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Tropical herbivorous phasmids, but not litter snails, alter decomposition rates by modifying litter bacteria

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Abstract. Consumers can alter decomposition rates through both feces and selective feeding in many ecosystems, but these combined effects have seldom been examined in tropical ecosystems. Members of the detrital food web (litter-feeders or microbivores) should presumably have greater effects on decomposition than herbivores, members of the green food web. Using litterbag experiments within a field enclosure experiment, we determined the relative effects of common litter snails (*Megalomastoma croceum*) and herbivorous walking sticks (*Lamponius portoricensis*) on litter composition, decomposition rates, and microbes in a Puerto Rican rainforest, and whether consumer effects were altered by canopy cover presence. Although canopy presence did not alter consumers' effects, focal organisms had unexpected influences on decomposition. Decomposition was not altered by litter snails, but herbivorous walking sticks reduced leaf decomposition by about 50% through reductions in high quality litter abundance and, consequently, lower bacterial richness and abundance. This relatively unexplored but potentially important link between tropical herbivores, detritus, and litter microbes in this forest demonstrates the need to consider autotrophic influences when examining rainforest ecosystem processes.

Key words: ecosystem process; enclosure; herbivory; light gap; litter; litterbags.

INTRODUCTION

Decomposition is a crucial flux in recycling organic matter and nutrients, and 'thus' is inherently tied to understanding our changing global carbon cycle (van Groenigen et al. 2014). Litter decomposition in tropical forests may be particularly important to the global carbon cycle because of the rapid litter turnover and high rates of carbon sequestration that occur there. Biotic control of decomposition may have disproportionately large effects in tropical systems compared to their temperate counterparts because of optimal climatic conditions for decomposition to occur and some geologic factors that usually have an important role in decomposition being negligible (e.g., high amounts of iron that render clays inactive for adsorption of nutrients) (Lavelle et al. 1993, Fonte and Schowalter 2005). However, studies examining how the biota affects decomposition in these systems have largely focused on members of the detrital "brown" food web (Milton and Kaspari 2007, Richardson et al. 2010). With so much litterfall year-round and high litter turnover rates, this dynamic brown food web is thought to regulate many ecosystem processes, including decomposition. This assumption has left the potential effects of interactions within the autotrophic "green" food web largely ignored. Moreover, the potential for cascading, interactive effects between macro-consumers in green and brown food webs have not been examined, especially in tropical rainforests.

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Macro-consumers may have differential effects on decomposition, depending on their trophic level, and these effects are largely context-dependent, thus making predictions about the effect of any one species in an ecosystem difficult. Litter-dwelling consumers may have positive or negative effects on decomposition depending on their dominant food. Generally, as members of the brown food web, litter-dwelling consumers can facilitate decomposition through their comminution of litter that occurs from feeding directly on litter (detritivores) or on litter microbes (microbivores), and, thus, increasing surface area available for microbes (Heneghan et al. 1998, González and Seastedt 2000, González et al. 2001). Detritivores are largely assumed to have positive effects on the rates of decomposition by directly processing litter. Microbivores may also affect decomposition through their selective feeding on functional groups within the litter layer (Moore et al. 1988, Wardle et al. 2002). Additionally, highly labile invertebrate frass is often stimulating to decomposition, but can also inhibit microbial activity thereby reducing decomposition rates, particularly if the frass is tightly compacted and has a low internal porosity (e.g., Hanlon and Anderson 1979). Litter-dwelling organisms may also alter microbial communities by acting as dispersal agents for fungal and bacterial propagules that colonize and, subsequently, decompose litter (Behan and Hill 1978).

Herbivore effects can also stimulate or inhibit decomposition due to context dependent mechanisms. Herbivores' frass has been shown to increase nitrogen cycling (Sirotnak and Huntly 2000, Rinker et al. 2001), stimulate microbial activity (Frost and Hunter 2004), and ultimately increase decomposition rates (Fonte and Schowalter 2005), but these effects may also be influenced by frass quality and internal porosity.

Their selective feeding can stimulate or reduce decomposition, partially depending on the herbivores' preferred foliage. For example, if herbivores prefer to consume faster decomposing plants, slower decomposing, poorer quality plants increase in abundance, resulting in poorer resources for decomposers, and thereby decreasing decomposition rates (Pastor et al. 1988, Brown and Gange 1992, de Mazancourt and Loreau 2000, Schmitz et al. 2000, Feeley and Terborgh 2005). The opposite pathway can also occur where herbivore preference for slower decomposing plants can increase rates of decomposition (McNaughton 1985, Holland 1995, Belovsky and Slade 2000, 2002). The effects of herbivores on decomposition may also be specific to the type of defensive strategy that the plant species involved use (inducible vs. constitutive defenses), and this trait can be plastic depending upon microclimatic conditions under which the plant is growing (Cardenas et al. 2014, Bixenmann et al. 2016). Because of the differential effects of these different trophic levels, the combination of herbivores and detritivores could result in either synergistic or negative feedbacks on the process of decomposition (Wardle et al. 2002, Wardle and Bardgett 2005).

Microclimatic differences in moisture, temperature, and light, such as those that result from disturbances like tropical storms or hurricanes, may alter the effects of herbivores, detritivores, and microbivores on decomposition. These microclimatic differences that are associated with canopy removal in light gaps can alter microbial activity and decomposition rates (Zhang and Zak 1995, Vasconcelos and Laurance 2005). However, plant and consumer populations also respond to these changes moisture, temperature, and light. For instance, in light gaps, plants grow rapidly due to release from light limitation. New foliage and high leaf turnover rates provide abundant resources for herbivores (Angulo-Sandoval and Aide 2000, Angulo-Sandoval et al. 2004), and litter-dwelling consumers, although the dry conditions often found in light gaps can hinder their feeding. Additionally, higher herbivore consumption rates are often found in light gaps because of an increase in fast growing, highly palatable plants (Coley and Barone 1996). Thus, if canopy removal that accompanies disturbances results in increased feeding, consumers' alteration of litter quality and quantity and decomposition may also be enhanced.

No studies that we are aware of have examined how tropical forest herbivores' selective feeding affects decomposition rates. Most research testing the effects of tropical rainforest herbivores on decomposition thus far have not manipulated herbivore presence directly, but have used leaves that have experienced herbivory (Cárdenas et al. 2015), been correlational in nature (Metcalf et al. 2014), or focused on how herbivores may mechanically prime decomposition (Cárdenas and Dangles 2012). The studies that have manipulated green macro-consumers directly have examined only how consumers' frass or feces affects decomposition in tropical forests with small-scale enclosure experiments (predators: Beard et al. 2003, herbivores: Fonte and Schowalter 2005). A more natural experimental test of these mechanisms (frass and selective feeding) in concert may enable a better understanding of consumer effects on decomposition in tropical forests. Additionally, although some studies have examined the role of litter macro-consumers on decomposition in tropical forests (Heneghan et al. 1998, González and Seastedt

2000, Richardson et al. 2010, González et al. 2014), the interactive effects of herbivores and litter macro-consumers have seldom been examined. In nature, litter-dwelling consumers would constantly experience any potential effects of herbivores on litter quality, and the possibility for interactions between these trophic levels would be high.

We hypothesized that: (1) macro-consumers from both green and brown food webs will alter decomposition rates; (2) these consumers will have interactive effects; and (3) canopy cover presence will alter consumer influences. We used a field enclosure experiment to test how common brown macro-consumers (litter snails, *Megalomastoma croceum* Gmelin) and green macro-consumers (walking sticks, *Lamponius portoricensis* Rehn) alter litter production, decomposition rates, and microbial communities in a disturbance-driven rainforest in Puerto Rico. We carried out this experiment in both light gaps and closed canopy sites to determine how increased light inputs that accompany disturbances may alter these influences. Specifically, we predicted that the litter snail, *M. croceum*, would increase decomposition rates by fragmenting litter and increasing surface area available for microbial activity. Because the focal herbivore, *L. portoricensis*, prefers faster decomposing plants with more nutritious foliage (Prather 2011), we predicted that these walking sticks would slow decomposition by decreasing the ratio of fast decomposing to slow decomposing plants, and thus lowering the quality of resources provided to decomposers. We predicted that herbivores would mediate detritivores' effects on decomposition because herbivore selective feeding may modify litter composition. Lastly, we predicted that canopy absence would amplify the effects of consumers on decomposition by increasing the abundance of high quality plants and litter available.

METHODS

Study site and species characteristics

This study was conducted at the Luquillo Long Term Ecological Research site (LUQ LTER; described in Odum and Pigeon 1970, Reagan and Waide 1996, Brokaw et al. 2012). LUQ is located in the Northeastern corner of Puerto Rico (18°19' N, 65°45' W) and has an average annual precipitation around 3,500 mm (Thompson et al. 2004). Puerto Rico is frequently hit by tropical storms and hurricanes that create large light gaps, and consequently the forest is in a constant state of secondary succession (Waide and Reagan 1996, Brokaw et al. 2012). For this enclosure experiment, we chose plants and consumers that are abundant in the understory, commonly studied, and easy to transport and manipulate. *Piper glabrescens* and *Miconia prasina* were chosen as relatively fast-decomposing and slow-decomposing representatives of the understory plant community, respectively, for this experiment because these genera are abundant across the Neotropics (Molina and Alemany 1997) and have been studied together in several Neotropical rainforests (Denslow et al. 1987, Baldwin and Schultz 1988). The invertebrate consumers used in this experiment were *M. croceum*, which is the most abundant litter snail at LUQ (Prather 2011) and *L. portoricensis*, which is the most abundant generalist herbivore in the forest (Willig et al. 1986).

Enclosure experimental design

We used a factorially designed, fully-crossed, enclosure experiment that manipulated herbivore, detritivore, and canopy cover presence (2 levels of herbivores \times 2 levels of detritivores \times 2 levels of canopy cover \times 3 replicates of each treatment = 24 total enclosures), and used unenclosed controls ($n = 3$ in both canopy and light gaps) to test the effect of the enclosure on decomposition (Appendix S1: Figs S1, S2). Mesh enclosures (0.15 mm openings, Bioquip) were supported by a 3.34 \times 3.34 \times 3.34 m PVC frame. All litter and visible organisms were removed from enclosures and controls, and we added 1,050 g (\pm 50 g) of a homogenized litter collected near study sites to initially create a similar litter layer in all enclosures. Plants of both species were grown for at least 3 months under similar conditions, and five individuals of each species were planted in each enclosure. We measured the natural abundances of consumers by surveying plants for walking sticks and litter for snails close to each experimental site, and the average biomass of these organisms added in August of 2005 (Prather 2011). We added \approx 3.6 fresh g of walking sticks per enclosure (six individuals: two adult males, one adult female, two juveniles and one nymph individual) and \approx 11.4 fresh g of snail per enclosure (nine individuals across a range of size classes). We maintained the biomass of consumers over the course of the experiment; some enclosures experienced mortality and individuals had to be added, while some were able to replace individuals through reproduction to maintain biomass. We monitored survival and reproduction rates, but these were not associated with our treatments. Non-target plants and animals in cages were removed throughout the experiment.

Litter decomposition rates, quantity, and quality

We began our first litterbag experiment in 2006 within our experimental enclosures when treatments had been established for 1 yr to determine overall consumer effects on decomposition rates using six sets (two different mesh sizes with three different types of litter) with four litterbags each (retrieved at four times: 0, 2, 5 and 8 months) per enclosure for a total of 24 litterbags in each enclosure or unenclosed control and 720 litterbags total (Appendix S1: Fig. S1). These different mesh sizes and litter types helped us to determine the potential mechanism of any herbivore and detritivore effects. We used different mesh sizes (1 large mm mesh, which allowed microarthropods access to litter, and small 500 μ m mesh, which excluded most micro-arthropods; Richardson et al. 2010) to see if herbivores and detritivores were altering decomposition rates via changes in microarthropod communities or microarthropod influences on decomposition. Using different types of leaf litter allowed us to see if the effects of herbivore and detritivore were altered by litter composition (two single species, *M. prasina* and *P. glabrescens*, and mixed-species litter composed of natural litterfall composition from Zalamea and González 2008, Table 1), employed similarly to previous litterbag studies at LUQ (González et al. 2014). Each litterbag (8 \times 16 cm) contained 4 g (\pm 0.5 g) of newly senescent litter. Upon retrieving litterbags, any live plant material or soil was carefully removed from bags. The litter was dried for at least 24 h at 60°C until

TABLE 1. Litter composition of mixed species litterbags composition, based natural litterfall from Zalamea and González (2008).

| Plant species | % of litter | Amount in litterbag (g) |
|---------------------------------|-------------|-------------------------|
| <i>Dacryodes excelsa</i> | 34.37 | 1.37 |
| <i>Manilkara bidentata</i> | 19.96 | 0.68 |
| <i>Prestoea montana</i> | 12.95 | 0.52 |
| <i>Buchenavia tetraphylla</i> | 9.13 | 0.37 |
| <i>Homalium racemosum</i> | 7.97 | 0.32 |
| <i>Rourea surinamensis</i> | 3.55 | 0.14 |
| <i>Sloanea berteriana</i> | 3.29 | 0.13 |
| <i>Cyrtilla racemiflora</i> | 2.96 | 0.12 |
| <i>Tetragastris balsamifera</i> | 2.96 | 0.12 |
| <i>Schefflera mortorioni</i> | 2.96 | 0.12 |
| <i>Matayba domingensis</i> | 2.74 | 0.11 |

Note: All of these plants are trees, except for *R. surinamensis*, which is a common liana.

reaching a constant weight, then weighed to determine leaf mass lost from each bag. k values for each litterbag set were determined using Olson's k : $X_t/X_0 = e^{-kt}$, where X_0 is the initial mass of litter, X_t is the mass of litter at time t , and k is the decay rate constant (Olson 1963).

We measured litter quantity and composition in each enclosure and control once annually when litterbag experiments were not occurring (each May, 2006–2008) by carefully removing, sorting, and weighing all litter from each plot. All litter was subsequently added back into each plot. To determine the quality of both litter used in decomposition experiment and herbivore frass, we performed chemical analyses on senescent leaves (time zero in litterbag experiment) and frass (from feeding trials with *L. portoricensis* feeding on *P. glabrescens* and *M. prasina*; Prather 2011). We determined C:N on an Elemental Analyzer at the University of Notre Dame (Costech Elemental Analyzer 4010, Valencia, California, USA), and fiber content (percent non-fibrous material, cellulose, hemicellulose and lignin) was measured by M. Strickland at University of Georgia, Athens.

Litter microbial communities

To further determine the mechanism of any significant consumer effects, we identified consumer treatments that significantly altered decomposition rates in our previous litterbag experiment, and determined the effects of these treatments on litter microbial communities with a second litterbag experiment beginning in 2007. We used two sets of 3 large mesh litterbags (14.5 \times 14.5 cm) containing 10 g of our two focal plant species retrieved at 2, 5, and 8 months in all treatments and controls besides detritivore only treatments (2 litter types \times 3 bags \times 4 possible treatments \times 2 disturbance treatments \times 3 replicates = 144 bags total). Upon retrieval, litterbags were placed in a cooler and kept chilled until DNA extraction. Litter from each litterbag was thoroughly homogenized, and DNA was extracted from 0.3 g of litter using a MoBio Ultraclean DNA Soil Extraction Kit (Mo Bio Laboratories, Inc., Carlsbad, California, USA). DNA concentration and quality was determined for each extract with a biophotometer (Eppendorf, Westbury, New York, USA). The bacterial 16S rDNA and the fungal ITS1-5.8S-ITS2 rDNA were amplified using universal

Eubacteria primers 27F-FAM/1525R (Lane 1991) and ITS1-FAM/ITS4 (White et al. 1990). PCR was performed using a mixture of 25 µL JumpStart REDTaq ReadyMix (Sigma-Aldrich, Saint Louis, Missouri, USA), 0.5 µmol/L of each primer and 10–50 ng DNA, and the following cycling parameters: initial cycle of denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1.5 min, and lastly a final cycle with an extension time of 10 min at 72°C. Positive and negative controls were used for quality assurance. PCR products were checked for amplification on a 1% agarose gel.

Terminal restriction fragment length polymorphism (TRFLP) was completed for each sample (Osborn et al. 2000, Buchan et al. 2003, Haynes et al. 2003). Amplicons were enzymatically digested with *Hae*III (fungi) and *Mn*I (bacteria) following the manufacturer’s protocols. Samples were precipitated with ethanol to eliminate impurities and dried. The samples were re-suspended in formamide with a GenScan 500 Liz size standard (ABI, Warrington, UK) and run on an ABI 3130 Genetic Analyzer (ABI, Foster City, California, USA). Samples were analyzed and TRFLP profiles were generated using GeneMapper Software version 4.0 (ABI, Foster City). Bacterial and fungal richness were determined by counting the number of significant peaks over 50 FU (fluorescent units), and each significant peak was considered an operational taxonomic unit (OTU). Total abundance in each sample was estimated by the summation of the total area underneath all significant peaks in the electropherogram.

Statistical analyses

We performed Kolmogorov Smirnov and Levene’s tests to determine whether dependent variables and residuals were normally distributed. Of our dependent variables, only *k* values needed to be transformed to meet the assumptions of parametric tests; we arcsine square root transformed *k*-values. *P*-values < 0.05 were considered significant. All statistical analyses were completed with Systat 13.1 (SPSS, Chicago, Illinois, USA). Differences in *k* were examined using a factorial, fixed effects model ANOVA with five factors (canopy cover, litter type, mesh size, herbivore and detritivore presence), and differences between different litter types were determined with a posthoc Tukey’s test. Treatment effects on C:N were analyzed with a two-way ANOVA (with factors mesh size and litter type). We used repeated measures ANOVA with three factors (canopy cover, herbivore, and detritivore presence) and time as the repeated measure (*t* = 3, 2006–2008) to evaluate treatment effects on litter quantity and the ratio of fast: slow decomposing litter (*P. glabrescens*: *M. prasina* litter biomass) in each enclosure and control. We examined treatment effects on bacterial and fungal abundance and richness using a fixed-effects ANOVA

with three factors: canopy cover, litter type, and herbivore presence. We could not use repeated measures ANOVA for this data because amplification of DNA for TRFLP analysis was not sufficient at all time points. This lack of amplification is not uncommon for litter microbial communities for several reasons, including the often degraded nature of DNA from leaf litter or because compounds found in litter material may inhibit PCR (e.g., Yang et al. 2007).

We determined the effect that enclosures had on each response variable measured by comparing cageless control plots to the herbivore + detritivore treatment, which should most closely represent the whole forest because natural abundances of consumers were added to treatments, but enclosures excluded canopy inputs and kept out other organisms. Consequently, each statistical test described above was repeated, replacing consumer treatment factors (herbivore and detritivore presence) and with an enclosure factor (enclosure presence).

RESULTS

Consumers altered decomposition rates and litter quality, independent of canopy cover, litter type, or microarthropod presence

Verifying many of the assumptions that were made in developing our hypotheses: *P. glabrescens* litter did indeed decompose ~35 % faster than *M. prasina* litter, and mixed

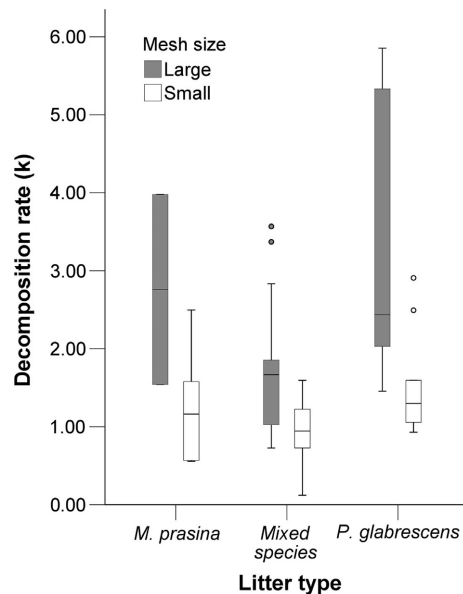


FIG. 1. Boxplots showing the effects of different litter types and mesh sizes on decomposition rates (*k*) from the first litterbag experiment. Circles above or below box indicate outliers.

TABLE 2. Frass and initial litter chemistry of different litter types (Means with SE).

| | C (%) | SE | N (%) | SE | C:N | SE | Non-fibrous (%) | SE | Hemi-cellulose (%) | SE | Cellulose (%) | SE | Lignin (%) | SE |
|-----------------------|-------|------|-------|------|-------|------|-----------------|------|--------------------|------|---------------|------|------------|------|
| <i>P. glabrescens</i> | 37.48 | 0.57 | 1.99 | 0.19 | 19.26 | 1.54 | 56.98 | 1.63 | 13.99 | 1.17 | 11.16 | 0.45 | 17.87 | 0.36 |
| <i>M. prasina</i> | 38.76 | 0.72 | 1.14 | 0.08 | 34.43 | 2.30 | 55.92 | 1.17 | 24.56 | 1.44 | 6.28 | 0.96 | 13.25 | 0.36 |
| Mixed | 45.93 | 2.18 | 0.91 | 0.09 | 50.95 | 3.41 | — | — | — | — | — | — | — | — |
| Frass | 37.89 | 0.08 | 2.43 | 0.04 | 15.60 | 0.25 | 61.60 | 1.60 | 8.65 | 1.03 | 11.98 | 0.44 | 17.76 | 0.66 |

litter decomposed the slowest (Table 2, Fig. 1). Litter quality also varied among different litter types (Table 2). The C:N of the faster-decomposing *P. glabrescens* was significantly lower than the slower decomposing species (Table 3), and *P. glabrescens* was also lower in hemicellulose ($t = 4.517$, $df = 5$, $P < 0.01$). Canopy cover (Fig. 2A) or enclosure presence did not affect decomposition rates ($F = 2.246$, $df = 1, 26$, $P > 0.05$), and this result was consistent for different litter types or mesh sizes (all $P > 0.1$).

The focal organisms had surprising effects on decomposition that did not depend on canopy cover presence, microarthropod presence or litter type: herbivores significantly

TABLE 3. Effects of canopy cover, mesh size, and herbivore, detritivore, and canopy cover presence on k values (arcsine square-root transformed) from the first litterbag experiment as determined by a fully-crossed ANOVA.

| Source | ss | df | MS | F | P |
|--------------------------|-------|----|-------|-------|------------------|
| Canopy cover (C) | 12.60 | 1 | 12.60 | 3.92 | 0.08 |
| Mesh size (M) | 41.85 | 1 | 41.85 | 15.09 | <0.001 |
| Herbivore presence (H) | 16.10 | 1 | 16.10 | 5.80 | 0.04 |
| Detritivore presence (D) | 5.09 | 1 | 5.09 | 1.84 | 0.18 |
| Litter type (L) | 25.78 | 2 | 12.89 | 4.65 | 0.04 |
| C × M | 7.40 | 1 | 7.40 | 2.67 | 0.11 |
| C × L | 15.47 | 2 | 7.74 | 2.79 | 0.08 |
| M × D | 10.90 | 1 | 10.90 | 3.93 | 0.06 |
| M × L | 39.23 | 2 | 19.61 | 7.07 | 0.003 |
| D × L | 15.52 | 2 | 7.76 | 2.80 | 0.07 |
| C × M × H | 6.59 | 1 | 6.59 | 2.40 | 0.13 |
| C × M × D | 8.10 | 1 | 8.10 | 2.92 | 0.07 |
| C × H × D | 9.77 | 1 | 9.77 | 3.52 | 0.07 |
| M × D × L | 9.04 | 1 | 9.04 | 3.26 | 0.08 |
| C × H × D × L | 13.49 | 1 | 13.49 | 4.86 | 0.08 |
| Error | 99.86 | 36 | 2.77 | | |

Note: Only interactions with $P < 0.2$ are reported; bolded values show significant effects.

reduced rates of leaf decomposition, whereas detritivores had no significant effect. Litter in enclosures with herbivores decomposed over twice as slowly as litter in enclosures without herbivores (Table 3, Fig. 2B). Although rates of litter decomposition were slightly higher with detritivores, this trend was not statistically significant (Table 3, Fig. 2C). There were also no significant herbivore + detritivore interactions on decomposition rates. Neither consumers' effects were altered by canopy cover and did not differ for different litter types. Additionally, although litter in the large mesh litterbags, which allowed microarthropod access, decomposed about twice as fast as litter in the small mesh litterbags, which excluded most non-microbial biota, consumer presence did not alter mesh size effects on decomposition.

Both consumers altered litter quality (i.e., the relative abundance of fast:slow litter) but not litter quantity

Although neither consumer affected the quantity of litter (Table 4), both altered litter quality (i.e., the relative abundance of litter from our two focal plant species that differed in litter quality): detritivores increased the ratio of fast: slow litter, while herbivores decreased litter quality (Table 5). Without herbivores, the ratio of fast: slow litter was near 1 (1.07 ± 0.28), but fell significantly below one with herbivores (0.5 ± 0.16 ; Table 5, Fig. 3). Additionally, there were no significant herbivore + detritivore interactions or the total litter quantity or quality. Canopy and enclosure presence did affect the quantity of litter: litter quantity was greater in closed canopy sites ($56.3 \pm 7.78 \text{ g/m}^2$) than light gap sites ($34.4 \pm 8.98 \text{ g/m}^2$; Table 5). Controls had almost eight times more total litter ($318.3 \pm 41.62 \text{ g/m}^2$) than enclosures ($39.8 \pm 16.8 \text{ g/m}^2$; $F = 11.43$, $df = 1, 13$, $P < 0.01$) because enclosures excluded canopy inputs. Canopy cover affected the ratio of fast:slow litter. This measure of litter quality was lower in light gaps (0.26 ± 0.22 ;

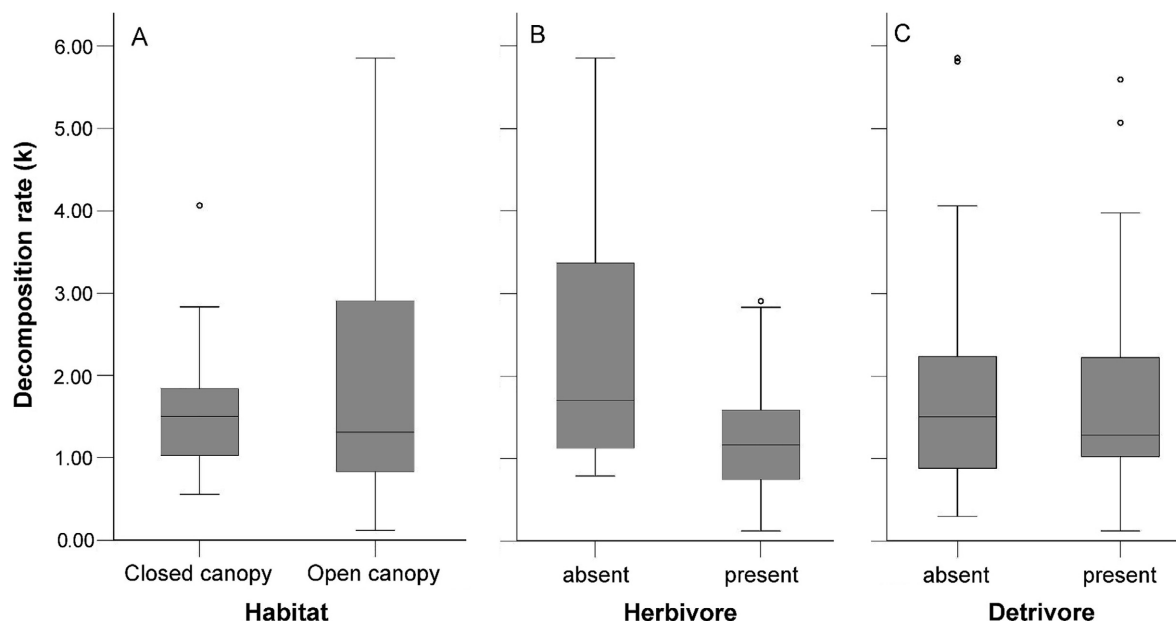


FIG. 2. Boxplots showing the effects of (A) disturbance, (B) herbivores, and (C) detritivores on decomposition rates (k) from the first litterbag experiment. Circles above or below box indicate outliers.

TABLE 4. The effects of time and canopy, herbivore, and detritivore presence on total litter on the forest floor inside enclosures (g/m² dry weight) as determined by a fully crossed repeated-measures ANOVA.

| Source | ss | df | MS | F | P |
|------------------|-----------|----|-----------|-------|--------------|
| Within subjects | | | | | |
| Time | 905.57 | 2 | 905.57 | 0.86 | 0.37 |
| Time × Canopy | 12,659.59 | 1 | 12,659.59 | 11.96 | 0.004 |
| Error | 13,766.50 | 13 | 1,058.96 | | |
| Between subjects | | | | | |
| Canopy | 8,782.21 | 1 | 8,782.21 | 3.02 | 0.02 |
| Herbivore | 2,065.31 | 1 | 2,065.31 | 0.71 | 0.42 |
| Detritivore | 4,291.41 | 1 | 4,291.41 | 1.48 | 0.25 |
| Error | 37,852.71 | 13 | 2,911.75 | | |

Note: Only interactions with *P* < 0.2 are reported; bolded values represent significant effects.

TABLE 5. The effects of time and canopy, herbivore, and detritivore presence on fast: slow litter on the forest floor inside enclosures as determined by a repeated-measures ANOVA.

| Source | ss | df | MS | F | P |
|-----------------------|-------|----|-------|-------|--------------|
| Within subjects | | | | | |
| Time (T) | 10.36 | 1 | 10.36 | 8.59 | 0.01 |
| T × Canopy (C) | 4.90 | 1 | 4.90 | 4.06 | 0.07 |
| T × Detritivore (D) | 2.79 | 1 | 2.79 | 2.32 | 0.15 |
| T × C × D | 4.15 | 1 | 4.15 | 3.44 | 0.09 |
| T × Herbivore (H) × D | 5.70 | 1 | 5.70 | 4.72 | 0.05 |
| T × C × H × D | 3.70 | 1 | 3.70 | 3.07 | 0.10 |
| Error | 15.70 | 13 | 1.21 | | |
| Between subjects | | | | | |
| C | 12.20 | 1 | 12.20 | 13.10 | 0.003 |
| H | 3.289 | 1 | 3.289 | 3.53 | 0.04 |
| D | 5.01 | 1 | 5.01 | 5.38 | 0.08 |
| C × H | 2.82 | 1 | 2.82 | 3.03 | 0.11 |
| C × D | 6.54 | 1 | 6.54 | 7.03 | 0.02 |
| H × D | 8.13 | 1 | 8.13 | 8.74 | 0.01 |
| Error | 12.10 | 13 | 0.93 | | |

Note: Only interactions with *P* < 0.2 are reported; bolded values represent significant effects.

Table 5) than closed canopy sites (1.34 ± 0.21). However, enclosures did not affect litter quality ($F = 1.25$, $df = 1, 13$, $P > 0.05$).

Herbivorous walking sticks altered litter microbial communities

Because only herbivores affected rates of decomposition, we only tested for herbivore effects on litter microbes. Total litter DNA concentration (i.e., the total amount of all DNA extracted from the litter) began low, peaked at 5 months, and declined at 8 months ($F = 8.30$, $df = 2, 6$, $P = 0.02$). Adequate DNA concentrations to conduct TRFLP analyses were only obtained for one time point each for fungi (2 months) and bacteria (5 months). Although fungal richness and abundance were not altered by canopy cover presence, litter types or herbivore presence at 2 months ($P > 0.05$), herbivores reduced bacterial abundance (Fig. 4A) and richness (Fig. 4B), with about twice as few bacterial OTUs when

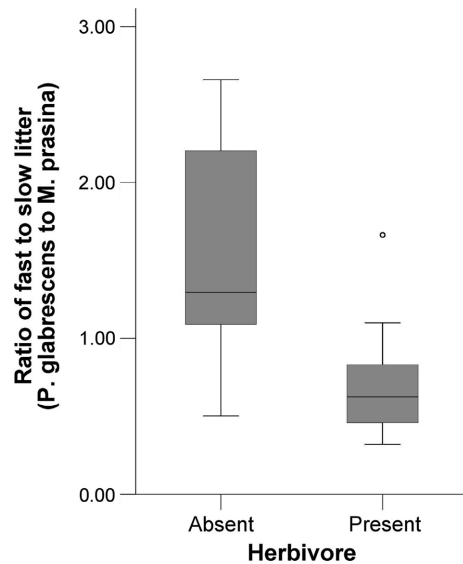


FIG. 3. Boxplots showing the effects of herbivores on ratios of fast (*P. glabrescens*) litter to slow (*M. prasina*) litter on the forest floor in each enclosure. Circles above or below box indicate outliers.

herbivores were present. Bacterial richness at 5 months was about 3.5 times higher on *P. glabrescens* litter (61 ± 7.0 OTUs) than *M. prasina* litter (17.6 ± 6.2 OTUs). Bacterial abundance was ~30% higher in control plots ($8,405 \pm 534$ FU) than in enclosures ($6,543 \pm 756$ FU; $F = 123.43$, $df = 1, 13$, $P < 0.001$). Canopy cover did not affect litter DNA concentration or bacterial richness (at the 5 month time period; $P < 0.7$, Table 6).

DISCUSSION

If members of the autotrophic food web alter detrital food web processes through by changing the quality and quantity of detritus, they may exert consumer control over tropical forest processes (Wardle and Bardgett 2005). Indeed, our results suggest that this control may be occurring in the simplified rainforest food web that we examined. Although decomposition in tropical forests is thought to be driven by members of detrital food webs (Milton and Kaspari 2007), we demonstrate here that selective feeding by consumer from the green food web (a tropical herbivorous walking stick) of this rainforest affects decomposition to a stronger degree than members of the brown food web (litter snails) by altering the quality of resources available to the microbial community. Here, we discuss the potential mechanisms of consumer effects, the role of canopy cover presence on consumer effects, and the implications for the understanding of tropical forest functioning.

Hypothesized mechanisms of consumer effects and interactions (and the lack thereof)

This herbivore’s frass does provide higher quality material than plant litter (i.e., lower C:N ratios in frass than litter). Additionally, greenfall from messy eating and frass from this herbivore had been previously documented to enhance decomposition rates (Fonte and Schowalter 2005). However,

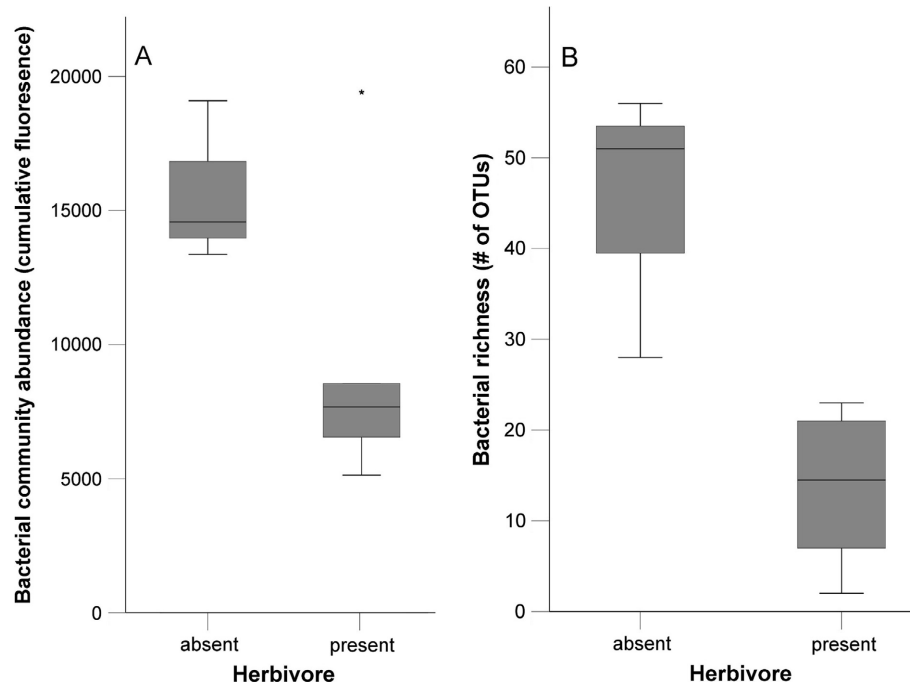


FIG. 4. Boxplots showing the effects of litter type and herbivores on bacterial richness and abundance from the litter in the second litterbag experiment. Richness (A) was measured as the total number of significant TRFLP peaks (over 50 FU) in a given sample, and abundance (B) was measured as the total area underneath significant peaks of the electropherogram. Circles above or below box indicate outliers.

TABLE 6. The effects of litter type and canopy cover and herbivore presence on bacterial TRFLP richness and abundance from litter in the second litterbag experiment as determined by a 3×2 ANOVAs.

| Source | Ss | df | MS | F | P |
|------------------|---------------|----|---------------|-------|-------------|
| Richness | | | | | |
| Canopy | 16.07 | 1 | 16.07 | 0.04 | 0.76 |
| Herbivore | 4,848.64 | 1 | 4,848.64 | 10.98 | 0.01 |
| Litter | 3,180.07 | 1 | 3,180.07 | 7.20 | 0.04 |
| Error | 7,507.29 | 17 | 441.61 | | |
| Abundance | | | | | |
| Canopy | 120,128.14 | 1 | 120,128.14 | 0.03 | 0.83 |
| Herbivore | 40,481,003.02 | 1 | 40,481,003.02 | 10.37 | 0.02 |
| Litter | 20,980,234.01 | 1 | 20,980,234.01 | 5.37 | 0.07 |
| Error | 66,376,497.92 | 17 | 3,904,499.88 | | |

Note: Only interactions with $P < 0.2$ are reported; bolded values represent significant effects.

our results suggest that the longer term alterations on litter quality via walking sticks' selective feeding may overwhelm these effects. We do not believe that the walking sticks' effects on litter decay were a result of any alterations to the micro-arthropod community because there was no significant interaction effect between herbivores and mesh size on litter decay, so these herbivores likely do not significantly alter the functionality of the litter micro-arthropod community. Therefore, the proposed mechanism of the herbivores' reduction of decomposition is due to changes in the litter bacterial community (Fig. 4).

Herbivore alterations of microbial communities have been documented in other ecosystems, including grasslands (Prather et al. 2017), non-tropical forests (Pastor et al.

1988, Frost and Hunter 2004, Classen et al. 2007), and agricultural systems (Holland 1995). The decrease in bacterial OTUs when herbivores are present could occur through different, non-exclusive mechanisms. A general decrease in diversity could occur because of poorer quality food when herbivores are present (C:N is much lower for *P. glabrecens* than *M. prasina*). Relatedly, herbivores could bring about a decrease in lignocellulytic bacterial groups because of the increases in *M. prasina*, which is lower in cellulose and lignin. The latter would be consistent with recent findings that herbivore alterations of the amount of lignocellulytic material can change microbial functioning in other ecosystems (Prather et al. 2017).

Another potential mechanism that might explain walking sticks' alterations of litter bacteria is that plant production of secondary compounds was enhanced in response to herbivory, negatively affecting the bacterial community. In general, even though about 90% of leaves on average escape herbivory globally (Gessner et al. 2010), we still have limited understanding about the prevalence of constitutive vs. inducible defenses (Bixenmann et al. 2016). We therefore know little about whether or not defensive compounds in leaf litter may be affected by herbivory, and thus future studies on the effects of herbivores in tropical forests should focus on this potential mechanism, in addition to feces and selective feeding.

The lack of an effect on decomposition by the litter snail, *M. croceum*, may be explained by several, non-exclusive mechanisms. First, snails may be primarily microbivores, but we know little about snail feeding in this forest. Lodge (1996) suggested that fungal biomass might be an important food source for many litter and soil invertebrates because

fungi concentrate many nutrients that are essential to invertebrate physiology, including calcium, which is essential for snail growth and abundance (Johannessen and Solhoy 2001, Hoptopp 2002). Microbivores in other ecosystems have been shown to preferentially consume fungi (Moore et al. 1988). Because decomposition rates in this experiment seemed to be driven by changes in bacterial communities, this lack of effect by *M. croceum* may make sense if this organism does prefer fungi. Second, if litterbags limited snail access to the litter by limiting the surface area available for snails' radulas to scrape litter, then the design of the bags themselves could inhibit the snails' feeding on litter. Most likely, some combination of snail fungivory and litterbag design led to little effect of the snails on decomposition.

Canopy cover and consumer effects on decomposition

Although canopy cover did not alter walking sticks' effects on decomposition, the results of this experiment suggest that the influence of herbivores on decomposition may be amplified by disturbance under natural conditions. Because we controlled plant abundance and herbivore biomass in this experiment, plants and herbivores did not naturally respond to the release from light limitation in light gaps. However, *L. portoricensis* tends to aggregate on plants in light gaps, resulting in patchy distributions of this herbivore in this forest (Willig et al. 1993). This preference is most likely driven by large numbers of highly palatable host plants in light gaps, a mechanism seen with herbivores in other forests (Coley and Barone 1996). Therefore, these herbivores' influences on decomposition could create spatial heterogeneity in decomposition rates, and, consequently, nutrient availability and plant biomass based on forest gap dynamics. Additionally, the similar ratio of fast: slow litter between the enclosures with herbivores and controls, which received overstory litter inputs, may indicate that canopy herbivores may also preferentially consume faster decomposing plants. Preferences for fast decomposing plants have been commonly shown for generalist herbivores (Grime et al. 1996, Wardle 2002). If generalist herbivores from both the understory and overstory herbivores preferentially consume faster decomposing plants, then canopy herbivores could affect litter decomposition by mechanisms similar to those shown for *L. portoricensis* in this study.

Conclusions and implications for understanding tropical rainforest functioning

We demonstrated that autotrophic consumers can have important effects on detrital processes that control nutrient cycling in this complex, highly productive rainforest. These patchily distributed herbivores may create spatial heterogeneity in decomposition rates in this forest because of their preference for plants often found in light gaps. Also, because this herbivore's effects on decomposition at LUQ seem to be driven by their selective feeding, studies of tropical decomposition and rainforest ecosystem models may benefit by explicitly considering autotrophic consumer inputs to the slow cycle of decomposition in addition to detrital food web effects, such as those proposed by DeAngelis (1992) and McCann (2011).

Understanding the potential effects of factors affecting decomposition are extremely important in these tropical forests that are so crucial to our global C cycle. Given our findings, it is possible that the effects of autotrophic consumers on rainforest ecosystem processes could be widespread to other rainforest ecosystems as well, but this has seldom been shown (Feeley and Terborgh 2005). However, it is worth noting that this forest has unique biogeochemistry influenced by marine inputs of Na (Kaspari et al. 2009, Medina et al. 2013). Decomposition, often limited by Na, is likely faster here than in other tropical rainforests (Dudley et al. 2012). As this forest may represent an extreme rate of decomposition, the effects of consumers on decomposition could be unique to this system, and experiments in inland sites need to be conducted to make in order generalizations. An understanding of these autotrophic influences on ecosystem processes, such as decomposition, may be especially important in these tropical systems where the majority of consumer species and the large biomass they may obtain are still unknown (Ellwood and Foster 2004) and their extinctions often go undocumented (Dunn 2005).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.2169/supinfo>

DATA AVAILABILITY

Data associated with this study are available from Figshare: <https://doi.org/10.6084/m9.figshare.c.3968499>