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Effects of canopy opening and debris deposition on fungal connectivity, phosphorus movement between litter cohorts and mass loss ☆

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ABSTRACT

Fungi are important for maintaining fast rates of decomposition in low quality tropical leaf litter via immobilization and translocation of limiting nutrients from sources to sinks and conserving nutrients after disturbance. Tropical trees often have low nutrient to carbon ratios. Disturbances such as hurricanes and logging transfer a large mass of green leaves with high nutrient concentrations to the forest floor, but the associated opening of the canopy dries the litter, inhibiting basidiomycete fungi that play critical roles in lignin degradation and nutrient conservation. We conducted a replicated block factorial experiment designed to disentangle the individual and interactive effects of canopy opening and green debris deposition on phosphorus (P) content, mass loss and fungal connectivity in decomposing leaf cohorts in subtropical wet forest in the Luquillo Mountains of Puerto Rico. Though green leaves had higher P concentrations they did not decompose significantly faster than senesced leaves. Mass loss differed among treatments after 14, 40.5 and 53 weeks decomposition. Mass loss at 7 weeks was predicted by P concentration at 7 weeks; mass loss in senesced leaves at 14 weeks was predicted by abundance of fungal connections between the senesced litter cohort and forest floor at 7 weeks. Fungal connectivity and P accumulation at 7 weeks and mass loss of senesced leaves beginning at 14 weeks were significantly different from and lower in plots with trimmed canopy and no debris than in the untrimmed plots with debris. Litter moisture was previously found to be significantly lower under open than closed canopy, and we found that moisture was a significant predictor of fungal connectivity in both senesced and green leaves. Deposition of green leaves ameliorated the inhibitory effect of canopy opening on fungal connectivity between litter cohorts by retaining moisture; consequently fungal connectivity and mass loss in senescent leaves did not differ between the Trim + Debris and the control treatments. Phosphorus content of senesced leaves increased significantly by 7 weeks in both trimmed and untrimmed plots with added green debris and in the control plots. Based on mass balance calculations, both the underlying forest floor and overlying green leaves likely contributed P to the decomposing senesced leaf cohort. Fungal translocation of P through hyphal connections between litter cohorts explains some of the changes in P content. Though fungi were important in conserving P, most of the P that was likely leached from green leaves was not retained in the litter layer.

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1. Introduction

Nutrient cycling through decomposition of organic matter is important for maintenance of soil fertility and forest productivity (Schlesinger, 1991; Ostertag and Hobbie, 1999; Sayer and Tanner, 2010). At a global scale climate is the main driver of leaf decomposition rates (Meentemeyer, 1978) while litter quality factors become more important at local to regional scales, including concentrations of limiting nutrients and nutrient to lignin ratios in senescent leaves (Aerts, 1997). Tropical and subtropical trees often have low nutrient to carbon (C) ratios (Vitousek, 1984) either because they have low nutrient requirements and hence low concentrations in their mature leaves (Escudero et al., 1992; Aerts, 1996; Parsons and Congdon, 2008) or they have a high efficiency of nutrient resorption before leaf abscision (Aerts, 1996; Koerselman and Meuleman, 1996; Herbohn and Congdon, 1998; Weerakkody and Parkinson, 2006; Huang et al.,

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2007; Wood et al., 2011; Mayor et al., 2014). Resorption efficiency varies by nutrient as well as among tree species (Wood et al., 2011; Reed et al., 2012). Nitrogen (N) is the most commonly observed limiting nutrient in temperate forests whereas in tropical forests phosphorus (P) is often more limiting for leaf production (Vitousek, 1982; Vitousek and Sanford, 1986; Vitousek et al., 1994; Reed et al., 2012; Sayer and Tanner, 2010; Sayer et al., 2012) and decomposition, at least during the early stages of leaf decomposition (Parsons and Congdon, 2008; Wieder et al., 2009; Cleveland et al., 2011).

Disturbances such as logging and hurricanes open forest canopies and often deposit debris on the forest floor. Such disturbances may increase nutrient losses from the forest depending on how the debris is managed, how the microbiota respond to the disturbance and the nutrient exchange capacity and nature of the soil (Miller and Lodge, 1997). While P limitation and movement are the focus of this paper, previous studies of N retention or loss in response to debris addition and removal are more abundant than those on P and demonstrate the basic principles in nutrient cycling. For example, Vitousek and Matson (1984, 1985) found in logged southeastern loblolly pine plantations that microbial biomass conserved most of the N via immobilization if the forest floor remained intact in logged sites (chopping of debris in place), but not when the forest floor was removed by shearing and piling into windrows followed by disking the soil. Presence of fine debris (leaves and twigs) was primarily responsible for short-term nutrient immobilization observed in the two-year study of logging site preparation by Vitousek and Matson (1984, 1985). Zimmerman et al. (1995) found a similar pattern of N immobilization by microbial biomass in Puerto Rico when debris from Hurricane Hugo was left on the forest floor as compared to adjacent debris removal plots. Hurricane debris removal in Puerto Rico reduced microbial competition with trees for limiting nutrients, which resulted in more available N in soil solution and a corresponding short-term increased rate of overstory tree canopy closure (Zimmerman et al., 1995). Removal of hurricane debris in Puerto Rico, however, had a negative long-term effect of slowing bole growth (Walker et al., 1996; Miller and Lodge, 1997). Based on modeling, the long-term effect of debris is related to a positive effect of decaying large woody debris on soil P exchange capacity (Sanford et al., 1991; Zimmerman et al., 1995).

Leaf decomposer fungi primarily fall into two broad functional groups – basidiomycetes that produce macroscopic mushroom (agaric) fruit bodies and mostly colonize multiple pieces of litter with their mycelia, and ascomycetes that reproduce mostly through microscopic asexual spores, produce macroscopic or microscopic sexual fruit bodies, and are typically restricted to a single piece of litter (termed unit-restricted). The former are often referred to as macrofungi while the latter (at least in their asexually reproductive stage) are referred to as microfungi. In mid-elevation wet forest of Puerto Rico, agaric basidiomycetes play a prominent role in early stages of leaf decomposition by employing the same nutrient translocation strategy as basidiomycete wood decomposers summarized by Boddy (1993). Biomass accumulates rapidly in freshly fallen leaf litter despite high C and lignin to P ratios because the fungi import P accumulated from their previous food bases and use them to build new biomass (Lodge, 1993; Lodge et al., 2008). Leaf decomposer basidiomycetes use root-like structures called hyphal strands, cords and rhizomorphs to colonize new substrata and translocate nutrients from old to new substratata; their activity further helps to conserve forest nutrients by binding litter into mats that reduce erosion on steep slopes (Lodge and Asbury, 1988; Lodge et al., 2008). In contrast, microfungi do not bridge gaps between pieces of debris nor translocate nutrients among food bases. In addition to immobilizing nutrients and importing them from previous food bases to overcome nutrient limitation in senesced leaves, basidiomycetes accelerate decomposition by producing enzymes that degrade lignin (Cromack and Caldwell, 1992; Osono and Takeda, 2002; Sinsabaugh et al., 2002). In contrast, few microfungi can degrade lignin. In Puerto Rico, Santana et al. (2005) found using a microcosm experiment that ligninolytic basidiomycetes accelerated mass loss of leaf litter by 22% above that caused by microfungi that lacked ligninolytic enzymes, and Lodge et al. (2008) found similar rates of accelerated mass loss (16%) associated with basidiomycetes in a three month field experiment. The dominant agaric fungi that maintain rapid leaf decomposition despite nutrient limitation in Puerto Rico were inhibited by canopy opening caused by Hurricane Hugo (Lodge and Cantrell, 1995). Paradoxically, a study of forest floor mass changes following Hurricane Georges found that forest floor mass rapidly returned to pre-hurricane levels (Ostertag et al., 2003), suggesting a homeostatic or compensatory mechanism potentially related to the higher nutrient concentrations in green leaves in the hurricane debris. Ostertag et al. (2003) suggest acceleration of forest floor decomposition from addition of nutrient-rich green leaves whereas observations by Lodge and Cantrell (1995) suggest that inhibition of ligninolytic agaric fungi in response to canopy opening might slow leaf decomposition after hurricanes. None of the previous studies, however, examined rates of mass loss or nutrient dynamics in specific leaf cohorts so the responses to the multifactor disturbances wrought by hurricane damage and the mechanisms involved have remained unknown.

In this study, we use a factorial experiment to separate the individual and interactive effects of the two key components of a severe hurricane – canopy opening and deposition of green litter – on agaric fungal connectivity between litter cohorts, P concentrations and content in senesced and green leaf cohorts, and mass loss. Our analyses focus on early stages of leaf decomposition when fine litter was shown to have the greatest effect on nutrient conservation or loss after disturbance (Vitousek and Matson, 1984, 1985), and when lignin degrading agaric fungi are active in colonization and P translocation at our site (Lodge, 1993; Lodge and Asbury, 1988; Lodge et al., 2008) and in Amazonian rain forest in Brazil (Luizão et al., 1998). We focus on P because it was found to be more limiting than N in early leaf decomposition in comparable tropical forests in Australia, Brazil, Costa Rica and Panama (Luizão et al., 1998; Parsons and Congdon, 2008; Wieder et al., 2009; Reed et al., 2012), as well as in pan-tropical meta-analyses (Cleveland et al., 2011; Reed et al., 2012), and because our site has higher green to senesced leaf ratios for P than for N (1.7–3.3:1 vs. 1.1–1.3:1 in Lodge et al. (1991); 6:1 vs. 2:1 for leaf species used in this study) which indicates that P is less available than N.

2. Methods

2.1. Research site

The Canopy Trimming Experiment (CTE) was conducted in subtropical wet forest at the El Verde Research Area (18°20′N, 65°49′W) of the Luquillo Experimental Forest where mean temperature ranges from 22° to 25 °C (Brown and Lugo, 1983). Rainfall (ca. 3500 mm per year) is considered non-seasonal, but January through early April are usually drier (Brown and Lugo, 1983). The plots were established on ridges and upper slopes between 340 and 485 m asl where the dominant canopy trees are *Dacryodes excelsa*, *Sloanea berteriana* and *Manilkara bidentata* (Shiels and González, 2014). Although ectomycorrhizal fungi can inhibit leaf decomposition, tree species in the plots were almost exclusively associated only with arbuscular mycorrhizal fungi (Lodge, 1996; J. Zimmerman http://luq.lternet.edu/data/luqmetadata144). Only two trees that have ectomycorrhizal fungal symbionts occurred

in the CTE plots, one 1.2 cm dbh *Coccoloba diversifolia* and one 6.7 cm dbh *Coccoloba pyrifolia*, both in the untrimmed plot with added debris in Block A, and neither sapling was located in the litter decomposition subplots. Species of *Inga* are sometimes reported as having ectomycorrhizal associations, but clearing and staining of fine roots from three individuals from our site of each of our two species, *Inga lauracea* and *Inga vera*, revealed no evidence of ectomycorrhizae, peritrophic mycorrhizae or ectendomycorrhizae (Lodge, 1996). Eight *Inga* trees were present in CTE plots, all but two in the untrimmed plot without added debris in Block A, all but one were small saplings and none were located in the decomposition subplots.

2.2. Plot treatments

Three blocks (A, B and C) were established in the research area (see map in Shiels and González, 2014), each with four 30 × 30 m plots. A factorial 2 × 2 experimental design was used with two levels of canopy trimming (Trim and No trim) and two levels of debris addition (added Debris and No debris) in all combinations. Treatment combinations were randomly assigned to plots within each block. All branches less than 10 cm diameter were trimmed in the canopy removal plots and carefully lowered to the forest floor. The debris was weighed, stored in piles off of the plots, and then applied to the debris addition plots. Details of trimming and debris application are published in Richardson et al. (2010). Measurements were confined to the inner 20 × 20 m of each plot, and the decomposition experiments were confined to five randomly assigned 5 × 5 m subplots.

2.3. Senesced and green leaf litter cohorts

This decomposition experiment was designed to mimic as closely as possible post-hurricane conditions in order to follow mass loss and nutrient content of the senesced and green leaf cohorts. To this end, 10 g surface air dried freshly fallen *D. excelsa* leaves (identified by fresh abscission scars that turn brown within 24 h, as in Zou et al., 1995) were used for the senesced leaf cohort; 100 g fresh green leaves of *D. excelsa*, *M. bidentata* and *S. berteriana* equal to mean hurricane green leaf litter per unit area (as determined in Lodge et al. (1991)) were used for the green leaf cohort. Debris added to plots as part of the CTE experiment had been stored in piles before being distributed; consumption of green leaves by invertebrates (primarily moth larvae) and decomposition resulted in the debris treatments being comprised primarily of woody debris and insect frass (Richardson et al., 2010), except in block C that retained green leaves in the debris. For this reason, we added fresh green leaves to the litter decomposition baskets in the two Debris addition treatments in the following proportions in all blocks: 25 g *D. excelsa*, 33 g *S. berteriana*, and 42 g *M. bidentata*. Three randomly selected bags of green leaves per block were used for determination of mean fresh to oven-dried weight ratios (0.373 in Block A, 0.367 in Block B and 0.366 in Block C) and initial nutrient concentrations (0.058%, 0.056% and 0.058% P in Blocks A, B and C, respectively; 1.12%, 1.24% and 1.23% N in Blocks A, B and C, respectively; 46.24%, 48.52% and 47.14% C in Blocks A, B and C, respectively).

For the senesced leaf cohort, freshly fallen leaves of *D. excelsa* were collected and air dried in nets for 24–36 h, then sorted into oven dried, tared paper bags to obtain a net weight of 10 g. Oven drying was avoided as it was previously found to slow leaf decomposition (Santana and Lodge, unpublished data; W. Silver, pers. com.). Three randomly selected bags of air dried senesced leaves per block were oven dried at 40 °C to obtain a mean conversion factor for adjusting the initial air dried weight to an estimated oven dried weight (conversion factors of 0.69 in Block A, 0.63 in Block B and 0.68 in Block C). These samples were used to determine initial nutrient content in senesced leaves: 0.0110%, 0.0090% and 0.0089% P in Blocks A, B and C, respectively; 0.67%, 0.60% and 0.56% N in Blocks A, B and C, respectively; 46.88%, 48.03% and 45.96% C in Blocks A, B and C, respectively.

2.4. Leaf litter decomposition baskets

Open-mesh plastic baskets 35 × 25 cm were modified by cutting out the solid bottom and replacing it with 2 mm nylon mesh (Fig. 1). The existing non-woody forest floor litter was transferred intact to the bottom of the basket using a flat metal sheet, the basket placed on the soil where the litter had been removed, and a 1 mm mesh plastic window cap screen was placed over the forest floor layer. All baskets then received 10 g surface air-dried freshly fallen (senesced) leaves of *D. excelsa* that covered 75–85% of the forest floor cap screen, and a second cap screen was then installed (Fig. 1). The two treatments that had received canopy debris (Trim + Debris, and No Trim + Debris) received an additional layer comprised of 100 g fresh weight of green leaves of three co-dominant tree species collected from the understory. These were placed on top of the senesced leaf cap screen. The green leaf layer was followed by a cap screen to separate this cohort from all subsequent (natural) litterfall. Natural litterfall cohorts that accumulated during the first 14 weeks and during subsequent three-month intervals were demarcated by placement of additional screens.

2.5. Experimental design

We implemented a repeated measures randomized block design where five measurement subplots were nested within the two factorial treatments (canopy trim and debris addition, with two levels in each treatment). Treatment combinations were assigned randomly within each of the three replicate blocks (Richardson et al., 2010; Shiels et al., 2010; Shiels and González, 2014). Some plastic baskets that were installed for this litter decomposition experiment were lost to photodegradation so some plots in blocks B and C had four rather than five measurement replicates. One basket was collected from each subplot at about 3 month intervals for one year (14, 28, 40.5 and 53 weeks) except for an additional collection at 7 weeks to capture dynamics of nutrient leaching and fungal translocation. Litter in baskets recovered after 1.5 years was not analyzed because most of the litter was gone and soil contamination was too great in some plots.

After the canopy trimming (October 2004–April 2005) and debris treatments were completed (January–June 2005) in all three

Fig. 1. Litter basket construction with screens separating forest floor, senesced leaf and green leaf cohorts.

blocks (see detailed methodology in Richardson et al., 2010; Shiels et al., 2010; Shiels and González, 2014), baskets were placed during the first week of July 2005, 10–12 days after litterbag placement in the same decomposition subplots used by Richardson et al. (2010). Except for our additional 7-week harvest, basket retrieval coincided with litterbag collections by Richardson et al. (2010) and González et al. (2014).

2.6. Analytical methods

The number of fungal connections between litter cohorts was counted in each basket after harvest as the screens separating the cohorts were carefully rolled starting at one end (Fig. 2). Each rhizomorph, cord and hyphal strand, was counted as it was broken; for fungi forming diffuse webs of hyphae, the number of 1 mm squares penetrated by wefts of hyphae were counted. The litter in each cohort was placed in tared paper bags, weighed, dried at 40 °C and reweighed. To adjust weights to dry weight at 60 °C (as in González et al., 2014), five samples of litter were dried at 40 °C, weighed, redried at 60 °C and reweighed to yield a conversion factor of 0.9915 times dry weight at 40 °C = dry weight at 60 °C. Percent moisture was calculated as weight of the water lost on drying divided by the wet weight of the litter. Because 2 g wet weight was removed from the senesced and green leaf cohorts beginning at 14 weeks for microbial analyses by Cantrell et al. (2014), final dry weight was adjusted to account for the estimated dry weight litter removed.

Only the pre-weighed senesced and green leaf cohorts that were placed in the litter decomposition baskets were analyzed for nutrients. Oven dried samples collected at 0 and 7 weeks were ground using a fine mesh Wiley Mill and analyzed for C, N and P concentrations to determine patterns of nutrient immobilization, mineralization and translocation. Ground litter was digested using alkaline persulfate (Patton and Kryskalla, 2003) followed by analysis for P using robotic colorimetry (Unity Smartchem 200, Brookfield, CT, USA) with the ascorbic acid method (EPA 365.3) at the University of New Hampshire. Litter mass (adjusted for mass when dried at 60 °C) times P concentration was calculated to obtain initial and 7-week P contents of the green and senesced leaf litter cohorts. Though nitrogen is not the focus of this study, C and N were measured in samples dried at 110 °C using a Perkin Elmer CHN Series II 2400 Elemental Analyzer at the University of New Hampshire (weight of litter dried at 110 °C = 0.89 times weight dried at 60 °C). These values were used in comparisons of litter quality.

Fig. 2. White rot and fungal cords in partly decomposed leaves in one of the litter cohorts from the decomposition baskets. Hyphal strands of agaric basidiomycete fungi that are responsible for the abundant mycelia and delignification are visible on the leaf surfaces and bridging between pieces of litter (photo by María Ortiz).

2.7. Statistical analyses

Probability values <0.05 are considered significant while those between 0.05 and 0.1 are considered suggestive. Percent mass remaining was analyzed using Proc Mixed model with repeated measures in SAS (ver. 9.3, 2011) with canopy trimming (2) and debris addition (2) treatments as fixed effects and blocks (3) as random effects; subplots nested within blocks were also treated as random effects. The Kenward Rogers denominator degrees of freedom method was used for the heterogeneous covariance model ARH(1) because the Levine's test showed the homogeneity of variance assumption was not met. This covariance structure had lower Bayesian Information Criterion values than a model using the spatial power function; therefore, it better accounted for unequally spaced repeated measures. Green and senesced leaf cohorts were analyzed separately because green leaves were decomposed in only two of the four treatment combinations that correspond to the debris-addition plot treatments. The model for green leaves was the same as for senesced leaves without the factorial portion; the autoregressive order (1) covariance structure was used because the homogeneity of variance assumption was not met. Differences in percent mass remaining between green and senesced leaves in the same basket were analyzed as above except that the analysis was a nested design. Least square means (LSM) were used to test the hypothesis that the difference in percent mass remaining between green and senesced leaves was zero.

Proportion of initial P remaining was analyzed using a Mixed model with repeated measures in IBM SPSS (ver. 20, 2011) with canopy trim (2) and (green leaf) debris deposition (2) treatments as fixed effects and blocks (3) as random effects; subplots nested within blocks were also treated as random effects. Green and senesced leaf cohorts were analyzed separately for P as noted above. The same model was repeated with the addition of covariates: arcsine of percent moisture at harvest on a wet-weight basis, and number of fungal connections to the litter layers above and below. The Levine's test ($p > 0.05$) showed the homogeneity of variance assumption was met in all tests, and PPlot showed the data were normally distributed.

Linear regression was used to determine the relationship of mean percent mass remaining at 14 weeks with mean number of fungal connections between senesced leaves and the forest floor at 7 weeks; data used for both variables were treatment means derived from block means which normalized the data and equalized the variances. Robust regression analyses were used on raw data to determine the relationship between P concentration and percent mass remaining, and between number of fungal connections between litter cohorts and arcsine of percent moisture after 7 weeks decomposition within treatment combinations. We used robust regression because of its stability in the presence of outliers and influential statistics in both the x and y direction (SAS, 2011). We used the Method of Moments (MM) estimation procedure because of its high breakdown values and its ability to detect outliers and influential data points.

3. Results

3.1. Percent mass remaining

Mass loss differed significantly with canopy trimming, debris deposition and time, but did not differ between the green and senesced leaf cohorts. Mass loss was generally fastest under closed canopy with added debris (No Trim + Debris), slowest in the

trimmed canopy without debris (Trim + No debris) treatment, and intermediate in the other two treatments (Fig. 3). For the senesced leaf cohort, the Mixed model repeated measures analysis for percent mass remaining showed that the main treatment effects of time, canopy trimming and debris addition were all highly significant ($p < 0.0001$ for both time and trim; $p = 0.0002$ for debris). None of the interactions were significant ($p > 0.05$). The analysis was repeated as a spatial power function to account for the unequally spaced samples (caused by the additional 7-week sampling) and resulted in similar fit statistics. There were significant differences in mass between all of the time steps as well as between some treatments within sampling times beginning at 14 weeks (Fig. 3). For the green leaf cohort, which was only placed in the two debris-addition treatments, time and canopy trimming were both highly significant ($p < 0.0001$) and the time × trim interaction was also significant ($p = 0.0002$). Although percent mass remaining in the green leaf cohort was always greater in the plots with trimmed canopy, differences between canopy treatments were only significant at 7 and 14 weeks. The estimated Least Square Means and Standard Errors based on the restricted maximum likelihood technique, and significant treatment differences are shown in Fig. 3.

3.2. Changes in phosphorus content

The P content of the senesced leaf cohort increased significantly from time 0 to 7 weeks in all but the open canopy without debris (Trim + No debris) treatment whereas the P content of the green leaf cohort decreased in both trimmed and untrimmed debris addition treatments (Fig. 4). The Mixed model analysis of percent of initial P remaining at 7 weeks was significantly different from zero (i.e., percent P differed between 0 and 7 weeks) in both the senesced and green leaf cohorts ($p = 0.006$ and $p = 0.002$, respectively). Debris addition had a significant effect on P in the underlying senesced leaf cohort ($p = 0.007$), while Block had a significant effect in the green ($p = 0.006$) but not the senesced leaf cohort. In this simple model (without covariates), the effect of canopy trimming was significant for the green and suggestive for the senesced leaf cohort ($p = 0.005$ and $p = 0.057$, respectively). When the same model was used for senesced leaves but with the added covariates of arcsine of percent moisture and number of fungal connections to the litter layers above and below, there was a significant canopy Trim × Debris deposition interaction ($p = 0.005$); fungal connections to the layers above and below were both suggestive ($0.1 > p > 0.05$) but percent moisture was not significantly different ($p = 0.517$). Phosphorus content of the senesced leaf cohort did not differ between canopy treatments when green leaves were placed over them in the baskets, but did differ between canopy treatments if no green leaves were added (No debris treatments; Fig. 4), resulting in the significant interaction. Senesced leaf P content remained the same in the trimmed canopy without debris (Trim + No debris) treatment, but increased significantly in closed canopy without debris (No Trim + No debris) control treatment (Fig. 4). The values presented for mass balance in Fig. 4 are P content of each leaf cohort but the letters denoting significances among treatments are based on the analyses of percent of initial P remaining at 7 weeks as reported above to account for slight differences in initial P content among Blocks.

Where no debris was added, the senesced leaf cohort had a mean of 0.242 mg more P when the canopy was untrimmed as compared to comparable trimmed plots. Using the natural leaf litterfall P concentrations during the first quarter following plot treatments from Silver et al., 2014 times the leaf litterfall mass that accumulated above the top screen in each basket during the first 7-week period in this experiment, the natural post-treatment litterfall cohort in plots with untrimmed canopy had a mean P

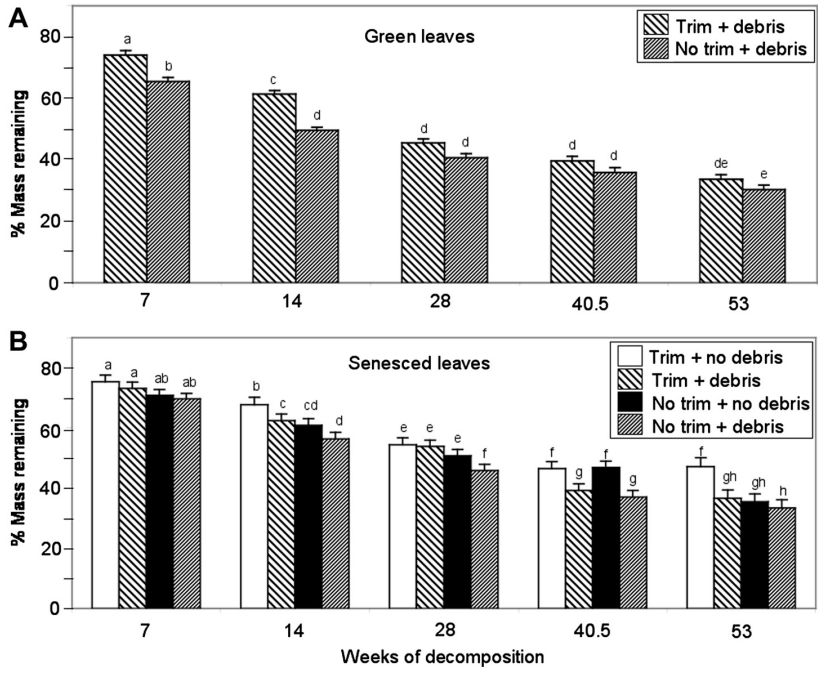

Fig. 3. Changes in percent mass remaining in decomposing green (A) and senesced (B) leaf cohorts over one year in a factorial design litter basket decomposition experiment in subtropical wet forest at El Verde in the Luquillo Mountains of Puerto Rico. The values shown are estimated Least Square Means and Standard Errors based on the restricted maximum likelihood technique, and significant treatment differences based on Bayesian estimates from the Mixed model results rather than arithmetic calculations.

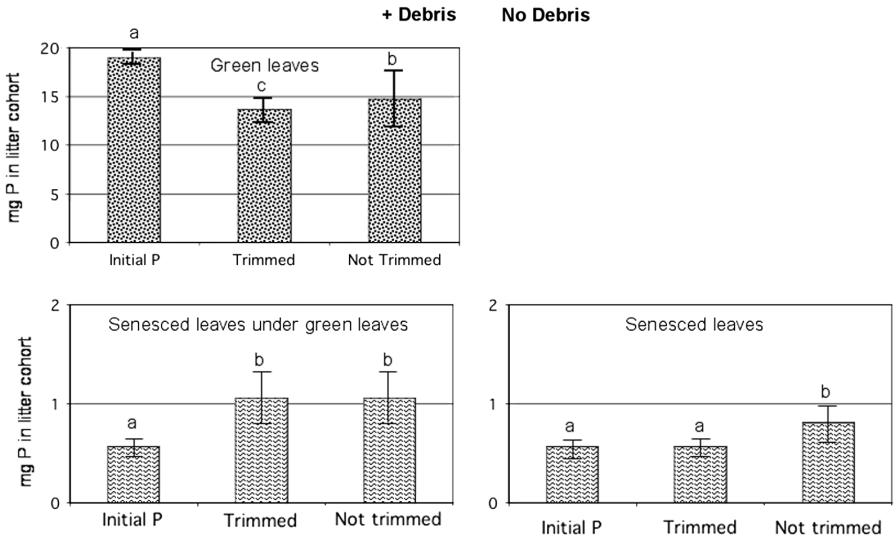

Fig. 4. The values presented in Fig. 4 are mg total phosphorus in the leaf cohort but the letters denoting significant differences among treatments are based on the analyses of percent of initial P remaining at 7 weeks.

content of 0.34 mg (0.21, 0.38 and 0.42 mg in Blocks A, B and C, respectively), while mean P content of trimmed canopy plots was 0.25 mg (0.04, 0.48 and 0.23 mg in Blocks A, B and C, respectively). Thus in the treatments without added debris, the estimated P input per basket via natural litterfall was 0.09 mg greater when the canopy was left intact than when it was trimmed.

3.3. Regression analyses of fungal connectivity, litter moisture, mass loss and P

Robust regression analyses showed that arcsine transformed litter moisture at 7 weeks was significantly predictive for the number of fungal connections to the litter layer above for both the senesced and green leaf cohorts ($p < 0.0001$ and $p = 0.0032$, respectively; Fig. 5A and B). The relationship between moisture and number of fungal connections was positive for both leaf types, but the R^2 value was higher in senesced than in green leaves (0.295 vs. 0.072, respectively; Fig. 5A and B). Robust regressions with percent mass remaining at 7 weeks as the dependant variable showed that P concentration was a significant predictive factor for green leaves ($p < 0.0001$) but only suggestive for senesced leaves ($p = 0.095$; Fig. 5C and D). LSM regression showed that after 14 weeks of decomposition, mean percent mass remaining in senesced leaves for the four treatments was significantly predicted by mean number of fungal connections to the forest floor for those treatments at 7 weeks (Fig. 6). The data shown in Fig. 6 are based on means of the three block means for each treatment combinations ($R^2 = 0.026$, $p < 0.001$).

3.4. Agaric basidiomycetes in the samples

Only a few agaric species can be identified by their mycelia, cords or rhizomorphs at El Verde, but these include *Gymnopus johnstonii* A.W. Wilson, Desjardin and E. Horak – the dominant leaf decomposer under closed canopy at our site, the abundant *Marasmius guyanensis* Mont., *Marasmius crinisequi* F. Muell. ex Kalchbr. and *Micromphale brevipes* (Berk. & Ravenel) Singer. *G. johnstonii* was abundant in plots with closed canopy and a few baskets in trimmed canopy plots with added debris. *M. guyanensis* was identified by fruit bodies and rhizomorphs in two baskets in one control plot and one basket each in a closed canopy plot with added debris, and a trimmed canopy plot without added debris. Fruit bodies of *Marasmius opacus* were found in one basket where the canopy was trimmed and no debris was added. *Mycena* sp. was fruiting in one basket in a control plot. Two species of *Resinomycena* and *Marasmius* sp. aff. *rotalis* were identified by María Ortiz Hernández (unpublished data) using cloning of the nuclear ribosomal ITS region in the two treatments with canopy trimming (i.e., with and without added debris), but samples were not analyzed from the closed canopy treatments.

4. Discussion

4.1. Mass loss in relation to P concentration and basidiomycete fungal connectivity

Agaric basidiomycete fungi play a key role in litter decomposition and P-translocation following canopy and understory disturbances. Early mass loss in the senesced leaf cohort was directly related to number of agaric fungal connections ($R^2 = 0.26$, $p > 0.001$, Fig. 6) to the underlying forest floor and P concentration at 7 weeks (Fig. 5C and D). Except where the canopy was trimmed and no debris was added, senesced leaf P concentrations and content exceeded 100% of initial at 7 weeks and may be viewed as a proxy for immobilization of P in fungal biomass together with importation of P from other litter cohorts via basidiomycete fungal connections. No net immobilization of P and slow decomposition in senesced leaves where the canopy was trimmed and no debris was added corresponds to low abundance of fungal connections to the forest floor in response to dryer litter moisture (Richardson et al., 2010), and reduced post-treatment litterfall inputs where the canopy was trimmed (Silver et al., 2014).

Many tropical agaric decomposer fungi in the litter layer are sensitive to reduced moisture associated with canopy opening (Lodge and Cantrell, 1995; Lodge et al., 2008). *G. johnstonii* is the most abundant leaf decomposer at El Verde under closed canopy, but it disappeared from exposed ridges when the canopy was opened by Hurricane Hugo (Lodge and Cantrell, 1995). Similarly, in this experiment, *G. johnstonii* was abundant in our baskets under

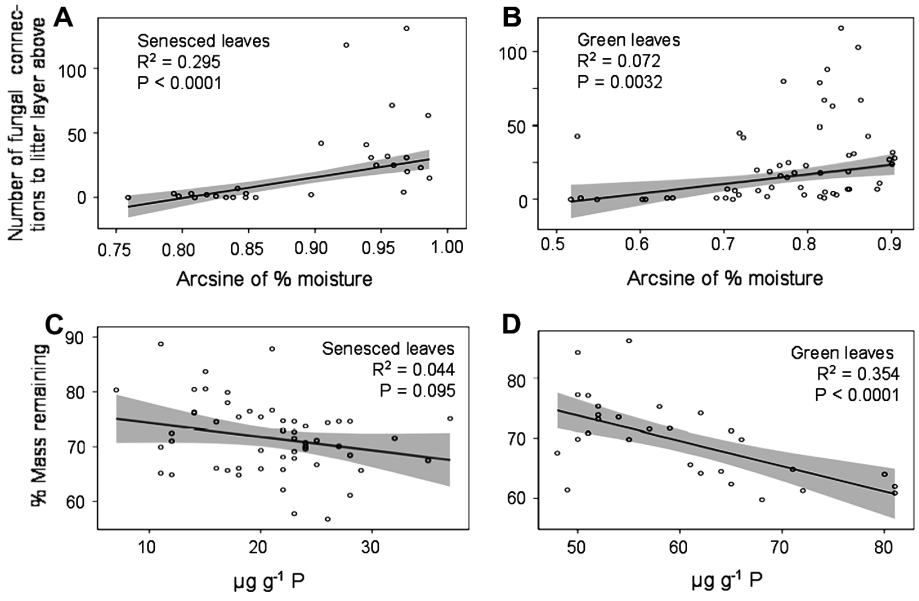

Fig. 5. Number of fungal connections (A and B) and percent mass remaining (C and D). Results of robust regression analyses of data from 7 weeks of decomposition in litterbaskets are shown with 95% confidence intervals in blue. R^2 values and probability that the regression slope is equal to zero are also shown. (A and B) Number of fungal connections from the litter cohort to the layer above at 7 weeks as predicted by arcsine transformed percent litter moisture in the senesced (A) green and (B) green leaf cohorts. (C and D) Percent mass remaining as predicted by the concentration of phosphorus at 7 weeks in senesced (C) and green (D) leaf cohorts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

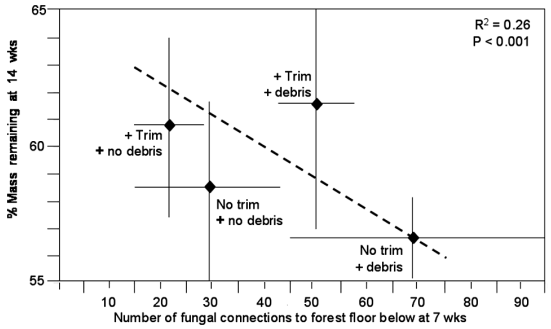

Fig. 6. Least Square Means regression of mean percent mass remaining in senesced leaves for the four hurricane simulation treatment combinations at 14 weeks as predicted by the mean number of fungal connections to the forest floor for those treatments at 7 weeks. The means and standard deviations shown are based on arithmetic means of the three block means for each treatment combination.

closed canopy, infrequent in plots where the canopy was trimmed and debris was added, and rare in plots where the canopy was trimmed and debris was removed. Lodge (1993) found using ^{32}P tracer in a microcosm experiment that *G. johnstonii* had the highest rate of ^{32}P translocation into unlabeled senesced *D. excelsa* leaves followed by *M. brevipes*, a species that increased its abundance on ridges at El Verde after Hurricane Hugo, so loss of *G. johnstonii* in response to litter drying would likely reduce P translocation by fungi between litter cohorts. Although *M. brevipes* has high P translocation rates and was observed in plots with trimmed canopy, it was not detected in our litter decomposition baskets, possibly because it occurs almost exclusively on small twigs and large petioles (Lodge, 1996). *M. opacus*, a species often found in drier microsites including the subcanopy, was found in on basket where the canopy was trimmed and no debris was added. In this experiment, *M. guyanensis* was the most widespread agaric, but in tests for possible use in erosion control it formed two thirds fewer rhizomorph attachments to leaves when it was exposed to partial shade along an entrance road as compared to the full shade of the forest (Lodge et al., 2008), and Lodge (1993) showed it had the lowest rates of ^{32}P translocation among the three species tested. Several representatives of the Mycenaceae were detected, *Mycena* sp. fruiting in a control plot and DNA sequences of *Resinomycena* spp. in trimmed canopy treatments. We can also infer the presence of decomposer agarics species of *Mycena* and *Gymnopus* by the presence of an achlorophyllous orchid, *Wullschlaegelia calcrata*. Martos et al. (2009) and Sellose et al. (2010) identified the saprotrophic mycorrhizal symbionts of *W. calcrata* as almost entirely species of *Mycena* and *Gymnopus* using DNA sequencing, though the orchid they studied on the island of Guadalupe was apparently misidentified as *Wullschlaegelia aphylla* using Feldmann and Barré (2001), a species that does not occur there according to a more recent monograph by Born et al. (1999; Sellose, 23 February 2014, pers. com.). *W. calcrata* was very abundant in the debris addition with no canopy trimming, had low abundance in the closed canopy without debris (control) and the trimmed canopy with debris (hurricane) treatments, but was absent from the trimmed canopy without debris treatment. The fully developed orchids were rooted in the senesced, green or post-treatment natural litter fall cohorts after 1.5 years, so their agaric decomposer fungal symbionts would have been active earlier in decomposition.

Agaric leaf decomposer fungi degrade lignin resulting in white rot (Hintikka, 1970; Cromack and Caldwell, 1992), and they were previously shown to accelerate mass loss of senesced leaves by 16–23% above that caused by microfungi during early stages of decomposition at our site (Santana et al., 2005; Lodge et al., 2008). The abundance of agaric macromycete fungal connections between litter cohorts was significantly related to percent moisture in the litter. Agarics that bind litter into mats on the forest

floor were previously found to be sensitive to drying from wind and sun associated with canopy opening (Lodge and Cantrell, 1995). Canopy opening significantly decreased litter moisture in the CTE (Richardson et al., 2010, using data generated from our no added debris baskets in this study), but addition of green leaves to the baskets allowed the underlying senesced leaf cohort to retain moisture and stimulated fungal growth such that mass remaining and number of agaric fungal connections to the forest floor did not differ significantly between the trimmed canopy with added debris (hurricane simulation treatment) and the control treatment (No trim + No debris). Richardson et al. (2010) found fungivorous arthropods that preferentially consume microfungi over basidiomycete (agaric) macrofungi were significantly more abundant in trimmed than in closed canopy treatments. The results of Richardson et al. (2010) suggest a greater abundance of the microfungal food base for arthropods in the more exposed, drier litter. We thus infer from the decrease in agaric fungal connections and the increase in the microfungal-based food web that opening the canopy caused a shift in fungal dominance from agaric fungal to microfungal dominance. We attribute this shift in fungal dominance to inhibition of basidiomycete macrofungi and favoring growth of microfungi because of dryer conditions with canopy gaps. These results support the prediction based on the observed loss of agaric mycelia on ridges following canopy opening associated with Hurricane Hugo (Lodge and Cantrell, 1995) that rates of leaf litter mass loss should slow when the canopy is opened because of inhibition of lignin-degrading agaric fungi that decompose leaves more rapidly than microfungi, but the effects were stronger for the more exposed green leaf cohort than for the senesced leaf cohort lying below the green leaves. The fast return of forest floor mass to pre-hurricane Georges levels that was observed by Ostertag et al. (2003) in Puerto Rico was not found in our study, but the forests they studied differed in elevation, exposure, successional status and litter quality from our site. The inhibitory effects of gaps on agaric fungal connections, P accumulation and mass loss were greatest in the absence of green debris overlying the forest floor. Lodge et al. (1991) showed there was high spatial heterogeneity in green leaf deposition caused by Hurricane Hugo, so protection of basidiomycete decomposers; consequently, spatial variation in decomposition rates are likely accentuated after a real hurricane, making estimation of forest floor mass difficult.

4.2. Phosphorus limitation and movement

Similar to many other tropical forests, P concentrations are low in senesced tree leaves of subtropical wet forest of Puerto Rico (Lodge, 1993). Low nutrient concentrations in senesced leaves can be produced by plants that have low nutrient requirements and hence low concentrations in their mature foliage (Escudero et al., 1992; Enright and Ogden, 1995; Aerts, 1996) and/or by high efficiency of nutrient resorption before leaf abscision (Vitousek, 1984; Schlesinger et al., 1989; Aerts and Chapin, 2000; Huang et al., 2007; Wood et al., 2011; Reed et al., 2012). Estimates of P resorption in the Luquillo Mountains are high, ranging from 43% to 72% (Medina et al., 1981; Weaver et al., 1986; Lodge et al., 1991), and 67.5% at our study site (Lodge et al., 1991). In contrast, estimates of N resorption are lower, ranging from 5% to 18% in the Luquillo Mountains (5% at our study site; Lodge et al., 1991). According to the classification of deciduous plants by Killingbeck (1996), the co-dominant canopy tree species on ridges used in this study (*D. excelsa*) is (highly) P-proficient (i.e., senescent leaf P of 0.008–0.011% < 0.05% threshold), and (marginally) N-proficient (i.e., senescent leaf N of 0.56–0.67% < 0.7% threshold). Litter P concentration was previously found to be more limiting than N in early leaf decomposition in a comparable tropical forest in Costa Rica (Wieder et al., 2009) as well as in a pan-tropical meta-analysis of lowland rain forests (Cleveland et al., 2011). Similarly, Xuluc-Toloso et al. (2003) found in a tropical dry forest in Mexico that the slower rates of leaf decomposition observed in later stages of succession, as compared to earlier stages, were directly related to P but not N concentrations. A study in wet tropical forests of Queensland, Australia however, showed that both N and P limitation were related to leaf decay rates, but that in contrast to Xuluc-Toloso et al. (2003) study, early successional forest had lower litter quality and slower rates of mass loss than undisturbed primary forest (Parsons and Congdon, 2008).

The high rates of P resorption before abcission at our study site results in P concentrations of senesced leaves that are so low that they resemble those of wood (Lodge, 1993). Wood decomposer fungi have previously been found to overcome nutrient limitation by translocating up to 81% of the N and P from an older food base and incorporating it in new biomass in a fresh piece of wood (Watkinson, 1984; Boddy, 1993). Similarly, a previous experiment in which ^{32}P tracer was added to forest floor material in microcosms showed that cord structures of the dominant leaf decomposer agaric fungus in subtropical wet forest, *G. johnstonii*, spanned a 5 mm air gap and tripled the P content of the freshly fallen senesced *D. excelsa* leaves above during the first three weeks of decomposition (Lodge, 1993, 1996).

In the absence of added debris, the senesced leaf cohort in this study accumulated an additional 0.242 mg P where the canopy was untrimmed vs. trimmed; the difference between treatments was significant. These results are concordant with a significantly higher mean number of fungal connections in the corresponding untrimmed vs. trimmed canopy treatments at 7 weeks (69 vs. 29, respectively). These results are also consistent with the agaric fungal translocation of P from forest floor to senesced leaves of *D. excelsa* documented by Lodge (1993) using a ^{32}P tracer microcosm experiment. An alternative source of some of the additional P is the natural litterfall since there was an estimated mean of 0.09 mg more P in new (post-treatment) litter that collected in each basket when the canopy was untrimmed vs. trimmed. Fresh litterfall, however, is unlikely to be the source of P augmentation in the senesced leaf cohort because it has low P concentrations (mean 0.1 mg g^{-1}; C:P ratio of 4000–6000 in *D. excelsa*) causing it to act as a net sink rather than a net source of P during the first few weeks of decomposition (Lodge et al., 2008). Luizão et al. (1998) found a similar peak in P content of 120–170% of initial at 61 days, and a change from net immobilization to net mineralization of P at around 90 days. Consequently, the percent of initial P exceeds 100% during the early stages of decomposition under intact canopy at our site (in this study a mean of 143% of initial P content at 7 weeks if no debris was added, and 186% of initial in Debris-addition treatments). Thus we deduce that in treatments without added debris that the increment in P content of the senesced leaf cohort in the untrimmed vs. trimmed canopy came primarily from the forest floor cohort via agaric fungal translocation rather than from natural litterfall.

We assume that as in the control treatment (No trim + No debris), some of the increase in P content of senesced leaves in the debris-addition treatments also came from the forest floor via fungal translocation. The disappearance of ca. 5 mg of P from green leaf cohort paired with an increase of ca. 0.5 mg P in the underlying senesced leaf cohort per basket in both debris-addition treatments (Fig. 4) suggests that the green debris was also a source of P that accumulated in the senesced leaves when debris was added. It is less clear how P moved from the green to the senesced leaf cohort. According to source-sink models, fungi might have translocated P from the green leaves, which have higher P concentrations than senesced leaves (0.057% vs. 0.01% in this study), in order to build biomass in the P-deficient senesced leaf cohort. In the CTE, however, there was only a slight, non-significant difference in

P content of the green leaf cohort between the canopy (trimmed vs. not trimmed) treatments (Fig. 4), whereas there was a modest but significant decrease in number of hyphal connections between the senesced and green leaf cohorts at 7 weeks in the plots with trimmed canopy. A more likely explanation is that P moved from the green to senesced leaf cohort via leaching. Cleveland et al. (2006) found in a wet Costa Rican forest that P was leached from leaf litter in the dissolved organic matter (DOM) and that rates of mass loss throughout decomposition were dominated by leaching of DOM rather than evolution of CO_2. Schreeg et al. (2013) also found high rates of P leaching from various leaf litter species in Panama (mean 35% P loss) but as inorganic orthophosphate rather than an organic form. Both Cleveland et al. (2006) and Schreeg et al. (2013) show high rates of P leaching when senesced leaves were placed in water for a brief period, which indicates the leaching process is largely driven by abiotic processes.

4.3. Phosphorus conservation following disturbance

Wet lowland tropical forests on highly weathered soils often have low soil P availability (e.g., low total P in white sands, or low available P where phosphorus-fixing clays occur). These forests thus often have high foliar and/or senesced litter C:P and N:P ratios, and P availability may limit annual net primary production as well as decomposition (Vitousek, 1984; Reed et al., 2012; Mayor et al., 2014). Exposure of the forest floor to drying following canopy disturbance, whether from natural events such as hurricanes or anthropogenic events such as logging, reduces fungal biovolume (primarily agaric basidiomycetes) in wet tropical forest and impairs the ability of litter decay fungi to retain P in the forest floor (Lodge, 1993; Lodge et al., 1994; Miller and Lodge, 1997). Immediately following disturbance events, wet tropical forests are especially at increased risk of nutrient loss if there is asynchrony between nutrient mineralization and fine root uptake (Silver and Vogt, 1993). It is therefore important to understand nutrient conservation mechanisms as nutrient limitation may control subsequent forest productivity (Sayer and Tanner, 2010). For example, Zimmerman et al. (2014) found that adding debris to our plots with intact canopies increased basal area by 10% above control plots seven years after applying the treatments. On the other hand, Walker et al. (1996) found that removal of forest debris from other plots at our site following Hurricane Hugo resulted in a significant decrease in bole increment as compared to control plots four years later, even though debris removal stimulated overstory canopy closure in the short-term by alleviating microbial competition for nutrients in the soil (Zimmerman et al., 1995). Sayer and Tanner (2010) also found a short-term stimulation of litterfall (2–3 years) in litter removal plots, in contrast to a longer-term (4–7 years) decrease in litterfall production in the same litter removal plots in Panama (Sayer et al., 2012). Sayer and Tanner (2010) and Sayer et al. (2012) found in Panama that the litter layer was important in supplying phosphorus to trees, probably directly to tree roots as the pools of available soil P remained unchanged with litter addition but increased with fertilization. Parallel experiments in Panama (Sayer and Tanner, 2010; Sayer et al., 2012), and Costa Rica (Wood et al., 2009) showed a rapid increase in leaf litterfall production in response to a doubling of leaf litter inputs. Although addition of litter can act quickly in stimulating nutrient cycling via litterfall in wet tropical forests, removal or addition of forest floor debris can have longer-term affects on soil microbial activity and soil carbon dynamics (Sayer et al., 2012), the amount of soil carbon that provides exchange sites for mineral nutrients, and consequently, forest productivity (Sanford et al., 1991; Miller and Lodge, 1997; Walker et al., 1996).

Based on our results, fungi were important in retaining P in the litter layer, actively translocating it and immobilizing it in their biomass during early decomposition of senesced leaves. When the P immobilized in the litter was mineralized, it would have been available to tree roots present in the litter or at the litter–soil interface. Some of the P that accumulated in the senesced leaf cohort was imported from the forest floor via fungal connections while a larger amount was immobilized from the green leaf cohort, presumably as leachate. Deposition of green leaves lessened drying of the underlying senesced leaf and forest floor cohorts, thus ameliorating the inhibitory effect of canopy opening on fungal connectivity and retention of P in the senesced litter cohort below. Presumably, some additional P that was apparently leached from the green leaf cohort was also retained in the forest floor layer, some was likely taken up by tree roots present in and below the forest floor, and some may have entered the soil without being immobilized by litter microbes or taken up by plant roots. Sayer and Tanner (2010) and Sayer et al. (2012) surmised that P uptake by tree roots directly by mineralization from forest floor litter was more effective in increasing the amount of P-cycling through increased litterfall than application of fertilizer in Panama. Forest floor mass in wet tropical forests is low, however, compared to temperate forests owing to rapid decomposition rates. Mean forest floor mass is 282 g m^{-2} at our site (Zou et al., 1995). Furthermore, the forest floor contains litter in various stages of decomposition (ca. 0–14 months old at our site) and the inflection point at which leaf litter changes from being a net sink to a net source of P occurs between 1 and 2 months at our site (Lodge, 1993), and at 2–3 months in wet Amazonian forest (Luizão et al., 1998). In control plots in this study, mean weight of natural litterfall cohort that was captured per basket during the first 7 weeks was 6.56 g (similar to the weight of the added senesced leaf cohort of 6.3–6.8 g), which represents only 20–30% of forest floor mass. The forest floor layer would therefore not be expected to immobilize much of the P that had leached from the green leaves above. Because the forest floor litter mass is small and only about 20–30% is still in the immobilization phase (i.e., less than 2–3 months old), stoichiometrically most of the P lost from the green leaf cohort likely entered the soil or was taken up by roots rather than being immobilized in the forest floor by microbial biomass. Although this study focused on fungal P immobilization and agaric macromycete translocation of P, bacteria became more abundant in our senesced and green litter cohorts at later stages of decomposition and are also important in nutrient immobilization (Cantrell et al., 2014).

Large pulses of P from litter to soil may be beneficial to forest regeneration in wet tropical forests that have phosphorus fixing clays since the inputs can overwhelm the fixation sites as well as the nutrient absorption capacity of the soil microbial biomass, resulting in more P being available to plant roots (Lodge et al., 1994). Although live fine root biomass declined in forests at our site following small-scale removal of aboveground biomass and large-scale disturbance from Hurricane Hugo with an associated drought (Parrotta and Lodge, 1991; Silver and Vogt, 1993), the decline was delayed by two to six months after disturbance, and in the case of one of the aboveground harvest plots, was preceded by an increase. In addition to root uptake of nutrients by the existing vegetation, recruitment of new individuals can act as a buffer to nutrient loss following disturbances such as at our site after Hurricane Hugo (Silver and Vogt, 1993; Walker et al., 1996) and the simulated hurricane in this experiment (Shiels et al., 2010).

5. Conclusions

While early P concentration was a significant predictor of mass loss in this study, which is consistent with P control of litter decomposition found in many other tropical forests, the variation in P concentration at 7 weeks is best viewed as a proxy for

microbial activity, particularly agaric fungi that are able to import limiting nutrients from other litter cohorts and also accelerate decomposition by degrading lignin. We found mean values of percent of initial P remaining in senesced leaves all exceeded 100% (mean 140%) at 7 weeks under closed canopy, which has been interpreted as evidence of nutrient limitation in decomposition (Ostertag and Hobbie, 1999; Prescott, 1995). Some P was likely translocated to the senesced leaf cohort from the forest floor via rootlike structures of agaric basidiomycete fungi. Canopy opening dried the litter layer and significantly inhibited fungal connectivity between litter cohorts as well as mass loss, whereas debris addition as green leaves buffered the underlying litter from moisture loss and added P, thus stimulating fungal connectivity and mass loss. Green leaf deposition ameliorated the inhibitory effect of canopy opening, preserving fungal connectivity and moderately high rates of mass loss in the senesced litter below in the simulated hurricane treatment. Phosphorus was likely lost from the green leaf cohort via leaching, some was immobilized in the senesced leaf cohort below, and some likely taken up by tree roots. Although most of the P lost from the green leaf cohort probably exceeded the immobilization capacity of microbes in lower litter layers of the forest floor, fungi were important in conserving P in litter during the early stages of decomposition following disturbance. Based on these and previous results in wet tropical forests, retention of debris on the forest floor contributes to conservation of phosphorus in available pools by buffering the underlying forest floor material from moisture loss, which favors agaric litter decomposers fungi that are adapted to conserving limiting nutrients via recycling, and it may also favor growth and nutrient uptake by tree roots in litter layer and at the litter–soil interface. The buffering of forest floor from moisture loss and its positive effects on abundance and activity of agaric fungi and possibly tree roots above the mineral soil likely contributes to subsequent increases in forest regeneration and productivity following disturbance, whether the origin of the disturbances are anthropogenic, such as logging, or natural, such as cyclones.

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