling (Lodge et al., 1994). In addition to affecting soil oxygen and redox potentials, rain can influence soil microbial communities via leachates from organic debris. Cleveland et al. (2006) found that a decrease in the C:P ratio of dissolved organic matter (DOM) leached from leaf litter in a Costa Rican rain forest stimulated microbial mineralization of DOM in soil. Lodge et al. (2014) found that while fungi and other microbes immobilized a significant amount of the P that was obtained from the nutrient-rich green leaf debris, a larger fraction probably entered the soil directly without being immobilized. Soil microbial communities subjected to clear cutting of the forest shifted in microbial community structure but microbial biomass remained constant (Hynes and Germida, 2012). Microbial changes through time were also observed when soils were amended with forest floor material (Hahn and Quideau, 2013).

4.2. Leaf litter microbial community

Differences in abiotic factors such as moisture, light, nutrients and temperature between treatments may partly explain the differences observed in diversity among treatments but trimmed plots also have reduced litterfall (Silver, 2014). Litter moisture was lower in trimmed plots due to increased solar radiation and wind (Richardson et al., 2010; Lodge et al., 2014, this issue). The surprising absence of microbial succession we observed in senesced leaves in the T + ND treatment corresponds to the slowest rate of mass loss found by Lodge et al. (2014, this issue) and González et al. (2014). This observation reflects the susceptibility of microbial communities to the combined effect of canopy opening and debris removal. In our study, fungal and bacterial diversity decreased through time in senesced leaves, while there was decreased in fungal diversity and increased in bacterial diversity in green leaves. The fastest mass loss in the NT + D was also concordant with the highest rates of fungal connectivity between litter cohorts, presumably by basidiomycete fungi that have lignin degrading enzymes (Lodge et al., 2014, this issue) and biomass of litter invertebrates (Richardson et al., 2010). Lodge and Cantrell (1995) found that canopy opening caused by Hurricane Hugo resulted in basidiomycete colony extinctions on ridges. The methods used to detect fungal communities in this study, however, cannot distinguish between agaric macrofungi that can degrade lignin and translocate nutrients among litter cohorts versus microfungi that have limited extent and enzymatic abilities. The addition of debris in the form of green leaves increased moisture and the amount of nutrients available to the layers below (Richardson et al., 2010; Lodge et al., 2014, this issue). Green leaves have higher concentrations of P that can be immobilized by microbes in nutrient-poor senesced leaves (Fonte and Schowalter, 2004; Lodge et al., 2014, this issue). Sayer et al. (2012) found in Panama that the litter layer is important in supplying phosphorus to trees, probably directly via fine roots in the litter layer. Many tree species at our site, including the co-dominant on ridges, *D. excelsa*, produce fine roots primarily in the litter layer.

5. Closing remarks

The primary effect of hurricanes on detrital communities is through changes in microclimate associated with canopy opening (increase solar radiation, surface leaf litter drying and increase surface temperature) and changes in resource quality and quantity from the deposition of green debris on the forest floor (Miller and Lodge, 1997). Even though Lodge et al. (1994) and Miller and Lodge (1997) document the effect of disturbances in soil and leaf litter microbial communities, no previous studies have looked at the separate and combined effects of canopy opening and debris addition on soil microbial communities. In this study, we found a suggestive time by trimming interaction effect on richness of fungal TRFs and arrested microbial succession associated with stalled decomposition in the senesced leaf litter. Sayer and Tanner (2010) found that the leaf litter in Panama was an important source of phosphorus for trees, probably directly via fine roots in the litter layer. Many trees at our field site including the co-dominant on ridges, *D. excelsa*, produce fine roots primarily in the litter layer and are thus likely susceptible to loss of decomposition function associated with combined removal of canopy and debris. The only treatment effects we detected in soil was an increased bacterial TRF richness associated with debris deposition. Previously, Walker et al. (1996) found a long-term decrease in bole growth associated with removal of hurricane debris at our field site, so keeping debris on the forest floor following disturbance appears to be important for maintaining soil fertility and forest productivity.

Our fatty acids results did not show significant effects of canopy opening or debris addition on either soil or leaf litter microbial communities but instead show spatiotemporal changes related to inter-annual variation in rainfall and study area heterogeneity. The soil microbial community changed between pre- and post-treatment samplings including in the unmanipulated plots. The degree of difference among treatments decreased with time which suggests that our study may have started too late after the manipulations and therefore missed most of the effects of canopy opening and debris addition. In addition, the staggered application of treatments by block may have obscured treatment differences in the soil samples by contributing to the significant variation among blocks.

The interaction of plants and soil microbes under a climate change scenario is important to be able to understand the effects of climate change on ecosystem stability and carbon cycling (Simard and Austin, 2010). Ecosystem management practices have largely disregarded the importance of the forest floor microbes' ability to facilitate ecosystem regeneration following a disturbance (Simard and Austin, 2010). Our findings contribute to the understanding of how microbial community structures can be affected by hurricane disturbances and forestry management practices that remove canopy and debris from the forest floor, and shows the need to analyze the microbial community immediately after the disturbance. Short-term changes in microbial communities due to forest disturbances can have significant implications for litter decomposition, soil organic matter accumulation, nutrient cycling, and food web dynamics in tropical forests. All of these factors should be taken into consideration when selecting the appropriate forest management practice.

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