

Influence of millipedes on litter decomposition, N mineralization, and microbial communities in a coastal forest in British Columbia, Canada

H.A. Cárcamo, T.A. Abe, C.E. Prescott, F.B. Holl, and C.P. Chanway

Abstract: Laboratory experiments were conducted with the millipede *Harpaphe haydeniana haydeniana* Wood (Polydesmida: Xystodesmidae) to determine (i) its litter feeding preferences, (ii) rates of leaf litter consumption, (iii) feeding effects on available nitrogen, and (iv) functional microbial diversity. The millipede exhibited a preference for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and, to a lesser extent, Sitka spruce (*Picea sitchensis* (Bong.) Carrière) litter compared with western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western redcedar (*Thuja plicata* Donn ex D. Don) litter when given a choice. When only one litter type was provided, millipedes consumed considerably more western redcedar than Douglas-fir, Sitka spruce, or western hemlock. Among the six broadleaf species tested, paper birch (*Betula papyrifera* Marsh.), bigleaf maple (*Acer macrophyllum* Pursh), vine maple (*Acer circinatum* Pursh), and red alder (*Alnus rubra* Bong.) were consumed at much higher rates than swordfern (*Polystichum munitum* (Kaulf.) Presl.) or salal (*Gaultheria shallon* Pursh). Daily rates of conifer litter consumption ranged between 10 and 20% of the millipede's fresh biomass and may translate to 36% of the annual litter fall. Our results suggest that transformation of conifer litter into millipede frass can increase rates of litter decomposition and N mineralization, as well as influence microbial activity and diversity in coastal forests.

Résumé : Des expériences en laboratoire ont été réalisées avec des millipèdes (*Harpaphe haydeniana haydeniana* Wood, Polydesmida : Xystodesmidae) en vue de déterminer (i) la litière qu'ils préfèrent comme source de nourriture, (ii) leur taux de consommation de litière de feuilles, (iii) les effets de leur consommation sur l'azote disponible et (iv) la diversité microbienne fonctionnelle. Lorsqu'ils avaient le choix, les millipèdes ont manifesté une préférence pour la litière du douglas de Menzies (*Pseudotsuga menziesii* (Mirb.) Franco) et, dans une moindre mesure, pour celle de l'épinette de Sitka (*Picea sitchensis* (Bong.) Carrière), comparativement à la litière de la pruche de l'Ouest (*Tsuga heterophylla* (Raf.) Sarg.) et du thuya géant (*Thuja plicata* Donn ex D. Don). Lorsqu'on leur fournissait seulement un type de litière, les millipèdes consommaient beaucoup plus de thuya géant que de douglas de Menzies, d'épinette de Sitka ou de pruche de l'Ouest. Parmi les six espèces feuillues testées, le bouleau blanc (*Betula papyrifera* Marsh.), l'érable à grandes feuilles (*Acer macrophyllum* Pursh), l'érable circiné (*Acer circinatum* Pursh) et l'aulne rouge (*Alnus rubra* Bong.) étaient consommés à un taux beaucoup plus élevé que le polystic épée (*Polystichum munitum* (Kaulf.) Presl.) ou la gaulthérie salal (*Gaultheria shallon* Pursh). Les taux journaliers de consommation de litière de conifères se situaient entre 10 et 20% de la biomasse fraîche des millipèdes et pouvaient représenter 36% de la chute annuelle de litière. Les résultats obtenus suggèrent que, dans les forêts côtières, la transformation de la litière de conifères en déjections de millipèdes peut augmenter le taux de décomposition de la litière et de minéralisation de l'azote et influencer l'activité ainsi que la diversité microbiennes.

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Introduction

Soil fauna play an important role in soil ecosystems through numerous interactions with microbes (Lussenhop 1992; Ingham 1992). These interactions can influence microbial species composition (Visser 1985) and faunal activities can indirectly affect decomposition rates and nutrient cycles (Moore and Walter 1988). Litter fragmentation and passage through the gut of macroarthropods, such as millipedes and isopods, favour the establishment of soil bacterial populations (Anderson and Bignell 1980; Hanlon 1981a, 1981b; Tajovsky et al. 1991). Hanlon (1981b) hypothesized that this was due to the higher pH and mixing in the macroarthropod gut. Firstein and Alexander (1967) showed that bacteria outcompeted the fungus *Fusarium oxysporum* for carbon and nitrogen after passing through the digestive

track of animals. Undigested fragmented litter also appears to favour colonization by bacteria. Hanlon (1981a, 1981b) suggested that the reduction in size of fragments, resulting in reduced pore size and increased surface area were the main mechanisms favouring bacterial proliferation. Gunnarsson et al. (1988), however, observed highest bacterial activity in larger fragments after litter had been leached for 1 day and found no correlation with fragment size after 7 days of leaching. They suggested that fragment size alone was not as important as previously recognized (Hanlon 1981a, 1981b), but that the presence of toxic phenolics in litter was important. They suggested that the main benefit of comminution is the faster leaching of phenolics associated with finely fragmented litter. In addition to comminution, fauna can affect diversity of microbial communities by their movements, which disrupt mycelial thalli and alter soil structure and chemistry (Visser 1985).

Millipedes are common inhabitants of the forest floor in coastal rainforests of the Pacific Northwest and British Columbia (Buckett and Gardner 1968). In a cedar-hemlock association in northern Vancouver Island, millipedes were the most dominant invertebrate group comprising 31% of total invertebrate biomass of the forest floor and reached densities of 20–90 individuals per square meter (Battigelli et al. 1994). Setälä et al. (1996) also reported large numbers of millipedes in microcosms prepared with soil from a Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand near Nanaimo, B.C., but they did not provide field estimates. *Harpaphe haydeniana haydeniana* Wood is one of the largest millipedes in the west coast with individual adults reaching 3–4 cm in length and a fresh biomass of up to 450 mg. This species has a widespread distribution in the coastal forests of Alaska, British Columbia, Washington, and Oregon (Buckett and Gardner 1968) and has been observed in massive aggregations at various sites (A. Moldenke and C. Prescott, personal observations). This has led to speculation that this species may have an important role in litter decomposition and nutrient cycling in these ecosystems (Moldenke 1990). In this study we attempted to quantify the role of *H. haydeniana* in litter decomposition and nitrogen mineralization in a series of experiments addressing the following questions:

- (1) Which litter types are exploited by *H. haydeniana*?
- (2) How much litter is consumed, assimilated and egested?
- (3) Does litter comminution influence pH and release of inorganic nitrogen?
- (4) Is microbial activity and functional diversity influenced by the conversion of litter into frass?

Methods

Litter collections

All litters for the experiments were collected from the Malcolm Knapp Research Forest of the University of British Columbia, located 60 km east of Vancouver. The site was in the Coastal Western Hemlock biogeoclimatic zone characterized by mild, cool, wet winters and very short dry summers. A detailed description of the area can be found in Prescott and Preston (1994).

Four types of feeding trials were conducted to investigate litter exploitation by *H. haydeniana*: (i) feeding preferences among four conifers or four broadleaf species, (ii) feeding choice on a mixture of four conifers and four broadleaf litters, (iii) feeding on

monospecific litter, and (iv) feeding on Douglas-fir litter to assess effects on functional microbial diversity. Four conifer litters were tested in the first three feeding trials: Douglas-fir, western redcedar (*Thuja plicata* Donn ex D. Don), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carrière). Litter used in the first feeding trial with conifers was collected on November 23, 1997, from trees of each species within mature mixed forests. To facilitate the subsequent sorting of conifer litter for the other feeding trials, litter was collected from monospecific plantations at the Malcolm Knapp Research Forest. Recently abscised needles or broad leaves on the soil surface were avoided, and aged, dark, softer litter was selected.

Selection of broadleaf species varied to some extent for each of the feeding trials. For the first trial, collections were made on November 18, 1997, and were composed of paper birch (*Betula papyrifera* Marsh.), bigleaf maple, (*Acer macrophyllum* Pursh), vine maple (*Acer circinatum* Pursh) and swordfern (*Polystichum munitum* (Kaulf.) Presl.). Although rare in coastal forests, paper birch was used to test previous findings that millipedes have a preference for this species (Neuhauser and Hartenstein 1978). Red alder (*Alnus rubra* Bong.) used in subsequent trials is the dominant early successional species in coastal British Columbia. Bigleaf maple and vine maple are found interspersed in mixed coastal forests, while swordfern is one of the dominant ground plants in mature coniferous stands. Field observations and collection of *H. haydeniana* by the senior author suggested that millipedes aggregate under decaying litter at the base of swordferns. Salal was used in the second feeding trial; this ericaceous shrub is also widespread throughout coastal British Columbia.

Millipede collection and cultures

Millipedes used in litter choice experiments (trials 1 and 2) were collected in October 1997 at the bases of swordferns in a mature mixed conifer stand at the Malcolm Knapp research forest described above. For studies of litter comminution (trial 3), animals were collected in late May 1998 from a mature mixed stand dominated by *A. macrophyllum* near the University of British Columbia campus in Vancouver. For both collections, millipedes were kept in 4-L opaque containers lined with a moist layer of mineral soil (10–20 mm thick) and fed Douglas-fir litter and (or) bigleaf maple. Less than 20 individuals were kept per container to avoid overcrowded cultures. A lid equipped with a breathing tube stuffed with cotton was placed on each container to prevent moisture loss. Millipedes were kept at variable temperatures and light regimes that approximated 20–22°C and 10–12 h of light. Little mortality was observed over a 6-month period and judging from the large amount of frass accumulated in the containers, the millipedes remained physiologically active.

Experimental protocols

Trial 1: litter feeding preferences

The first feeding trial was designed to assess litter feeding preferences by *H. haydeniana* among conifer and broadleaf species. After starving for 48 h, a female millipede was isolated in a 0.5-L plastic tub without soil lining and presented with a choice of litter types as follows: (i) 500-mg (fresh weight) bundles each of Douglas-fir, western redcedar, western hemlock and Sitka spruce (conifer choice) and (ii) 500-mg bundles each of bigleaf maple, vine maple, swordfern, and birch (broadleaf choice). As a control, 250 mg (fresh weight) of each of the eight litters were kept under the same conditions in a similar tub without millipedes. Five replicates were run for each of the two experimental treatments and the controls. After 9 days, remaining litter and frass were sorted and oven-dried at 78°C for 24 h. It should be noted that distinguishing the broadleaf species other than fern after partial feeding by millipedes was difficult because of the similar appearance of fragments.

To estimate litter consumed by each millipede, weight loss in the control treatments was subtracted from total losses observed in the experimental treatments. Litter consumption of each species was expressed as the percent of the total initial dry mass of litter that was consumed.

Trial 2: litter feeding preferences

The objective of the second experiment was to determine the feeding preferences of *H. haydeniana* when offered a mix of conifer and broadleaf litters. The same four conifer species used in trial 1 were used; however, alder and salal were substituted for birch and swordfern which were not eaten by *H. haydeniana* in trial 1. Two millipedes (male–female pair) were housed in each of five, 4-L pails during the experiments. The bottom of each pail was lined with a moist layer (1 cm) of mineral soil. Approximately 300 mg (dry weight equivalent) moist litter of each species was presented inside screw cap vials (38 × 27 mm) placed sideways and arranged around the edge of each pail. In each of the five pails, litter species were selected at random and their position alternated between conifer and broadleaf types. This design facilitated the sorting of litter types at the end of the experiment. Five replicate trials were run with and without millipedes from March 5 to 25, 1998. Upon completion of the experiment, the contents of each vial were washed through a 0.5-mm mesh to remove the frass. The remaining litter was oven-dried and weighed to determine the amount eaten by each millipede pair. Actual litter consumption was estimated by subtracting the average dry mass losses in control containers from the estimated initial dry mass for each litter in the experimental containers.

Trial 3: comminution rates and N release

The four conifer species were selected to further assess rates of litter consumption, defecation, and the effects of comminution on pH and release of mineral nitrogen. Litter was washed under tap water in a sieve and extraneous particles were removed before allowing it to air-dry overnight to remove excess moisture. Five subsamples (less than 1 g) were taken from each of the four litters to measure moisture content at the start of the experiment. Approximately 9–10 g (fresh weight) of conifer litter were added to each of ten, 1-L containers. This amount covered the bottom of each container and allowed millipedes to bury themselves when inactive. The bottom of each container had drainage holes for collection of leachates but a fine mesh (54 µm) glued to the inner surface prevented passage of litter or fecal matter. A lid equipped with a breathing tube stuffed with cotton was placed on each tub to maintain high humidity.

Millipedes were starved for 24 h before each experiment. Individual weights of millipedes were recorded and one male–female pair was added to five containers for each litter species. Another five pails contained litter but no millipedes (controls). The experiment ran from May 11 to 25, 1998, except for Douglas-fir which started on May 7. On May 25, most of the litter had been consumed in one or more of the replicates and the millipedes were removed and weighed. Two hundred millilitres of tap water was added to each of the microcosms to measure ammonium and nitrate released from litter and millipede frass during the incubation period. Leachates were stored at 0°C for less than 24 h before colorimetric analysis of ammonium (NH₄-N) and nitrate (NO₃-N) (Mulvaney 1996). Litter was allowed to air-dry for 7 days before sorting frass. To facilitate sorting of frass from conifer needles, these were lightly crushed with a mortar to dislodge frass prior to sieving using a 0.5-mm screen. The same procedure was applied to litter from millipede-free pails to quantify the small amount of litter fragmented along with frass. These values were used to adjust the fecal production estimates. Frass and litter materials were oven-dried for 24 h at 78°C before weighing. The pH of each material was measured in three replicates of a 1:10 solution of frass or

litter material to distilled water. In addition, to assess millipede effects on pH under more natural conditions, i.e., without leaching or oven-drying, three subsamples of frass and its food source (Douglas-fir litter) were collected from a millipede mass culture.

Trial 4: microbial activity and functional diversity

The effects of millipedes on microbial activity and functional diversity were investigated using Douglas-fir litter only. Litter and millipedes were collected from the Malcolm Knapp Research Forest on November 5, 1998. Animals were maintained in mass cultures at room temperature and fed Douglas-fir litter until needed. Litter used in the experiment was removed from cold storage (5–10°C), and cleaned by removing nonfoliage particles (cones, woody material, stones, etc.). Litter (10.0 ± 0.02 g; range) was added to ten 0.5-L plastic tubs but was not washed to maintain a natural microbial community. Five tubs received two adults and three or four juvenile *H. haydeniana*. Dead millipedes were removed every 2 days until termination of the experiment after 10 days, when most of the litter had been consumed.

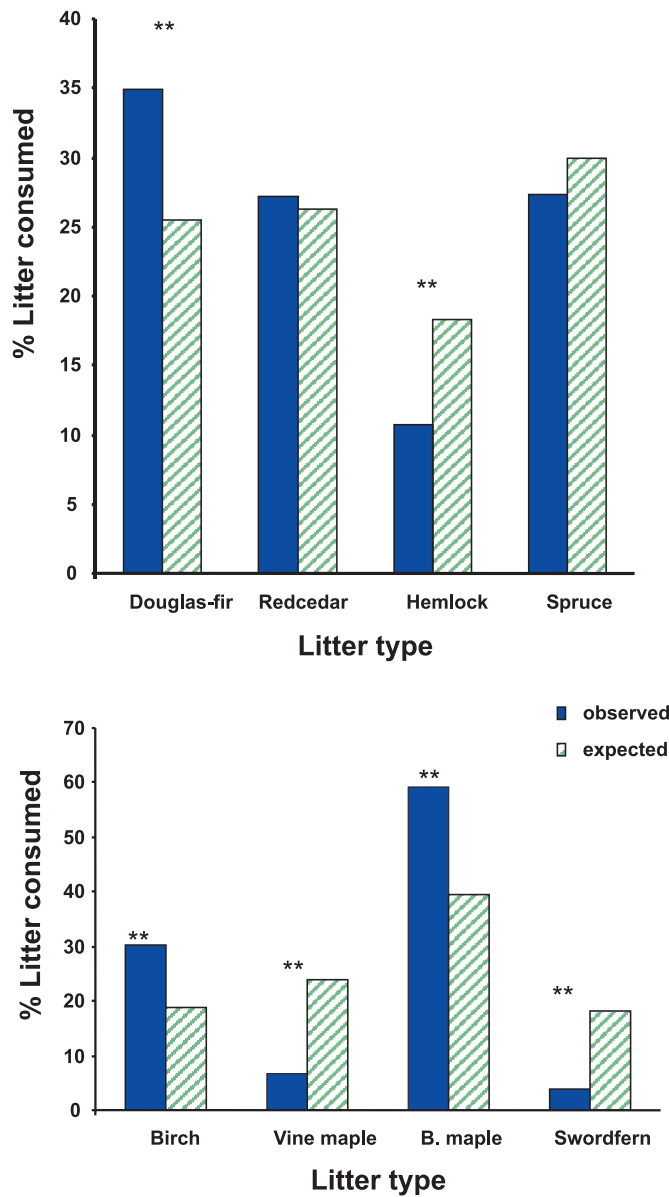
Microbial activity was inferred from basal respiration measured with a LI-COR™ infrared gas analyzer. Five grams of litter or frass (fresh weight) were added to sterilized glass jars, sealed, and incubated for ca. 1 h. Functional diversity of the microbial communities in litter and frass was quantified using the Biolog™ assay (Zak et al. 1994). This method allows for a rapid characterization of heterotrophic microbial communities (mostly bacteria) by measuring utilization of 95 carbon substrates, each in a separate well, of a microplate. The dye, tetrazolium violet, found in each well detects respiration activity (formation of NADH) resulting from microbial metabolism of the carbon substrates and results in varying color intensities. A subsample of litter or frass material (1 g) was added to 99 mL of quarter-strength Ringer's solution (2.25 g/L NaCl, 0.105 g/L KCl, 0.12 g/L CaCl₂·2H₂O, 0.05 g/L NaCO₃) and shaken manually for 20 min. This solution was further diluted to 1000-fold, and 150 µL aliquots were added to each of the 96 wells of five GN (gram negative) and GP (gram positive) Biolog™ microplates. Light absorbance was measured at 595 nm after 72 h using a Titertek™ Multiskan plate reader.

Data analyses

To take into account differential masses of dry materials presented in trial 1 (broadleaf or conifer litter choice), each litter species eaten was expressed as a percentage of the total consumption for the four species (observed value). The initial amount of each litter species was also expressed as a percentage of the total litter presented (expected value). Significant differences between observed and expected values were analyzed using χ^2 statistics and used to determine litter preferences. In trial 2, the same amount of fresh litter (300 mg dry weight equivalent) for all species was used; therefore, the mass of litter consumed was compared directly using one-way ANOVA.

For trial 3 with monospecific litters, results for ingestion and egestion were converted to milligrams of oven-dried material ingested or egested per gram of fresh weight millipede biomass per day prior to statistical analysis. Concentrations of mineral N, basal and substrate induced respiration, total number of substrates metabolized per plate, Simpson's diversity (1/D), and sum of reactions per plate for litter and frass materials were compared using ANOVA. Principal components analysis (PCA) was used to compare changes in metabolic profiles, i.e., community similarity, between litter and frass. Before ANOVA in any of the above experiments, data were tested for homogeneity of variances using Bartlett's test and transformed (ln(x + 1)) where necessary. Kruskal–Wallis nonparametric analysis was used for data with heterogeneous variances.

Fig. 1. (a) Millipede feeding choices among conifer litter. (b) Millipede feeding choices among broadleaf litter. **, $P < 0.01$ for χ^2 test between observed proportions and expected based on initial litter compositions. B. Maple, bigleaf maple.



Results

Trial 1: litter feeding preferences

Millipede feeding preferences were observed within conifer and broadleaf litters (Figs. 1a and 1b). Among the conifers, consumption of Douglas-fir was significantly higher than expected ($\chi^2 = 21.26$; $P < 0.01$), whereas hemlock was lower ($\chi^2 = 25.58$; $P < 0.001$). For spruce and western redcedar, relative consumption values were not any different that what could be expected from random feeding by the millipedes ($\chi^2 = 2.73$ and 0.74 , respectively; $P > 0.05$ for both). Of the four broadleaf litters, swordfern was clearly avoided as suggested by consumption values of less than 4%

Fig. 2. Litter losses among eight plant species with and without millipedes. Means for experimental treatments with different letters are significantly different (LSD test, $P < 0.05$).

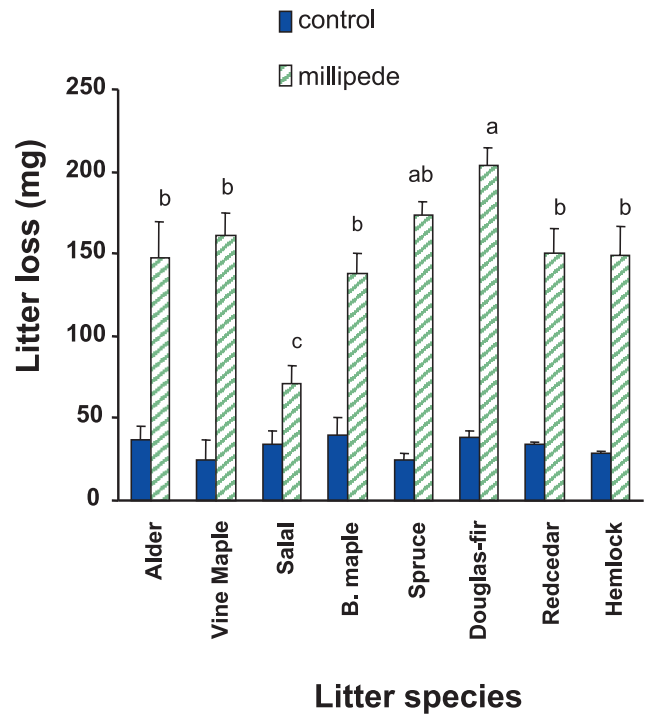


Fig. 3. Daily litter consumption and frass production in milligrams per gram of millipede biomass per day. Means for either series with different letters are significantly different (LSD test, $P < 0.05$).

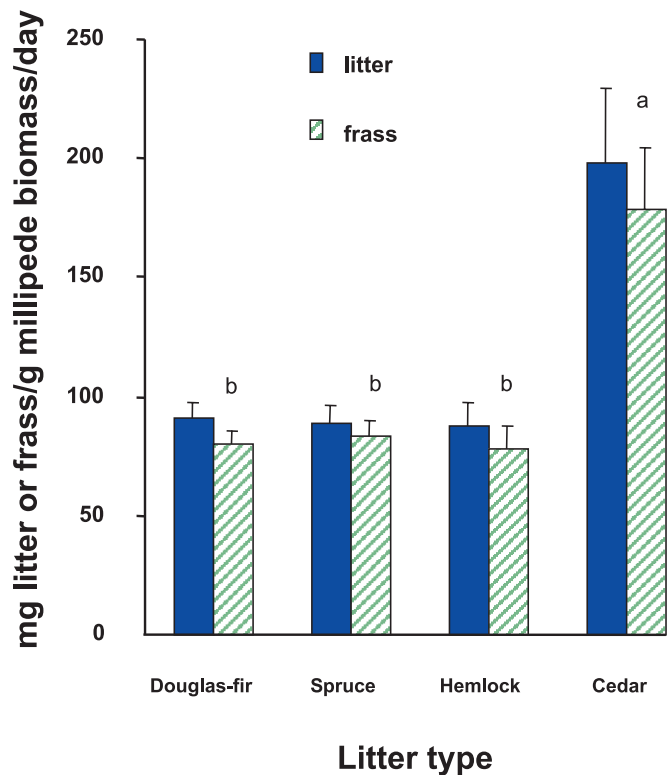


Fig. 4. Ammonium N concentrations in litter with and without millipedes. Asterisks indicate significant difference between control and millipede treatment; means for millipede treatments with different letters are significantly different (LSD test, $P < 0.05$).

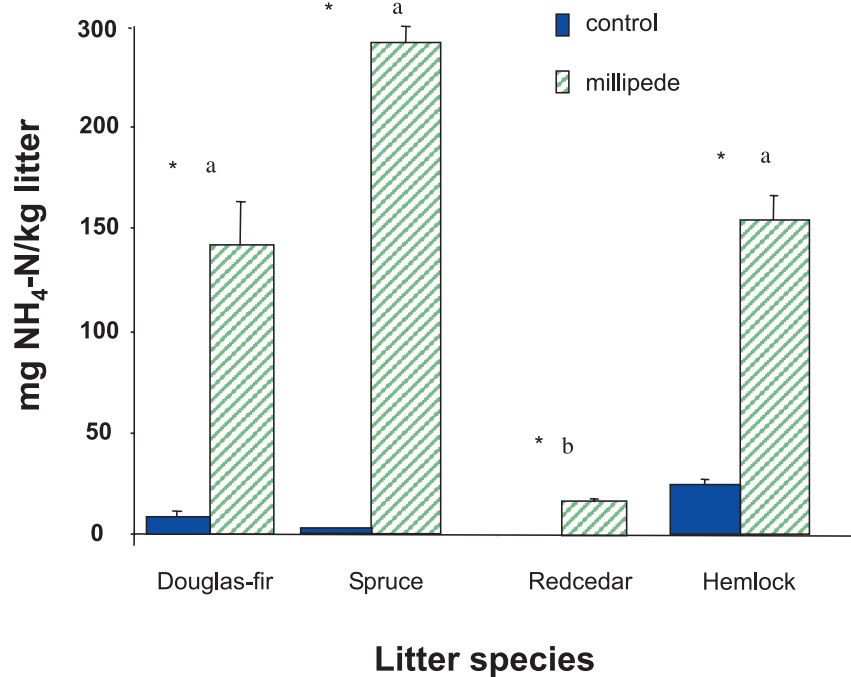
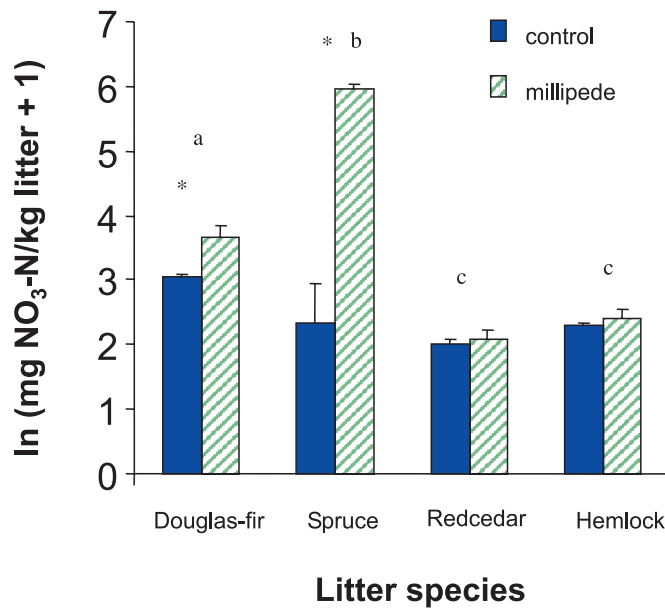


Fig. 5. Effect of millipedes on nitrate concentrations in litter. Each value is the mean \pm 1 SE for $\ln(x + 1)$ transformed data. Asterisks indicate significant differences between control and millipede treatment; means for millipede treatments with different letters are significantly different (LSD test, $P < 0.05$).



(Fig. 1b; $\chi^2 = 57.39$; $P = 0.001$). Bigleaf maple and paper birch were consumed at higher than expected values ($\chi^2 = 82.38$ and 79.22 , respectively; $P < 0.001$ for both), whereas vine maple consumption was much lower ($\chi^2 = 240.24$; $P < 0.001$).

Trial 2: litter feeding preferences

Consumption of Douglas-fir litter was greater than all other litters except Sitka spruce (Fig. 2; $df = 7, 32$; $F = 6.61$; $P < 0.001$; LSD: $P < 0.05$). *Harpaphe haydeniana* appears to contribute little to fragmentation of salal litter as the consumption values for salal were significantly lower than all other litters ($P < 0.05$).

Trial 3: comminution rates and N release

Daily litter consumption per gram of millipede biomass was higher for western redcedar than for any other species (Fig. 3; $df = 3, 16$; $F = 13.25$; $P < 0.001$). For Douglas-fir, western hemlock and Sitka spruce, consumption rates were very similar, around 90 mg/g of animal biomass per day (or ca. 36 mg/adult) and were not significantly different from one another. Patterns of frass production were similar to litter consumption (Fig. 3; $df = 3, 16$; $F = 15.56$; $P < 0.001$). Rates of matter assimilation were less than 10% for most litters as evident from high egestion values (Fig. 3). The amount of dry litter consumed on a daily basis was estimated to be equivalent to 10–20% of the fresh body weight of the millipedes.

Fragmentation of litter by millipedes significantly affected release of mineral nitrogen, particularly ammonium. For litter of each species, release of ammonium by leaching with tap water was significantly higher in the presence of millipedes (Fig. 4; $df = 1, 8$; range of F values = 80.60–315.81 for redcedar, Douglas-fir, and hemlock, $P < 0.001$; Kruskal–Wallis test for Sitka spruce: $H = 6.81$, $P < 0.01$). For nitrate, the results were inconsistent across litter species (Fig. 5). Douglas-fir and Sitka spruce were the only species where millipede presence caused increases in nitrate leached (Kruskal–Wallis test: $H = 6.82$, $P < 0.01$).

Fig. 6. Leaf litter pH of four conifer species before and after feeding by millipedes. Each value is the mean \pm 1 SE of five samples. †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$.

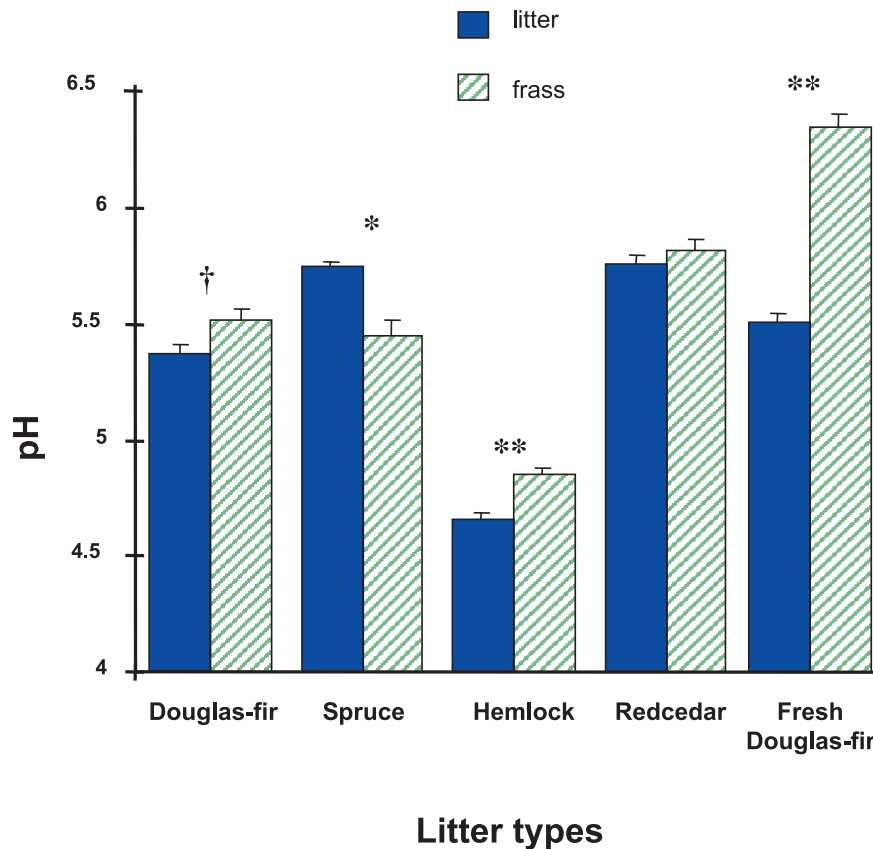


Table 1. Influence of millipede feeding on microbial activity and functional diversity of Douglas-fir litter.

Microbial parameter	Litter		Frass	
	Mean	SE	Mean	SE
Basal respiration ($\mu\text{L CO}_2/\text{h}$)	5.72*	0.11	4.99	0.12
Biolog™ assay				
Sum of activities (% light absorbance/plate)				
GN microplates	52.42*	3.64	65.76	2.98
GP microplates	39.80*	4.99	54.40	3.83
No. of substrates utilized				
GN microplates	91.80	1.24	94.20	0.20
GP microplates	81.00	4.18	91.40	1.29
Functional diversity (Simpson's index)				
GN microplates	72.81*	2.16	84.17	1.48
GP microplates	57.44*	5.50	74.05	2.11

Note: Entries are means and SEs of five samples. Asterisks indicate significant differences between litter and frass ($P < 0.05$).

Rates of nitrogen release from millipede frass were highest in Sitka spruce and lowest in western redcedar. Rates of $\text{NH}_4\text{-N}$ release in redcedar were lower than from all other litter species (Fig. 4; Kruskal–Wallis test: $H = 16.09$, $P < 0.01$). There was a strong trend to higher ammonium release

in spruce (Fig. 4) than in the three other types of litter, but the nonparametric test failed to detect significant differences ($P > 0.05$). The amount of $\text{NO}_3\text{-N}$ leached was higher for spruce relative to all other litters (Fig. 5; $df = 3, 16$; $F = 155.31$; $P < 0.0001$; LSD: $P < 0.05$). Redcedar was slightly lower than hemlock, and both of these were lower than Douglas-fir (LSD: $P < 0.05$).

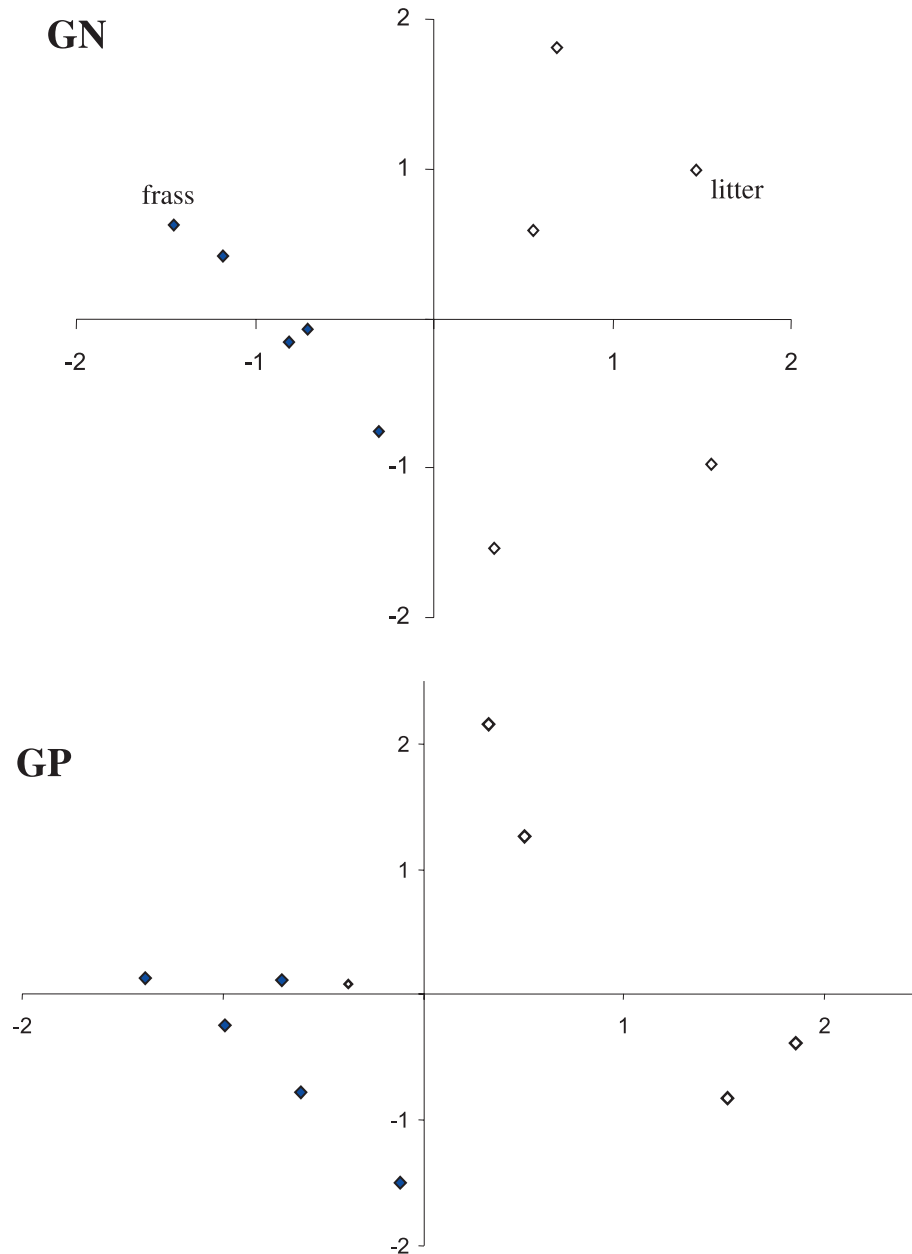
Conversion of litter into frass had small but significant effects on pH after materials had been leached and oven-dried. Increases in pH were observed in frass derived from hemlock litter (Fig. 6; $df = 1, 4$; $F = 22.36$; $P < 0.01$) and, to a lesser extent, for Douglas-fir ($df = 1, 4$; $F = 4.75$; $P = 0.095$). Spruce had a small decrease in pH during the transformation of litter to frass ($df = 1, 4$; $F = 18.62$; $P < 0.05$), whereas redcedar frass had a similar pH to litter. Changes in pH may be more substantial if measured on fresh material as was observed with frass collected from a mass culture of millipedes fed Douglas-fir litter (Fig. 6; $df = 1, 4$; $F = 134.3$; $P < 0.001$).

Trial 4: microbial activity and diversity

Microbial activity patterns measured with basal respiration and Biolog™ microplates in Douglas-fir litter and millipede frass derived from this tree litter are shown in Table 1. Basal respiration suggested higher microbial activity in litter than in frass ($df = 1, 8$; $F = 10.4$; $P < 0.01$).

The Biolog™ assay showed that total substrate utilization (inferred from color intensity in the microplates) in GN and

Fig. 7. Principal component analysis of litter and frass samples based on catabolism of carbon substrates in gram negative bacterial strain (GN) and gram positive bacterial strain (GP) microplates.



GP bacterial strain plates was significantly higher for millipede frass than litter (Table 1; $df = 1, 8$; $F = 8.05$ and 5.39 , respectively; $P < 0.05$). A similar pattern was observed for total number of substrates utilized for both bacterial strain microplates, although the differences were not statistically significant (Kruskal–Wallis test: $H = 1.81$ and 3.64 , $P = 0.18$ and 0.056 for GN and GP, respectively). Diversity of substrate utilization measured with the Simpson's index, which incorporates richness and evenness (Magurran 1988), was significantly higher in frass than in litter for both GN and GP plates ($df = 1, 8$; $F = 18.81$ and 7.95 , respectively; $P < 0.05$).

Principal components analyses compared litter and frass materials by their carbon utilization profiles in the GN and

GP microplates, which is analogous to species similarities (Fig. 7). For both bacterial types (GN and GP), frass samples clustered towards the negative portion of PCA axis 1, while litter samples were dispersed mostly on the positive side of axis 1 and along axis 2 (Fig. 7). The eigenvalues for the GN microplates were 0.43 for axis 1 and 0.20 for axis 2; for GP microplates the eigenvalues were 0.49 and 0.14 for axes 1 and 2, respectively. Analysis of variance for the PCA ordination scores of axis 1 showed differences between frass and litter samples for both GN and GP microplates ($df = 1, 8$; $F = 32.64$ and 11.13 for GN and GP, respectively; $P < 0.05$). Ordination of substrates showed little dispersion along any of the axes (data not shown). There were no consistent differences in the types of substrates that were utilized between

frass and litter samples. All substrates metabolized in plates inoculated with frass emulsions were also utilized in at least one of the replicates inoculated with litter emulsions.

Extrapolating laboratory millipede feeding rates to field conditions

The laboratory results reported above can be combined with field studies on millipede density and annual litter fall in conifer forests in coastal British Columbia to estimate the role of the millipede in litter breakdown. Studies of the distribution of millipedes in plantation forests on Vancouver Island have found millipede densities averaging 2 adults and 10 juveniles per square metre (Prescott and Cárcamo 1999). Millipede biomass for *H. haydeniana* per square metre can be conservatively estimated at about 2 g (mean fresh weights ($N = 30$): males = 342 mg; females = 448 mg; total juveniles ca. 1500 mg). Millipede activity in these forests, based on pitfall catches, occurs from May to October (H.A. Cárcamo, unpublished data). Annual rates of litter fall at northern Vancouver Island have been measured at ca. 2000 kg/ha for Douglas-fir coastal forests (Trofymow et al. 1991). Based on these values, the following approximations were used to calculate proportion of litter consumed by millipedes in the field: (i) 2000 kg/ha of needle litter fall; (ii) consumption rates of 20% of millipede fresh biomass per day; (iii) 180 days of millipede activity per year; and (iv) millipede biomass of 20 kg/ha (2 g/m²). Based on these values, we estimate that millipedes would consume 4 kg/ha per day or 720 kg/ha per year of needle litter, which equals 36% of the estimated annual aboveground needle litter input. Total faunal processing is probably even greater as other millipedes, isopods, snails, slugs, and a number of arthropods also feed on litter in these coastal forests.

Discussion

Feeding preferences and litter breakdown

This study demonstrated that *H. haydeniana* consumed conifer litter and preferred Douglas-fir over other common conifer and broadleaf species tested. These laboratory results support results of a field study that showed greater abundance of *H. haydeniana* in old-growth forests dominated by Douglas-fir than in regenerating forests dominated by alder (B. Matsuda, University of British Columbia, Vancouver, personal communication). Although millipedes fed on alder in the mixed-species choice trial in our study, a mass culture of 10 adults survived less than 2 weeks when fed only alder leaves. In another study on the effects of monospecific forest plantations on litter macroinvertebrates on Vancouver Island, we also found more millipedes in Douglas-fir plots than in western redcedar and western hemlock (Prescott and Cárcamo 1999). The presence of Douglas-fir may be an important determinant of habitat distribution of *H. haydeniana* and as discussed below, the millipedes may contribute to significant nutrient release in Douglas-fir forests.

It is not clear which aspects of litter quality determine its palatability to millipedes. Both swordfern and salal were rarely consumed by millipedes in this study. Many ericaceous shrubs, such as salal, are known to produce high levels of allelochemicals (Prescott and Preston 1994) that, if

retained in leaf litter, may deter feeding by fauna. However, Neuhauser and Hartenstein (1978) found no correlation between phenolic content and litter preferences of millipedes or isopods. In agreement with our results, these authors found a millipede preference for Douglas-fir over other conifer litters such as eastern white cedar, which had lower levels of phenolics. Western redcedar, the species tested in our experiments, also had lower levels of polyphenols than Douglas-fir as well as lower nitrogen concentrations (Prescott and Preston 1994). In the absence of other litter, redcedar was consumed at rates higher than any other conifer litter. Higher redcedar litter consumption may have been a consequence of its poorer nutrient value. Western hemlock, the least preferred species, had the lowest nitrogen concentration and also the highest tannin content (Prescott and Preston 1994). The combination of litter nutritive value and the inverse of polyphenol content may be a better predictor of litter palatability than phenolic content alone.

Our estimate of 36% of annual litter fall consumed by *H. haydeniana* is higher than the 10% estimated for *Glomeris marginata* Villers in European forests (Bocock 1963). This difference may be largely explained by the much higher volumes of litter fall in deciduous forests, ca. 12 000 kg/ha (Bocock 1963) versus 2000 kg/ha in our coniferous forest. Our estimates of 36–78 mg of litter consumption per millipede per day are within the range of 2–78 mg for a number of millipede species reviewed by Dangerfield (1994). The highest consumption rate of 78 mg/day reported for *Rossiulus kessleri* Lohmander represented 11–36% of the animal's dry body mass (Striganova and Prishutova 1990). We did not estimate dry body mass of *H. haydeniana*, but our consumption values of 20% of its fresh body weight would be higher than those of Striganova and Prishutova (1990) once converted to dry basis. The higher litter intake per body weight in *H. haydeniana* may be explained by the lower resource quality of conifer litter compared with the deciduous litter used in most European studies.

Extrapolations of laboratory results to field consumption rates provide only conservative approximations, as pointed out by Dangerfield (1994). Most controlled studies, including ours, may exaggerate levels of coprophagy and underestimate actual field consumption rates. Under laboratory conditions, millipedes have restricted feeding choices and may be forced to consume their own frass at greater rates than under natural conditions (Dangerfield 1994). Furthermore, our estimate of 36% of annual litter fall is likely very conservative, because millipede densities are usually underestimated by field surveys, so our values of 2 g/m² may be on the low side. In addition to *H. haydeniana*, other invertebrates including earthworms, molluscs, isopods, arthropods, and other millipedes are expected to feed on litter. For example, in Vancouver Island, another large xystodesmid millipede (*Tubaphe levii* Causey) was found on mesic sites (Shelley 1990, 1993) and likely plays a similar role in litter breakdown. We conclude, therefore, that at least 36% of the litter produced in coastal forests of British Columbia is probably consumed and thus transformed by soil macrofauna.

Effects on microbes and nutrients

The BiologTM assay and measures of CO₂ evolution as indicators of microbial activity produced contradictory patterns

in millipede frass and litter. There are no published studies relating these two techniques. However, in other microcosm studies, basal respiration and the Biolog™ assay suggested higher microbial activity in mixed litter–fermentation–humus materials incubated with animals than without animals (Prescott and Cárcamo 1999). We hypothesize that these discrepancies are related to the dominance of fungi in field-collected conifer foliage prior to ingestion (D. Parkinson, University of Calgary, Alta., personal communication). Under conditions of high grazing pressure (Hanlon 1981a) or reduced resource quality (Maraun and Scheu 1996), fungal biomass decreases after passage through the millipede gut. Furthermore, millipedes preferentially strip and absorb microbes from ingested litter (Bignell 1989). Lower respiration rates in millipede frass than in litter can be expected if fungal biomass is depressed directly by millipede feeding and respiration by bacterial colonies does not compensate for fungal losses in the short term. It is well established that bacteria become dominant in frass of several invertebrates including millipedes (Anderson and Bignell 1980; Ineson and Anderson 1985). Hence, the discrepancy of the two methods may be explained by the fact that Biolog™ microplates measure mostly bacterial activity, which is probably much higher in millipede frass.

Carbon utilization patterns were clearly affected by millipede feeding. Ordination analysis suggested that the functional diversity of the microbial community in millipede frass was very different from the litter. Frass samples clustered very close to each other in ordination space suggesting that passage through the millipede gut “homogenized” the microbial community’s biochemical potential. The difference in utilization patterns between frass and litter were presumably caused by variation in the degree of utilization of the carbon substrates, rather than lack of catabolism of particular substrates. Digestion of fungi from litter along with increased pH would give bacteria a competitive advantage over fungi (Hanlon 1981b) and may explain the greater functional diversity of frass materials. There are no published studies assessing functional diversity (Biolog™ assays) of invertebrate frass and litter but our study suggests that the technique is sensitive enough to explore this aspect of faunal–microbial ecological interactions.

Our study confirmed previous findings (e.g., Anderson et al. 1985) that millipedes enhance release of nitrogen by transforming litter into frass. This result was most pronounced in Sitka spruce, and to a lesser extent Douglas-fir, and can be explained by the high nitrogen concentration in litter of these two species (Prescott et al. 2000). The main likely mechanism explaining the immediate increase in available nitrogen is direct excretion of ammonium with frass. A similar mechanism was invoked by Teuben and Verhoef (1992) to explain increases of ammonium in frass of the isopod *Philoscia muscorum* Scolopi. Some of the inorganic nitrogen in the frass comes from digestion of fungal biomass that is stripped from litter (Bignell 1989; Maraun and Scheu 1996). Changes in litter chemistry also occur as a result of gut passage and nutrient assimilation. Carbon to nitrogen ratios are slightly lower in frass than litter (e.g., Bockock 1963; Marcuzzi 1970; Jambu et al. 1988; Bano 1992; Teuben and Verhoef 1992). Comminution can also cause greater leaching of labile nitrogen from the litter frag-

ments and ruptured microbial cells (Teuben and Roelofsma 1990).

In conclusion, we have shown that the millipede *H. haydeniana* readily consumed conifer litter and can potentially consume a substantial proportion of annual foliage litter fall. Availability of ammonium and, to a lesser extent, nitrate were enhanced in millipede frass relative to litter. Transformation of Douglas-fir litter into millipede frass was accompanied by an increase in pH and higher microbial functional diversity. The results indicate that this millipede contributes significantly to litter decomposition and N mineralization in forests of coastal British Columbia. More research on the distribution of *H. haydeniana* and its responses to forestry activities is warranted.

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