BIOTROPICA 45(6): 719–727 2013 10.1111/btp.12057

Influence of Terrestrial Molluscs on Litter Decomposition and Nutrient Release in a Hawaiian Rain Forest

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ABSTRACT

The roles of terrestrial molluscs in many important ecosystem processes are largely unknown, particularly in tropical forests. It has been suggested from studies in temperate forests that snails/slugs contribute to litter decomposition directly (by their own metabolism) and/or indirectly (by habitat modification enhancing micro-arthropod or microbial activity). Forty-two mesocosms were established at seven Hawaiian rain forest sites to examine the role of the five most abundant snail/slug species: the native *Succinea cepulla*, and the non-native *Arion intermedius*, *Deroceras laeve*, *Oxychilus alliarius*, and *Limax maximus*. Controls had no snails/slugs. To assess the contribution of each mollusc species separately, decomposition experiments were conducted in mesocosms containing litter bags and manipulated field densities of the mollusc species. Presence of molluscs increased litter decomposition rates, which were strongly correlated with mollusc biomass. While rates of release of some nutrients (C, K, Mg, and Mn) differed among treatments, multivariate analyses indicated that the different mollusc species influenced nutrient release in a similar way. No increases in smaller invertebrate abundances were observed in treatments containing molluscs, indicating that molluscs do not facilitate smaller invertebrate recruitment and probably influence these ecosystem processes primarily by microbe facilitation. If a major functional role of terrestrial molluscs is to facilitate microbial growth, then maintaining adequate mollusc biomass may be essential for maintaining healthy functioning ecosystems.

Key words: biodiversity; ecosystem services; invasion; microcosm; slugs; snails.

Dramatic human-induced changes in global biological diversity have the potential to alter the world's ecosystems significantly (*e.g.*, Chapin *et al.* 1997, 2000; Luck *et al.* 2003). Predicting such ecosystem consequences, however, is difficult because the roles of most invertebrates in many ecosystem processes are largely unknown, but recognized to be substantial. Terrestrial molluscs, for example, are a major component of terrestrial biodiversity (*e.g.*, Peterson & Luxton 1982) and, depending on species composition and abundances, litter-dwelling terrestrial molluscs can contribute to the cycling of nutrients directly (via metabolism) and indirectly (by modifying habitat to enhance micro-arthropod or microbial activity) (Jennings & Barkham 1976, Newell 1967, Theenhaus & Scheu 1996, De Oliveira *et al.* 2010). Molluscs consume only a small percentage (<1.5%) of the annual litter input (Mason 1970, Jennings & Barkham 1976), and so we expect their major effect on decomposition to be primarily a result of indirect mechanisms.

Understanding the influence of terrestrial molluscs on ecosystem processes is especially pertinent in Hawaii because terrestrial mollusc communities are undergoing major compositional changes. The native Hawaiian land snail fauna was exceptionally diverse (over 750 species) and exhibited high levels of archipelago endemism (over 99%) (Cowie *et al.* 1995), but 65–90 percent of these species are now estimated to be extinct (Solem 1990, Cowie 2001, Lydeard *et al.* 2004). They are being replaced by a smaller number of widely distributed non-native species of snails and slugs (Cowie *et al.* 2008, Meyer & Cowie 2010).

Examining the role of terrestrial molluscs in litter decomposition and nutrient release in the tropics has particular importance as a recent study conducted in 23 tropical forests concluded that the decomposer community made a substantially greater contribution to decomposition than did litter quality or precipitation (Powers *et al.* 2009). Because many of the non-native molluscs examined here have also become established on many other Hawaiian and Pacific islands (Cowie 2001, Cowie *et al.* 2008, Meyer & Cowie 2010), understanding the ecological role of these species is relevant to understanding the functioning of these ecosystems now and in the future.

We used a field mesocosm approach to examine three hypotheses: (1) presence of abundant terrestrial mollusc species, at current field densities, increases rates of leaf litter decomposition; (2) different terrestrial mollusc species influence the rates of nutrient release differently; and (3) terrestrial molluscs facilitate recruitment of mesoinvertebrates (between 100 μm and 2 mm in width), which can facilitate litter decomposition by fragmenting

Received 20 September 2012; revision accepted 5 May 2013.

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BIOTROPICA THE JOURNAL OF TROPICAL BIOLOGY AND CONSERVATION

leaf litter and thereby increasing the surface area available to microbes (Wardle 2002).

METHODS

Study site and study species.—The study was conducted on the eastern (windward) side of the island of Hawaii in the Upper Waiakea Forest Reserve (N 19.33479, W 155.15023), which is a wet forest (annual rainfall 2500–5000 mm/yr) with dense canopy cover (Juvik & Juvik 1998). The plant community is characterized by native tree species (predominantly *Metrosideros polymorpha*, *Cheirodendron trigynum*, and *Melicope* spp.), mid-canopy tree ferns (*Cibotium* spp.), and understory plants (*Broussaisia arguta* and *Peperomia* spp.). The invasive tree species *Psidium cattleianum* and *Morella faya*, both of which can have large ecosystem effects (Vitousek *et al.* 1987, Mascaro *et al.* 2008), were either absent or occurred at extremely low densities. Seven sites were chosen and ranged from ~ 1000 to 1250 m in elevation. All sites were located more than 50 m from any road and had a canopy of *M. polymorpha* and a *Cibotium* spp. mid-canopy.

The only common native mollusc species in the litter is *S. cepulla*, which has a flat shell into which the animal cannot fully retract. Other *Succinea* species occur in the area but have different shell morphology and are typically found on vegetation (Meyer 2009). Common non-native molluscs in the study area are the slugs *A. intermedius*, *L. maximus*, and *D. laeve*, and the non-native snail *O. alliarius* (Meyer & Cowie 2010). These five mollusc species are the most abundant in the leaf litter in our study area.

Terrestrial mollusc population densities.—Field densities of the five snail/slug species were estimated from trapping in July and October 2006 and January and April 2007. At six of the seven study sites a 7 × 7 grid of beer traps (a small cup partly filled with beer and partly embedded in the substrate when possible) was set up, with each trap 0.5 m apart. Traps were checked each day for 3 days after they were set, on each occasion removing, identifying and counting trapped snails and slugs and refilling the traps with fresh beer. The outermost grid rows might have trapped snails/slugs moving into the central area from outside the grid, so to avoid overestimating densities we included samples from only the inner 5 × 5 grid of traps (2 × 2 m). In most instances, all snails were trapped in the first 2 days, so each species' density could be determined from the total number of individuals captured. In some cases, linear extrapolation from the decline of the number of snails/slugs trapped per day allowed us to estimate the total number of individuals present in the grid (Krebs 1999).

Leaf litter decomposition experiment.—Decomposition experiments were conducted in mesocosms with manipulated densities of molluscs to assess the influence of each mollusc species separately on litter decomposition and nutrient release. It is important to assess the contribution of each mollusc species separately, because nutrient release can vary by an order of magnitude among species (Smith & Steenkamp 1992). At each of the seven sites, six mesocosms were randomly assigned to one of six treatments (one control, five experimental). Controls had no snails/slugs added. The experimental treatments were: (1) *S. cepulla* (native snail), (2) *A. intermedius* (alien slug), (3) *D. laeve* (alien slug), (4) *O. alliarius* (alien snail), and (5) *L. maximus* (alien slug), all at their estimated field densities.

The mesocosms (1.0 × 1.0 × 0.5 m high) were constructed using PVC poles. Mesh screen (2.0 mm mesh) covered each mesocosm on all sides, including the top and bottom. The mesh prevented snails and other macro-invertebrates (invertebrates >2.0 mm in width) from entering or escaping, but allowed access by smaller invertebrates. All mesocosms were placed on the ground and filled to a depth of 0.5 cm with soil and covered with a mix of leaves. The soil and leaves were from the area immediately underneath the eventual location of the mesocosm. To remove all naturally occurring molluscs prior to addition of soil and litter to the mesocosms, soil was passed through a 2 mm sieve in the field, while litter was taken to the lab and hand sorted. Two small plants (*Peperomia* sp.) were planted in each microcosm and were allowed to acclimate and grow for 1 mo prior to the addition of molluscs and litter bags.

To evaluate litter decomposition and nutrient release, litter was placed in bags, which were then deployed in the experimental treatments (mesocosms). Litter bags consisted of 4.0 ± 0.02 g (mean air-dry mass ± range) of leaf litter enclosed in a 10 × 10 × 8 cm plastic mesh food carton (to provide a 3-dimensional framework) covered with plastic screen (2.0 mm mesh). Leaves were collected just after abscission between June and December 2007 at all seven sites by placing plastic sheeting on the ground and collecting all fresh leaves every 2 weeks. Leaves were air dried for at least 2 weeks. *M. polymorpha* and *Cibotium* spp. were the most abundant species, but all litter species collected were used, thereby encompassing a broad range of decomposability. Leaves from all plant species and sites were mixed thoroughly to ensure that a random mixture of leaf litter was added to each bag. For *Cibotium* spp., the main rachis was removed and only the compound leaves were placed in the litter bags. Two holes (2.5 cm diam.) were cut in the mesh on opposite sides of the bags to allow the molluscs access.

After all mesocosms had been placed in the field, ten litter bags were placed in each and the appropriate snails/slugs were added. The experiment was run for 6 mo (20 January to 17 July 2008). At the end of the experiment, the litter bags and plants were removed for analysis. Fourteen litter samples (4.00 ± 0.02 g) that had not been put in litter bags or placed in the field, were used to determine the initial dry mass, initial ash-free dry mass (AFDM), and initial nutrient concentrations. The initial C:N ratio (±1 SE) of the mixed species litter was 49.2 (±7.3) with carbon and nitrogen constituting 45.1 (±0.9) and 0.93 (±0.11) percent of the litter respectively.

To determine if the mesocosms themselves influenced rates of litter decomposition, an additional control treatment was set up in which litter bags were placed outside mesocosms at the same sites. These bags did not have holes and thereby prevented macro-invertebrate access. Results from these bags were

compared with those from the control mesocosm treatments in which all macro-invertebrates were also excluded.

MEASUREMENTS.—Litter bags were used to determine differences in decomposition rates, nutrient release, and invertebrate densities among mesocosms. Seven of the ten litter bags from each mesocosm were used to examine differences in decomposition and nutrient release among treatments. The other three litter bags were used to extract invertebrates (see below). Prior to processing, leaf litter was sorted to remove invertebrates, roots and large pieces of soil. These litter samples were then dried at 50°C for 48 h and weighed to the nearest 0.001 g. AFDM was determined after grinding each sample in a Wiley mill (20 mesh) and then ashing one subsample (~0.5 g) at 500°C for 4 h. This accounted for any soil attached to the litter samples and permitted calculation of the mass of litter remaining in each bag. In the mesocosms, one subsample from three litter bags and one *Peperomia* sp. from each mesocosm were analyzed for the concentration of C, N, Ca, Mg, Mn, P, and K. Concentrations of C and N were determined on a Costech ECS 4010 Elemental Analyzer (Valencia, CA,USA). For the other nutrients, 0.25 g dried sample was first ashed at 500°C for 5 h, cooled, and then resuspended in 5 ml of 1N HCl and allowed to digest for 0.5 hr. Then 20 ml of reagent-grade water was added to each sample and samples were run on a Varian Vista MPX ICP-OES Spectrometer (Palo Alto, CA, USA). Peach leaves (NIST SRM 1547) were used as an international standard and recovery averaged 92 percent. Abundances of snails/slugs and other invertebrates in the mesocosms were determined as follows. Litter from the three remaining litter bags from each mesocosm was transferred into individual Berlese funnels for 48 h to extract litter invertebrates. Five beer traps were placed in each mesocosm for 2 days following litter bag removal to determine the final biomass of each snail/slug species in each mesocosm; this allowed us to determine whether molluscs survived during the entire experiment and whether snails/slugs of other species had been able to move into the mesocosms. In addition, all molluscs collected during processing of the litter were recorded.

STATISTICAL ANALYSES.—To examine differences among treatments in litter decomposition rate, the concentration and quantity of individual nutrients in the litter and the number of invertebrates we used permutation-based hypothesis testing (ANOSIM analyses) in the program PRIMER 5.2.9 (Clarke & Gorley 2001). These ANOSIM analyses used univariate data. Additional investigations of differences among treatments in the concentration and quantity of nutrients in the leaf litter (see next paragraph) also used ANOSIM analyses but with multivariate data. Prior to each univariate ANOSIM analysis, we created a similarity matrix among samples using the normalized Euclidean distance coefficient. Because there were significant differences among sites in all analyses, we used a two-way crossed ANOSIM (9999 permutations) using site and treatment as factors. This allowed us to test for differences among treatment groups averaged within blocks of sites, an analysis that is a permutation analogue to a complete randomized block ANOVA and does not require the assumption of equal variances. We report both the ANOSIM test statistic (*R*-values) and the permutation based *P*-values. The *R*-statistics are the average rank dissimilarities among and within groups, scaled so that *R*-values vary between roughly 0 and 1 (there may be some negative values); a value of 0 indicates that there are no differences among treatments, and a value of 1 indicates that all dissimilarities between samples in different treatments are larger than the average dissimilarity among samples within each treatment. The *P*-values test whether *R*-statistics differ significantly from zero (*i.e.*, that there are significant differences among treatments). Pairwise differences among treatments were examined only following a significant ANOSIM test and with *P*-values adjusted for multiple testing (15 pairwise comparisons) using a sequential Bonferroni procedure. We used the mass loss (%) in each litter bag to determine differences in litter decomposition rates among treatments. The output from the elemental analyzer and the spectrometer is the percentage of each nutrient in the total sample. To test the effect of treatment on litter nutrient concentrations, we used the percentage of each nutrient in each litter bag. We also used the percentages to calculate the quantity of each nutrient in a litter bag (in grams). Both the total quantity and percentage values were corrected based on the AFDM to exclude the inorganic material in the samples. Total quantity and the percentage of each nutrient were both analyzed because the quantity is the total amount of the nutrient remaining in the litter, whereas the percentage is an indication of the concentration of a nutrient in the remaining litter. Mean abundances of invertebrate groups collected in the three Berlese funnels from each mesocosm were used to determine whether the various mollusc species facilitated or inhibited recruitment of smaller invertebrates into the mesocosms.

To further investigate differences in the quantities and concentrations of nutrients in the leaf litter and to examine if there were differences in nutrient concentrations in the plants, we again used PRIMER 5.2.9 (Clarke & Gorley 2001) for permutation-based hypothesis testing but using a multivariate rather than univariate approach. This multivariate approach examined differences among treatments in all nutrient concentrations and quantities in the litter bags placed in the mesocosms. Prior to these ANOSIM analyses, we classified mesocosms based on the quantities and concentrations of nutrients in the leaf litter using the Bray–Curtis similarity coefficient, which is better than Euclidean distances for analyses with multiple variables with different scales of measurement and larger inter-sample differences (Legendre & Legendre 1983). A two-way crossed ANOSIM was again used to examine the differences in treatment groups averaged within blocks of sites. Pairwise differences among treatments were tested, after adjustment of significance values using the sequential Bonferroni procedure, only following a significant ANOSIM test. We also generated multi-dimensional scaling (MDS) plots to visualize differences among treatments in all litter nutrient concentrations and quantities. Initial litter concentrations and quantities are included in these MDS plots to permit assessing whether nutrient profiles in treatments moved in divergent or similar trajectories.

Divergent trajectories would suggest species-specific effects, while similar trajectories would suggest that species influence nutrient profiles in similar ways.

Correlations between biomass of each mollusc species ($\log_{10}$ g dry weight determined by collecting them at the end of the study and drying them at 50°C for 3 days) and $\log_{10}$ litter mass loss were analyzed using the mean values for each of the five mollusc species.

To determine if the mesocosm itself influenced rates of litter decomposition, a paired *t*-test was used to assess the difference in mass loss (at month 6) between the mesocosm control treatment (no mollusc species) and the treatment in which litter bags were placed outside the mesocosms.

RESULTS

The average densities (individuals/m^2 ± 1 SE) of the five most abundant mollusc species in the study area were: *S. cepulla* (0.33 ± 0.12), *A. intermedius* (1.88 ± 0.80), *L. maximus* (0.69 ± 0.27), *D. laeve* (1.85 ± 0.62), and *O. alliarius* (5.20 ± 1.03). These estimates were used to determine the number of individuals of each species to be placed in the mesocosms by rounding the mean value to the nearest whole integer, or in the case of *S. cepulla*, for which the density estimate was <0.5 individuals/m^2, to one.

There were significant treatment effects on litter decomposition ($R = 0.126$, $P < 0.001$; Fig. 1A). The greatest litter mass loss was in the *L. maximus* and *O. alliarius* treatments, which are the two treatments with the highest mollusc biomass (Figs. 1 and 2). In all mesocosms with molluscs, except one (*A. intermedius* at one site), mass loss was greater than in the control (Fig. 1B). Litter mass loss in the mesocosms was strongly correlated with mollusc biomass across species ($R = 0.99$, $P < 0.001$; Fig. 2) but not within species: *S. cepulla* ($R = -0.01$, $P = 0.98$), *A. intermedius* ($R = 0.197$, $P = 0.672$), *D. laeve* ($R = -0.41$, $P = 0.36$), *L. maximus* ($R = 0.35$, $P = 0.44$), *O. alliarius* ($R = 0.75$, $P = 0.06$).

A significant difference was found among treatments in the quantity of K, Mg, and Mn and in the percentages of C, K, Mg, and Mn in the litter (Table 1). Although significant differences in nutrient quantities were detected for K, Mg, and Mn, only Mn exhibited significant differences in pairwise comparisons following Bonferroni corrections. Less Mn was found in the *D. laeve* and *L. maximus* treatments than in the *S. cepulla* treatment, although none differed significantly from the control (Table 1), the corollary of this being that more Mn was released in the *D. laeve* and *L. maximus* treatments than in the *S. cepulla* treatment (Table 2). For C, the percentage was higher in the *L. maximus* treatment than in all other treatments except the *D. laeve* treatment. The *O. alliarius* treatment differed significantly from the control in percentage of K in the litter, and the *S. cepulla* treatment had higher percentage of Mn in the litter than the *D. laeve* treatment. Significant differences among treatments in the concentration of Mg in the litter were detected, but there were no significant differences in pairwise comparisons following Bonferroni corrections. In general, significant pairwise differences for individual nutrient concentrations (C, K, and Mn) and quantities (Mn) were between treatments with high mollusc biomass (*L. maximus*, *O. alliarius*, and *D. laeve*) and either the control treatment or treatments with low mollusc biomass (*S. cepulla* and A. *intermedius*).

The 2-dimensional MDS plots using both nutrient concentrations (stress = 0.02; Fig. 3A) and nutrient quantities (stress = 0.01; Fig. 3B) indicate that nutrient profiles among treatments are moving along similar trajectories suggesting that the different mollusc species influence total nutrient profiles in the litter in a similar way. While the trajectory is similar among treatments, the treatments with high mollusc biomass (*L. maximus*, *O. alliarius*, and *D. laeve*) are further away from the initial concentrations than treatments with lower mollusc biomass

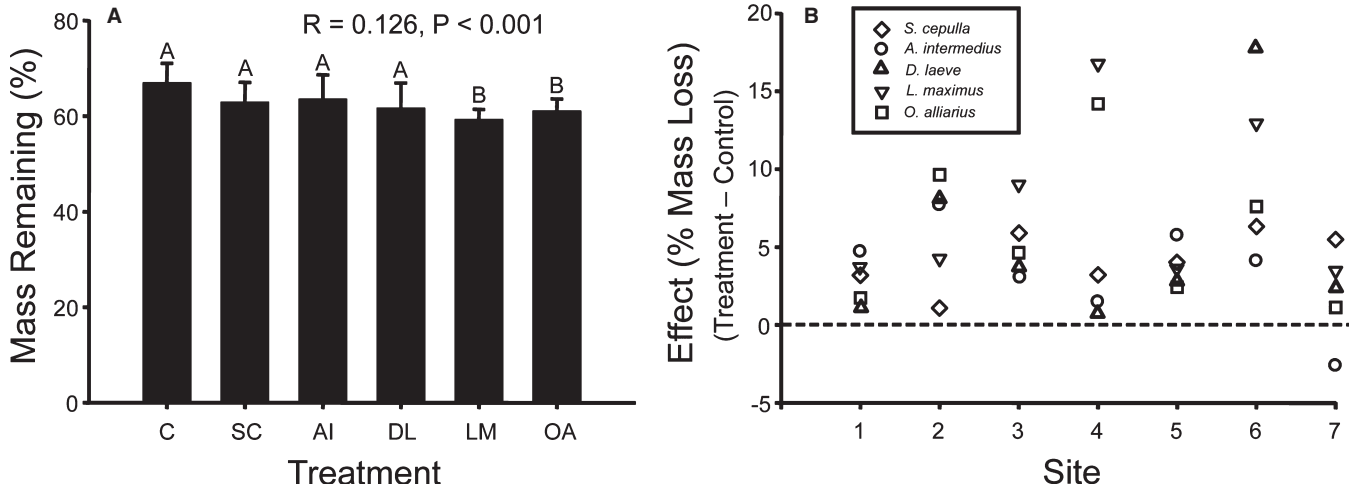

FIGURE 1. (A) Remaining litter mass and SE in mesocosms containing each mollusc species: C, control; SC, *Succinea cepulla*; AI, *Arion intermedius*; DL, *Deroceras laeve*; LM, *Limax maximus*; OA, *Oxychilus alliarius*. Means denoted with the same letter do not differ significantly. (B) Treatment effect (mass loss in treatment minus mass loss in control) among sites.

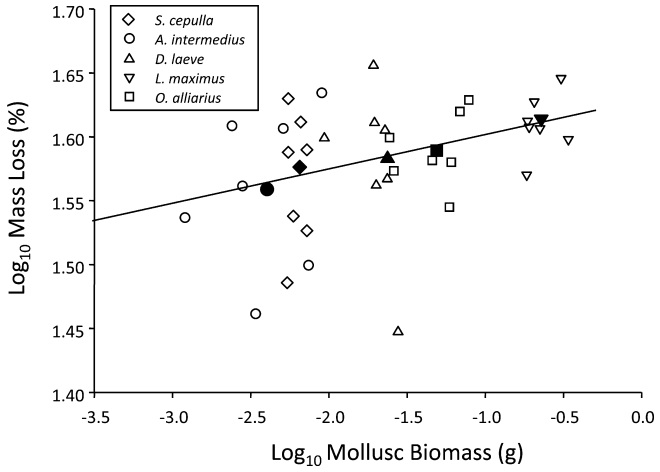

FIGURE 2. Litter mass loss as a function of final mollusc biomass. Filled symbols: mean mass loss versus mean biomass for each species across all mesocosms ($R^2 = 0.99$, $P \leq 0.001$). Open symbols: mass loss versus biomass of each species in individual mesocosms.

(*S. cepulla* and *A. intermedius*), suggesting that these differences may be influenced by differences in the biomass of these species in the different mesocosms.

Multivariate ANOSIM analyses confirmed these patterns and revealed that although significant differences among treatments in nutrient concentrations ($R = 0.215$, $P \leq 0.001$) and quantities of nutrients in leaf litter ($R = 0.132$, $P = 0.008$) were found, pairwise differences (Table 3) showed that nutrient profiles typically differed between treatments with high mollusc biomass and either the control or treatments with low mollusc biomass. For instance, nutrient concentrations differed between the control and the *L. maximus* and *O. alliarius* treatments, the two treatments with the highest biomass, and between the *L. maximus* treatment and the *S. cepulla* and *A. intermedius* treatments, the two treatments with the lowest mollusc biomass. Surprisingly, significant differences were also observed between the *O. alliarius* and *L. maximus* treatment, suggesting that there might be some species-specific differences between these two species. Nutrient quantities only differed between the *L. maximus* and the *A. intermedius* treatments. No treatment effect on nutrient concentrations in *Peperomia* sp. was found ($R = 0.019$, $P = 0.98$).

Various invertebrates were captured using the Berlese funnels, indicating that they could move in and out of the mesocosms. They included worms (Platyhelminthes and Annelida) and various arthropods (Diplura, Dermaptera, Coleoptera, Collembola, Acari, Isopoda, and Diplopoda). Only groups collected in over 75 percent of the mesocosms (Acari, Collembola, Isopoda,

TABLE 1. *Chemical properties of leaf litter after 6 mo in mesocosms: C, control; SC,* Succinea cepulla*; AI,* Arion intermedius*, DL,* Deroceras laeve*; LM,* Limax maximus*; OA,* Oxychilus alliarius. *Values in mg are the mean quantities and percentages are the mean concentrations of each nutrient per litter bag in each mesocosm. Values denoted with the same superscript letter across a row do not differ following pairwise comparisons using sequential Bonferroni corrections. Significant* P*-values are highlighted in bold type.*

	Treatment						Treatment effect	
Nutrient type	C	SC	AI	DL	LM	OA	*R*	*P*
C								
mg	1.264	1.251	1.213	1.231	1.218	1.187	−0.007	0.681
%	49.19^{A}	51.20^{A}	50.02^{A}	52.06^{AB}	55.02^{B}	51.11^{A}	0.217	**<0.001**
N								
mg	31.70	31.68	29.28	30.83	31.74	32.12	0.057	0.095
%	1.262	1.306	1.236	1.310	1.431	1.377	0.041	0.202
P								
mg	1.128	1.125	0.998	1.097	1.051	1.187	0.064	0.077
%	0.0449	0.0464	0.0422	0.0464	0.0474	0.0509	0.043	0.168
K								
mg	0.821^{A}	0.821^{A}	0.816^{A}	0.838^{A}	0.843^{A}	1.009^{A}	0.093	**0.021**
%	0.0302^{A}	0.0365^{AB}	0.0340^{AB}	0.0354^{AB}	0.0377^{AB}	0.0431^{B}	0.149	**0.002**
Mg								
mg	2.402^{A}	2.454^{A}	2.445^{A}	2.793^{A}	2.845^{A}	2.767^{A}	0.112	**0.016**
%	0.0926^{A}	0.1005^{A}	0.1001^{A}	0.1174^{A}	0.1316^{A}	0.1196^{A}	0.110	**0.010**
Mn								
mg	0.942^{AB}	0.977^{A}	0.955^{AB}	0.721^{B}	0.694^{B}	0.805^{AB}	0.060	**0.002**
%	0.0372^{AB}	0.0405^{A}	0.0400^{AB}	0.0307^{B}	0.0311^{AB}	0.0350^{AB}	0.034	**0.029**
Ca								
mg	30.18	30.97	29.39	30.67	28.65	33.86	0.020	0.317
%	1.200	1.279	1.212	1.299	1.308	1.471	−0.035	0.771

TABLE 2. *Estimates of nutrient release (kg/ha/yr) for the treatments: C, control; SC,* Succinea cepulla*; AI,* Arion intermedius*; DL,* Deroceras laeve*; LM,* Limax maximus*; OA,* Oxychilus alliarius. *Values denoted with the same superscript letter across a row do not differ following pairwise comparisons using sequential Bonferroni corrections.*

	Treatment						Treatment effect	
Nutrient type	C	SC	AI	DL	LM	OA	*R*	*P*
C	895.6	921.6	997.6	961.6	989.6	1,049	−0.007	0.681
N	7.232	7.272	12.072	8.972	7.152	6.392	0.057	0.095
P	0.952	0.958	1.212	1.014	1.106	0.834	0.064	0.077
K	34.90^{A}	34.90^{A}	34.92^{A}	34.88^{A}	34.86^{A}	34.54^{A}	0.093	**0.021**
Mg	12.53^{A}	12.43^{A}	12.44^{A}	11.74^{A}	11.76^{A}	11.80^{A}	0.112	**0.016**
Mn	0.340^{AB}	0.270^{A}	0.314^{AB}	0.782^{B}	0.836^{B}	0.614^{AB}	0.060	**0.002**
Ca	21.42	19.85	23.00	20.44	24.48	14.07	0.020	0.317

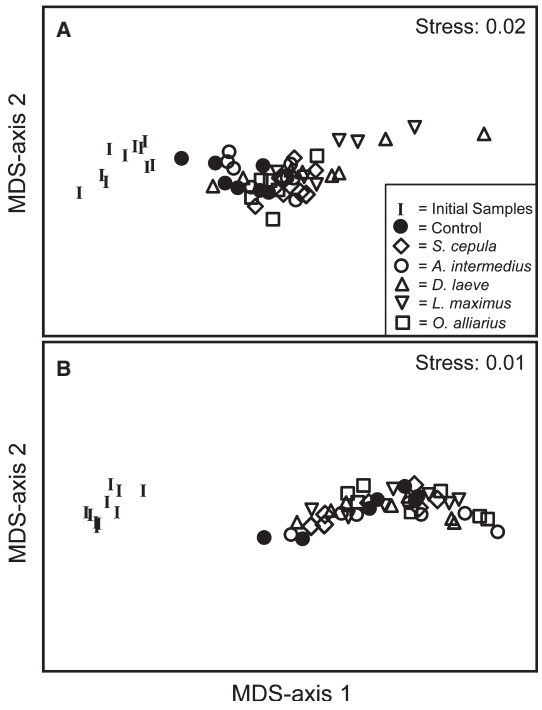

FIGURE 3. Multi-dimensional scaling (MDS) ordination plots illustrating differences in nutrient concentrations (A), and nutrient quantities (B) among treatments. Initial litter concentrations and quantities (*i.e.* nutrient profiles of the litter prior to being placed in litter bags) are included to permit assessment of whether nutrient profiles in the treatments moved in divergent or similar trajectories.

TABLE 3. *Results for pairwise ANOSIM tests examining differences in nutrient concentrations (% of each nutrient) and nutrient quantities among treatments. Significant differences following sequential Bonferroni adjustment are indicated by an*.*

	Nutrient percent		Nutrient grams	
Treatment	*R*	*P*	*R*	*P*
Control vs. *Succinea cepulla*	0.171	0.060	−0.197	0.979
Control vs. *Arion intermedius*	−0.067	0.779	0.047	0.032
Control vs. *Deroceras laeve*	0.311	0.011	0.072	0.219
Control vs. *Limax maximus*	0.495	<0.001*	0.064	0.274
Control vs. *Oxychilus alliarius*	0.289	0.002*	−0.016	0.494
S. cepulla vs. *A. intermedius*	−0.005	0.504	0.201	0.043
S. cepulla vs. *D. laeve*	0.040	0.336	0.228	0.048
S. cepulla vs. *L. maximus*	0.331	0.002*	0.038	0.354
S. cepulla vs. *O. alliarius*	0.164	0.065	−0.021	0.526
A. intermedius vs. *D. laeve*	0.212	0.022	0.360	0.005
A. intermedius vs. *L. maximus*	0.467	<0.001*	0.472	<0.001*
A. intermedius vs. *O. alliarius*	0.175	0.053	0.270	0.006
D. laeve vs. *L. maximus*	0.075	0.277	0.216	0.045
D. laeve vs. *O. alliarius*	0.190	0.069	0.201	0.035
L. maximus vs. *O. alliarius*	0.528	<0.001*	−0.027	0.586

Diplopoda) were used to assess whether snails/slugs facilitated recruitment of other macro-invertebrates. There was a significant treatment effect on millipede ($R = 0.114$, $P = 0.023$) abundance (Fig. 4). Contrary to our hypothesis, however, significantly fewer millipedes were found in the *S. cepulla*, *A. intermedius*, and *D. laeve* treatments than in the control and *L. maximus* treatments (Fig. 4).

Only once were molluscs collected in a mesocosm in which they did not belong; two small *A. intermedius* were found in one control mesocosm. No molluscs were found in any other control mesocosms and the other mesocosms only contained mollusc species originally added to them. Breeding was evident, but abundances of molluscs in mesocosms at the end of the experiment differed very little from those at the start: eggs were observed in some *L. maximus*, *S. cepulla*, and *A. intermedius* treatments; an additional (and noticeably smaller) *A. intermedius* was collected in each of two of the *A. intermedius* treatments; and in one mesocosm there was one fewer *D. laeve* than originally added.

No mesocosm effect (*i.e.*, the influence of the apparatus itself) on litter loss was observed ($t = 0.115$, df = 6, $P = 0.913$). Litter mass loss was 32.76 ± 5.91 percent for the treatment outside the mesocosms and 33.05 ± 4.16 percent for the mesocosm

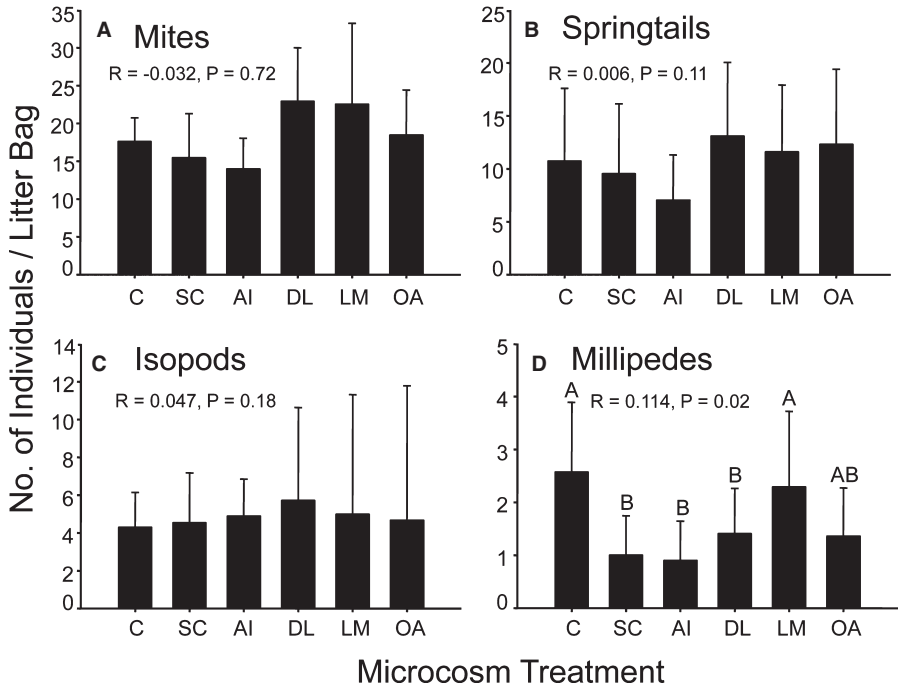

FIGURE 4. Mean number and SE of individuals of abundant invertebrate groups collected in mesocosms containing each mollusc species: C, control; SC, uccinea cepulla; AI, *Arion intermedius*; DL, *Deroceras laeve*; LM, *Limax maximus*; OA, *Oxychilus alliarius*. Means denoted with the same letter do not differ significantly.

control treatment. However, both treatments did exclude macroinvertebrates, which can significantly influence rates of litter decomposition and nutrient release (Hättenschwiler & Gasser 2005, Lensing & Wise 2006, Meyer *et al.* 2011).

DISCUSSION

The presence of molluscs increased rates of litter decomposition. Only one mesocosm out of 35 containing molluscs (*A. intermedius* at site 7) had less mass loss than the control. As such, our study demonstrates the importance of these molluscs, mostly introduced European species, in litter decomposition. Because each mesocosm had only one species at its mean field density, the total biomass in each mesocosm was well below natural levels of overall molluscan biomass. At the much higher densities at which these and other species often occur, *e.g.*, *O. alliarius* at 13–20 per m^2 in England (Mason 1970) and 22 per m^2 in the Canary Islands (Kappes *et al.* 2009), their influence is probably greater. Also, the combined role of the entire mollusc fauna, which elsewhere may number in the hundreds or even thousands of individuals per m^2 (Mason 1970), orders of magnitude greater than at our study sites, may be crucial to the functioning of many ecosystems.

In this experiment, litter decomposition was strongly correlated with the total mollusc biomass in each mesocosm suggesting that the effects of molluscs on litter decomposition is probably dependent on both the size and the abundance of the mollusc. For example, *L. maximus* was the largest mollusc in the experiment and the *L. maximus* treatment had the largest mass loss despite having only one slug per mesocosm, while other treatments with higher numbers of individuals (*e.g.*, *A. intermedius*, *D. laeve*, and *O. alliarius*) had less mass loss. The biomass of an individual snail or slug is probably correlated with its effect, because it is related to both the species' caloric requirement and the surface area of its foot, the main part of a mollusc that comes into contact with the litter.

The major effect of molluscs on decomposition is presumed to be enhancement of microbial activity (Newell 1967, Schaefer 1991). Estimates from temperate forests with much higher densities of molluscs than in our study indicate that terrestrial molluscs only consume between 0.35 and 1.5 percent of the annual litter input (Mason 1970, Jennings & Barkham 1976). These data combined with no observable leaf consumption or fragmentation in our litter bags suggest that direct ingestion of litter by terrestrial molluscs is probably less important than their indirect effects on litter via microbes. The two most plausible mechanisms by which molluscs can promote microbial abundance and activity are through (1) waste and mucus production, which modify the habitat in ways to facilitate microbial colonization and growth (Newell 1967, Theenhaus & Scheu 1996) and (2) consumption of microbes, which can lead to vigorous compensatory microbial growth (Lavelle 1997). Carcasses, which can provide increased local nutrient input and may facilitate microbial growth and activity may also be important at different spatial and temporal scales in ecosystems, but our short-term experiment did not capture the importance of these events because there was little turnover of molluscs. We also hypothesized that molluscs may modify the habitat in ways that increase recruitment of other invertebrates

that can also facilitate litter decomposition (Swift *et al.* 1979, Wardle 2002). None of our experimental treatments, however, had significantly higher abundances of non-mollusc invertebrates than the controls (Fig. 4). The *S. cepulla*, *A. intermedius*, and *D. laeve* treatments had significantly fewer millipedes than either the control or *L. maximus* treatment. In our study area, millipedes, *S. cepulla*, and *A. intermedius* are primary consumers of litter, while *D. laeve* (secondary consumer), *L. maximus* (scavenger) , and *O. alliarius* (facultative predator) feed, on average, higher in the food web (Meyer & Yeung 2011). Thus, the interactions of molluscs with other invertebrate soil fauna may be influenced by trophic position. Because there were no increases in abundance of other invertebrate groups, the differences among treatments in the mesocosms were probably a result of molluscs facilitating microbial growth. Because all mollusc species, regardless of trophic position or biogeographical origin, facilitated litter decomposition, it seems possible that commensalism among molluscs and microbes is driving increases in decomposition rates.

The presence of molluscs also influenced the nutrient concentrations (*i.e.*, quality) and to a lesser extent nutrient quantities in the litter, which can indirectly influence microbial biomass, activity, and community composition (Ndaw *et al.* 2009). Because the duration of an experiment can influence interpretation of the role of an organism in nutrient release (Meyer *et al.* 2011), the observed significant effects of different mollusc species on nutrient release might differ if this experiment were conducted over a longer period. While differences in nutrients released from litter exposed to different mollusc species have been recorded elsewhere (Smith & Steenkamp 1992), our findings suggest that different mollusc species have largely similar effects on nutrient concentrations and quantities. Although significant differences in nutrient concentration and quantities for a few individual nutrients were observed, most significant pairwise differences for individual nutrients concentrations and quantities were between treatments with high mollusc biomass and either the control treatment or treatments with low mollusc biomass. Similar differences between treatments with high mollusc biomass and either the control treatment or treatments with low mollusc biomass were also observed in the multivariate analyses of nutrient concentrations and quantities. Because biomass was not controlled for in the different treatments it is impossible to ascertain if these differences are species-specific or related to differences in biomass among the treatments. MDS plots (Fig. 3) demonstrate that nutrient profiles among the various treatments are changing along a similar trajectory relative to initial samples, suggesting that different mollusc species influence nutrient release in a roughly similar way; however, there are some differences among mollusc species. For example, the *L. maximus* and *O. alliarius* treatments differed significantly in their total nutrient concentrations, despite being the two treatments with the highest mollusc biomass. We suggest that further research that explicitly controls biomass is critical if we are going to understand the role of mollusc species and the mechanisms underlying these processes in more detail.

To what extent then does changing the mollusc composition have on the functioning of a Hawaiian rain forest? Terrestrial mollusc communities in Hawaii have changed dramatically since human colonization of the islands (Cowie 1998, Burney *et al.* 2001). For example, in our study site, the most abundant mollusc species are all introduced. In contrast, native snails are comparatively rare; for example, *S. cepulla*, the only native snail in this study, had the lowest density among the snails/slugs studied. This then raises the awkward conservation question: are these invasive mollusc species benefitting native ecosystems by maintaining important ecological processes that were once carried out by native species? Unfortunately, we are missing important pieces of information to answer this question (*e.g.*, species composition, richness, and densities of molluscs in historical communities). In this study, litter decomposition and nutrient release were correlated with mollusc biomass. As such, maintaining adequate mollusc biomass may be essential for maintaining a healthy functioning ecosystem.

ACKNOWLEDGMENTS

We thank the National Science Foundation (NSF grant DEB0710435 to R. H. Cowie and W. M. Meyer), the Ecology, Evolution and Conservation Biology program of the University of Hawaii (NSF grant DGE05-38550 to K. Y. Kaneshiro), Conchologists of America, Unitas Malacologica, the University of Hawaii Jessie Kay Memorial Fellowship and the Hawaiian Malacological Society for funding. R. Ostertag was facilitated by NSF grant CAREER0546868. Randi Schneider ran the elemental analyses at the UH Hilo Analytical Laboratory, which was supported by NSF EPSCoR 0237065 and 0554657 (J. R. Gaines, D. K. Price and K. Y. Kaneshiro, PI and co-PIs). Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of NSF. We also thank Pat Aldrich, Jeremy Claisse, Jeff Eble, Creighton Litton, Jodie Schulten, Derek Skillings, and Barbara Rowe for help in the field and lab, and Brian Bowen, Tad Fukami, and Andy Taylor for helpful comments on a draft of the manuscript. The Hawaii Department of Land and Natural Resources (permit FHM08-147) allowed access to our study sites.

LITERATURE CITED

Burney, D. A., H. F. James, L. P. Burney, S. L. Olson, W. Kikuchi, W. L. Wagner, M. Burney, D. McCloskey, D. Kikuchi, F. V. Grady, R. Gage, II, and R. Nishek. 2001. Fossil evidence for a diverse biota from Kaua'i and its transformation since human arrival. Ecol. Monogr. 71: 615–641.

Chapin, III, F. S., B. H. Walker, R. J. Hobbs, D. U. Hooper, J. H. Lawton, O. E. Sala, and D. Tilman. 1997. Biotic control over the functioning of ecosystems. Science 277: 500–504.

Chapin, III, F. S., E. Zavaleta, V. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Dias. 2000. Consequences of changing biodiversity. Nature 405: 234–242.

Clarke, R., and R. N. Gorley. 2001. PRIMER v5: User manual/tutorial. PRIMER-E, Plymouth.

Cowie, R. H. 1998. Patterns of introduction of non-indigenous non-marine snails and slugs in the Hawaiian Islands. Biodivers. Conserv. 7: 349–368.

Cowie, R. H. 2001. Invertebrate invasions on Pacific islands and the replacement of unique native faunas: A synthesis of land and freshwater snails. Biol. Invasions 3: 119–136.

Cowie, R. H., N. L. Evenhuis, and C. C. Christensen. 1995. Catalog of the native land and freshwater molluscs of the Hawaiian Islands. Backhuys Publishers, Leiden, The Netherlands.

Cowie, R. H., K. A. Hayes, C. T. Tran, and W. M. Meyer, III. 2008. The horticultural industry as a vector of alien snails and slugs: Widespread invasions in Hawaii. Int. J. Pest Manage. 54: 267–276.

De Oliveira, T., S. Hättenschwiler, and I. T. Handa. 2010. Snail and millipede complementarily in decomposing Mediterranean forest leaf litter mixtures. Funct. Ecol. 24: 937–946.

Hättenschwiler, S., and P. Gasser. 2005. Soil animals alter plant litter diversity effects on decomposition. Proc. Natl Acad. Sci. USA 102: 1519–1524.

Jennings, T. J., and J. P. Barkham. 1976. Quantitative study of feeding in woodland by the slug *Arion ater*. Oikos 27: 168–173.

Juvik, S. P., and J. P. Juvik. 1998. Atlas of Hawai'i. 3rd edn. University of Hawaii Press, Honolulu.

Kappes, H., J. D. Delgado, M. R. Alonso, and M. Ibáñez. 2009. Native and introduced gastropods in laurel forests on Tenerife, Canary Islands. Acta Oecol. 35: 581–589.

Krebs, C. J. 1999. Ecological Methodology. 2nd edn. Addison-Welsey Educational Publishers, Inc., Menlo Park.

Lavelle, P. 1997. Faunal activities and soil processes: Adaptive strategies that determine ecosystem function. Adv. Ecol. Res. 27: 93–132.

Legendre, L., and P. Legendre. 1983. Numerical ecology. Elsevier Scientific, New York.

Lensing, J. R., and D. H. Wise. 2006. Predicted climate change alters the indirect effects of predators on ecosystem processes. Proc. Natl Acad. Sci. USA 103: 15502–15505.

Luck, G. W., G. C. Daily, and P. R. Ehrlich. 2003. Population diversity and ecosystem services. Trends Ecol. Evol. 18: 331–336.

Lydeard, C., R. H. Cowie, W. F. Ponder, A. E. Bogan, P. Bouchet, S. A. Clark, K. S. Cummings, T. J. Frest, O. Gargominy, D. G. Herbert, R. Hershler, K. E. Perez, B. Roth, M. Seddon, E. E. Strong, and F. G. Thompson. 2004. The global decline of nonmarine mollusks. Bioscience 54: 321–330.

Mascaro, J., K. K. Becklund, R. F. Hughes, and S. A. Schnitzer. 2008. Limited native plant regeneration in novel, exotic-dominated forests on Hawai'i. For. Ecol. Manage. 256: 593–606.

Mason, C. F. 1970. Snail populations, beech litter production and the role of snails in litter decomposition. Oecologia 5: 215–239.

Meyer, III, W. M. 2009. Status and ecological importance of rainforest land snails on the island of Hawaii. University of Hawaii, Manoa. PhD Dissertation.

Meyer, III, W. M., and R. H. Cowie. 2010. Invasive temperate species are a threat to tropical island biodiversity. Biotropica 42: 732–738.

Meyer, III, W. M., R. Ostertag, and R. H. Cowie. 2011. Macro-invertebrates accelerate litter decomposition and nutrient release in a Hawaiian rainforest. Soil Biol. Biochem. 43: 206–211.

Meyer, III, W. M., and N. W. Yeung. 2011. Trophic relationships among molluscs and other invertebrates in a Hawaiian rainforest based on carbon and nitrogen isotopic ratios. J. Trop. Ecol. 27: 441–445.

Ndaw, S. M., A. C. Gama-Rodrigues, E. F. Gama-Rodrigues, K. R. N. Sales, and A. S. Rosado. 2009. Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. Can. J. Microbiol. 55: 1089–1095.

Newell, P. F. 1967. Mollusca. *In* A. Burges and F. Raw (Eds.). Soil biology, pp. 413–433. Academic Press, London, UK.

Peterson, H., and M. Luxton. 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39: 287–308.

Powers, J. S., R. A. Montgomery, E. C. Adair, F. Q. Brearley, S. J. DeWalt, C. T. Castanho, J. Chave, E. Deinert, J. U. Ganzhorn, M. E. Gilbert, J. A. González-Iturbe, S. Bunyavejchewin, H. R. Grau, K. E. Harms, A. Hiremath, S. Iriate- Vivar, E. Manzane, A. A. De Oliveira, L. Poorter, J. Ramanamanjato, C. Stalk, A. Varela, G. D. Weiblen, and M. T. Lerdau. 2009. Decomposition in tropical forests: A pan-tropical study of the effects of litter type, litter placement a mesofaunal exclusion across a precipitation gradient. J. Ecol. 97: 801–811.

Schaefer, M.. 1991. Secondary production and decomposition. *In* E. Rohrig, and M. Ulrich (Eds.). Temperate deciduous forests. Ecosystems of the world, vol 7, pp. 175–218, Elsevier, Amsterdam, The Netherlands.

Smith, V. R. M., and M. Steenkamp. 1992. Macroinvertebrates and nutrient release on a sub-Antarctic Island. S. Afr. J. Bot. 58: 105–115.

Solem, A. 1990. How many Hawaiian land snail species are left? and what we can do for them. Bishop Museum Occasional Papers 30: 27–40.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial environments. Blackwell Scientific, Oxford, UK.

Theenhaus, A., and S. Scheu. 1996. The influence of slug (*Arion rufus*) mucus and cast material addition on microbial biomass, respiration, and nutrient cycling in beech leaf litter. Biol. Fertility Soils 23: 80–85.

Vitousek, P. M., L. R. Walker, L. D. Whiteaker, D. Mueller-Dombois, and P. A. Matson. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. Science 238: 802–804.

Wardle, D. A.2002. Communities and ecosystems: Linking the aboveground and belowground components. Princeton University Press, Princeton.