AN ABSTRACT OF THE THESIS OF

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Abstract approved 

Andrew Moldenke

Comminution of forest leaf litter by millipedes affects litter decomposition and nutrient cycling. The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is common in low to mid-elevation forests on the Pacific Coast of North America.

In a series of experiments, the suitability of broadleaf and conifer tree litters for growth of juvenile *H. haydeniana* was investigated. First, in a 14 day feeding trial, feeding rates for adult and juvenile *H. haydeniana* on red alder (*Alnus rubra* Bong.), big-leaf maple (*Acer macrophyllum* Pursh) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) litter were measured gravimetrically. All three litters were consumed at similar rates.

Next, the three litters, along with two others and an equal-part litter mixture, were used to rear juvenile *H. haydeniana* for 99 days. Multiple regression was used to relate millipede growth and litter chemistry (%N and %Ca). Litter %Ca was significantly related to millipede growth. Within the three conifer litters, litter %N had no significant relationship to growth. Growth was poor in alder, possibly because of the phenolic content.

In a second experiment, the nutrient content of Douglas-fir litter was modified by adding N, Ca and cellulose. Growth of juvenile *H. haydeniana* was increased by both cellulose or Ca and decreased by N. Millipede carbon and

nitrogen stable isotope ratios suggested that cellulose increased millipede growth by increasing microbial biomass, while Ca increased millipede growth by increasing millipede assimilation of plant C and N. Although the low N content of leaf litter is generally cited as the reason for slow growth rates in detritivores, adding exogenous N did not increase millipede growth, nor were $\delta^{15}N$ values in millipedes consistent with N-limitation.

And finally, stable isotope ratios in millipedes and other detritivores in a natural system were described. Three age classes of millipede (*Nearctodesmus insulanus* (Polydesmida: Nearctodesmidae) were present. The $\delta^{15}N$ of adult *N. insulanus* was significantly higher than 6^{th} or 7^{th} stadium juvenile millipedes, suggesting that adults may have been feeding on a different resource than the juveniles, or that their N balance may differ from the juveniles.

NUTRITIONAL ECOLOGY OF MILLIPEDES IN PACIFIC NORTHWEST CONIFER FORESTS

By

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Nancy Charlotte Baumeister, Author

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NUTRITIONAL ECOLOGY OF MILLIPEDES IN PACIFIC NORTHWEST CONIFER FORESTS

1. INTRODUCTION

As detritivores, millipedes play a significant role in the regulation of plant litter decomposition and nutrient cycling (Anderson *et al.* 1985). When millipedes consume, process and egest litter, the particle size is reduced, pH increases, and N mineralization is enhanced. Millipedes have been estimated to consume from 20 to 100% of litterfall annually in various, mostly deciduous forests (Hopkin and Read 1992). The assimilation efficiency is usually low, around 5 to 10%, but can be as high as 70% (Kohler *et al.* 1991). The major role of millipedes in decomposition is in modifying the physical and chemical character of the litter. Millipedes feed by shredding litter with tooth-like structures on paired mandibles, and then crushing the shredded material between smooth molar plates (Kohler *et al.* 1991). The surface area of ash leaves was increased more than 7 orders of magnitude during feeding by a population of julid millipedes (Kheirallah 1990). The chemical characteristics of the ingested plant material also change during passage through the millipede gut; pH, moisture content and bacterial counts are higher in frass than in the ingested litter, while fungal populations decrease initially and then increase (McBrayer 1973; Tajovsky *et al.* 1992; Ineson and Anderson 1985).

Detritivore populations are generally believed to be resource limited (Swift *et al.* 1979); that is, the size of the population is limited by the amount and quality of the food available to the community, rather than by predation or abiotic forces. Thus, the quantity and quality of litterfall is probably an important factor

controlling the abundance and distribution of millipedes in Pacific Northwest forests.

In general, millipedes are most abundant in temperate deciduous forests on neutral soils, where total millipede biomass can be as high as 3400 mg dry mass m^{-2} (Petersen and Luxton 1982). The average millipede biomass in temperate conifer forests is much lower. However, the conifer forests of the Pacific Northwest have the highest millipede biomass (500 to 1000 mg dry mass m^{-2}) of all six conifer sites listed in Petersen and Luxton (1982) and in fact support a millipede biomass which is greater than the **average** of the deciduous forests. Carcamo *et al.* (2000) estimate the biomass of a single millipede species (*Harpaphe haydeniana*) in forests on Vancouver Island (British Columbia, Canada) at 2000 mg live biomass m^{-2} (580 mg dry mass m^{-2}, live mass / dry mass conversion factor of 0.29, N. Baumeister unpub. data).

On the average, conifer litter contains less N and mineral nutrients, and is higher in indigestible fiber than deciduous litter (Chabot and Hicks 1982). In the Pacific Northwest, the two common deciduous canopy species, alder (*Alnus rubra* Bong.) and bigleaf maple (*Acer macrophyllum* Pursh), have litter of higher quality (more N and Ca, and less lignin) than litter from the two dominant conifer species, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). Table 1 contains litter chemistry values for four important Pacific Northwest forest canopy tree species. In addition to species-level differences, litter chemistry varies naturally with soil pH and site fertility and considerable variability is possible, particularly in Douglas-fir litter. The quality of Douglas-fir litter is particularly significant, since Douglas-fir is the dominant canopy tree and therefore Douglas-fir litter is the dominant food resource available to detritivores.

Table 1.1. Litter chemistry in four common Pacific Northwest tree species[1]

Component	Douglas-fir	Western hemlock	Bigleaf maple	Red alder
N (%)	0.5 – 1.0	0.6 – 0.8	0.9 – 1.0	1.5 - 2.0
Ca (%)	0.3- 1.2	0.6 – 0.7	1.3 – 2.2	0.8
Lignin (%)	19	22	16	10
Cellulose (%)	21	30	23	15

[1] The lignin and cellulose data are from Harmon *et al.* 1991. The N and Ca data are composited from Edmonds 1980, Fried *et al.* 1990, Harmon *et al.* 1991 and Binkley 1995.

Globally, the Diplopoda consists of about 10,000 species (1700 genera) in 14 orders. The Pacific Northwest fauna is quite diverse; with the exception of the Glomerida, all the temperate millipede orders are represented (Table 1.2). Much of the taxonomy is in need of revision and some of the species names are outdated. The entire chordeumatid order and some of the julid families are in particularly poor condition. I have listed the species names which appear to be reliable, and omitted those that are uncertain, based on the comments in Hoffman (1999) and Shelley (1990). Fortunately, the orders containing the species most relevant to this dissertation (Polydesmida and Spirobolida) have received recent attention (Shelley 1990, 1994) and are well described.

Table 1.2. Millipedes of western Oregon, western Washington and British Columbia

Order	Family	Genera
Polyxenida	Polyxenidae	*Polyxenus*
Polyzoniida[1]	Polyzoniidae	*Bdellozonium*
	Hirudisomatidae	*Octaglena* (*Hypozonium*)
Spirobolida	Spirobolidae	*Tylobolus uncigerus* Wood
Spirostreptida	Cambalidae	*Cambala washingtonensis* Causey
Julida	Blaniulidae	No native species, several introduced species
	Julidae	No native species, several introduced species
	Nematosomatidae	Several genera
	Paeromopodidae	*Californiulus euphanus* (Chamberlin) *Aprophylosoma darceneae* Hoffman
	Parajulidae[1]	Many genera
Platydesmida	Andrognathidae	*Ischnocybe plicata* Cook & Loomis
Chordeumatida[1]	Six families	Many genera
Polydesmida	Xystodesmidae	*Chonaphe armata* (Harger) *Harpaphe haydeniana* Wood *Tubaphe levii* Causey
	Polydesmidae	*Scytonotus insulanus* Attems *Scytonotus simplex* Chamb.
	Nearctodesmidae	*Kepolydesmus anderisus* Chamberlin *Nearctodesmus insulanus* Chamberlin

Data summarized from Kevan 1993, Parsons *et al.* 1991 and Hoffman 1999.

[1] The validity of names is uncertain because the taxon is in need of revision.

The Pacific Northwest millipede biomass is dominated by two large species, *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) and *Tylobolus uncigerus* Wood (Spirobolida: Spirobolidae). Current knowledge of these two species is discussed below.

The large xystodesmid millipede *H. haydeniana* is a dominant species over much of the Pacific Northwest. Neither of the two syntopic xystodesmid genera, *Tubaphe levii* (Causey) or *Chonaphe armata* (Harger), are as abundant or as widely distributed as *H. haydeniana*. *Tubaphe levii* has a fairly restricted distribution in the northern part of the coastal rainforests of the Olympic Peninsula and Vancouver Island (Shelley 1994) and is closely associated with the deciduous component in those forests. *Chonaphe armata* appears to be uncommon, although it has been collected in eastern Oregon, along the Columbia Gorge, and in the Willamette Valley. Shelley (1994) found this species, like *Tubaphe levii*, in patches of deciduous litter within a conifer forest. Pitfall trapping at the H. J. Andrews Experimental Forest collected 2931 *H. haydeniana* and only two (possible) *Chonaphe* (Parsons and Moldenke, unpublished data).

The genus *Harpaphe* has a "distinct and somewhat unusual niche among western xystodesmids" (Buckett and Gardner 1968) in its association with alder and redwood trees, rather than oak. Of the three species in the genus, *H. haydeniana* has by far the widest distribution, occurring from coastal southeast Alaska to central California (with disjunct populations in both the Sierra Nevada mountains and Coast Range). Some populations of *H. haydeniana* are apparently isolated enough to have formed subspecies. Five of the sub-species are only found in either the California Coast Range or Sierra Nevada mountains: *H.h. scotia* (Monterey and Santa Cruz counties, CA), *H. h. lanceolata* (Napa County, CA), *H. h. maurogona* (Baxter County, NV), *H. h. inlignea* (Shasta County, CA), and *H. h. cummingensis* (Humboldt, Mendicino and Sonoma counties, CA).

The subspecies *H. h. haydeniana* has a much wider range than the other subspecies, occurring continuously from southern Oregon to southeast Alaska. It appears to be able to adapt to a wide range of forest environments (Parsons and Moldenke, unpublished data). Shelley (1990), in his survey of the millipede fauna of western Canada, describes *H. haydeniana* as "ubiquitous" and the large number of collecting locales he lists supports this designation.

In the Oregon Cascade Mountains, *H. haydeniana* occurs with the spirobolid millipede *T. uncigerus*, while only *H. haydeniana* (no *T. uncigerus*) were found in the Oregon Coast Range. In the warmer and drier forests of southern Oregon, *T. uncigerus* was the dominant millipede and *H. haydeniana* was uncommon (S. Madsen, personal communication).

The spirobolid millipede *T. uncigerus* is one of the largest millipedes in the Pacific Northwest. Adults can weigh up to 3.1 g live weight, up to 3 times greater than the live weight of adult *H. haydeniana* (N. Baumeister, unpublished data). *Tylobolus uncigerus* appears to be better adapted to warmer and drier conditions than *H. haydeniana*. The northern extent of its distribution is Klickitat County, in southwestern Washington (Hoffman 1999), and it has not been found in the ecologically similar, but cooler, oak woodlands of the Puget Trough in northwestern Washington. Several other species of *Tylobolus* occur further south in the oak woodlands of California.

In summary, surprisingly little is currently known about the ecology of any Pacific Northwest millipede. The work conducted as part of the International Biological Program and reported in Petersen and Luxton (1982) still represents the only published estimate for millipede biomass in the Pacific Northwest on an area basis. The species specific information in this literature review comes primarily from two sources: comments on ecology, habitat associations or distributional limits from papers whose primary focus is taxonomic, and recent ecological data from pitfall traps. Millipedes and isopods are frequently collected in pitfall traps,

but pitfall trap data cannot be directly converted to abundance or biomass per area. Thus, there is a great need for ecological research on millipedes.

The overall objective of this research is to investigate the nutritional ecology of millipedes in Pacific Northwest conifer forests by examining food choice, feeding rate and nutritional constraints on growth. In Chapter 2, I measured feeding rates of adult and juvenile *H. haydeniana* and *T. uncigerus* on common litter species. I also related the litter chemistry of five forest litters to growth of juvenile *H. haydeniana* and *T. uncigerus* (Chapter 3) and studied the effect of modifying the nutrient content of Douglas-fir litter on growth of *H. haydeniana* (Chapter 4). Finally, I used C and N stable isotope analysis to examine the mechanism of the growth response seen in Chapter 4 and to describe trophic relationships in a natural detritivore community (Chapter 5).

These results may help us understand how the distribution of two important millipede species is related to the plant community in Pacific Northwest forests. Forest management is growing increasingly sophisticated and perhaps in the future the soil fauna will be intentionally managed to optimize nutrient cycling and forest soil health.

2. MILLIPEDE FEEDING PREFERENCES AND FEEDING RATES

ABSTRACT

The feeding rate of adult and juvenile *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), bigleaf maple (*Acer macrophyllum* Pursh) and red alder (*Alnus rubra* Bong.) litter was measured. Relative consumption rates (RCR) for adult *H. haydeniana* were higher on broadleaf litters than the conifer litter. The RCR for juvenile *H. haydeniana* was greater than for adults and was equally high on all three litters. RCR for adults and juveniles of another native millipede species, *Tylobolus uncigerus* Wood (Spirobolida: Spirobolidae), were measured on a mixture of alder, maple and Douglas-fir litter . RCR for juveniles of *T. uncigerus* was higher than RCR for adults. These results suggest that both millipede species may play a significant role in the decomposition of Douglas-fir, alder and bigleaf maple litter in Pacific Northwest forests.

INTRODUCTION

Detritivorous soil fauna, such as millipedes, isopods and earthworms, have been shown to regulate litter decomposition and nutrient cycling rates (Anderson *et al.* 1985). Millipedes are particularly important detritivores in the forests of western Oregon. The native millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is widely distributed along the Pacific coast, from southern Oregon to southeast Alaska (Buckett and Gardner 1968). A second common native species, *Tylobolus uncigerus* Wood (Spirobolida: Spirobolidae) is found along the Pacific Coast from Santa Cruz County, California to southwestern Washington (Hoffman 1999). In Pacific Northwest forests, the conifer Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a dominant tree species. The distribution of the broadleaf species bigleaf maple (*Acer macrophyllum* Pursh) and red alder (*Alnus rubra* Bong.) is more limited. Bigleaf maple occurs in riparian corridors or as scattered trees in forests dominated by Douglas-fir. Alder also occurs in riparian corridors and in secondary succession in recently disturbed areas such as clear-cuts or burned areas. Thus, the forest could be described as consisting dominantly of conifer trees, but with a broadleaf component as well. The millipede *H. haydeniana* is commonly seen in these forests, with no clear habitat preference for conifer or broadleaf litter.

In feeding preference experiments, millipedes exhibit fairly consistent preferences for certain litter types over others. Deciduous litter is generally preferred over conifer litter (Neuhauser and Hartenstein 1978). However, *Apheloria virginiensis* (also in the family Xystodesmidae) preferred well-rotted white pine litter over sugar maple and beech litter (Romell 1935) and Eaton (1943) reports that *Fontaria trimaculata* (another xystodesmid) refused five deciduous litters (basswood, sugar maple, white ash, white oak and beech) and fed in preference on forest humus. Thus there may be precedence for an association of *H. haydeniana* with conifer, rather than deciduous litter.

Among the deciduous litters, maple, ash and birch are preferred to beech and oak (Lyford 1943, Neuhauser and Hartenstein 1978, Kheirallah 1979, Striganova and Prishutova 1990, Kohler *et al.* 1991). Millipede feeding rate on any given litter also varies with stage of decomposition of the litter (Kheirallah 1979). Feeding rates on most freshly-fallen litters were very low and most litters peaked in palatability between 10 and 11 months after litterfall. The increased palatability of well-decomposed litter may be due in part to leaching of water-soluble phenolics and in part to an increase in fungal and bacterial biomass. The chemical components of palatability were investigated by Sakwa (1974) who found that sugars were a feeding attractant, while tannins and other polyphenols were avoided by the millipedes. Neuhauser and Hartenstein (1978) found no correlation between millipede feeding rate and phenolic content of freshly-fallen litter. The mineral content of the litter may also be important. Lyford (1943) found that litter Ca content explained 55% of the variation in feeding rate on freshly collected green leaves of a number of tree and shrub species. However, many other aspects of leaf chemistry and physical structure co-vary with mineral content, so it is difficult to identify the factors that truly drive palatability.

The general objective of the experiments presented in this paper is to investigate the feeding behavior of two common native millipede species and to relate feeding rate to litter chemistry. In this laboratory study, feeding rates of two native millipede species on common forest litters were measured gravimetrically. Feeding rates of adult and juvenile *H. haydeniana* and *T. uncigerus* were measured for leaf litter from three common forest tree species which vary in their N and Ca content. I hypothesized that feeding rate would be positively correlated with the N and Ca content of the litters since both nutrients are likely to be important to millipedes. Additionally, I hypothesized that the feeding rate of juvenile millipedes would be higher than the feeding rate of adults since juvenile animals generally have a higher metabolic demand than adults.

MATERIALS AND METHODS

Forest litters were collected in the Oregon Coast Range, Benton County, Oregon. Alder and bigleaf maple litters were gathered in July from the surface of the paved portion of an unused logging road at an altitude of 300m in the Siuslaw National Forest. The alder and maple litters were predominately whole leaves from the litterfall of the previous year. Douglas-fir litter was collected from a second growth stand (800m). The Douglas-fir litter was the upper portion (L and F layers) of the forest floor. Air-dried litters were sieved through a 5 mm sieve to remove woody debris, oven-dried to constant weight at 65 °C and re-sieved through a 1mm sieve to remove fine material as well.

The C, N and Ca content of the litters were analyzed at the Environmental Protection Agency Environmental Research Laboratory in Corvallis, Oregon. The C and N content of the litters was analyzed by flash combustion (Carlo-Erba CHN analyzer EA 1108). Ca content was analyzed by atomic absorption spectroscopy (ICP, Thermo-Jarrell Ash).

Feeding rates of two species of millipedes were compared; all millipedes were collected in Benton County, Oregon. Juvenile and adult *H. haydeniana* were collected in a low-elevation (ca. 750 m) mixed Douglas-fir forest in the Oregon Coast Range. Adult *T. uncigerus* were collected from oak woodlands (ca. 150 m elevation) in the Coast Range foothills. Juvenile *T. uncigerus* were raised from eggs hatched in laboratory culture.

Feeding rates on alder, maple and Douglas-fir litter were measured. An equal-part litter mixture was also measured to test the hypothesis that the high N content of the alder litter and the high Ca content of the bigleaf maple litter would be complementary and that the millipedes would consume less litter *in toto* if all litters were available simultaneously. Adult male and juvenile *H. haydeniana* were tested on all three litters separately and the litter mixture. There were not enough

adult female *H. haydeniana* or adult and juvenile *T. uncigerus* available to test on all litters so they were tested only on the litter mixture.

The millipede biomass placed into each microcosm was standardized in order to keep the same ratio of millipede biomass to litter mass over all comparisons. Approximately 500 mg live biomass of millipede was used in each microcosm (1 adult or 7 juvenile *H. haydeniana,* 1 adult or 2 juvenile *T. uncigerus*). Based on an average feeding rate for millipedes (0.05 to 0.10 mg dry litter mg^{-1} dry millipede d^{-1}) taken from the literature (Hopkin and Read 1992), the 145 mg dry weight of millipede was expected to consume about 200 g of litter over the 14 day experiment. Thus, the 10g of litter supplied to the millipedes was far in excess (about 50-fold) of the projected food requirement. Because the adult *T. uncigerus* were larger than the adult *H. haydeniana*, the ratio of litter mass to millipede mass was lower for adult *T. uncigerus* but the amount of litter was still in excess of projected requirements.

Individual microcosms were made by placing 10 g litter into a 233 ml plastic cup. Two 5 mm airholes were punched into the side of the cup and covered with mesh to exclude competing fauna. Ten ml of de-ionized water was added to wet the litter. The microcosms were allowed to equilibrate for 14 days before millipedes were added. Three replicate microcosms were run for each litter/millipede combination tested.

Millipedes were allowed to feed on the litter in the microcosms for 14 days. The microcosms were kept at room temperature (20-22 ° C). The moisture content of the control and millipede microcosms was maintained at 100 ± 10% g water g^{-1} dry litter. Water was added twice during the two week experiment by misting the surface of the litter with distilled water.

After 14 days, millipedes were removed from the microcosms and re-weighed. Unconsumed litter in the microcosms was separated from frass by sieving under running water through nested 0.5 mm and 0.25 mm sieves. Particles retained

on each sieve were rinsed onto pre-weighed paper cone filters and oven-dried at 65° C to constant weight.

Frass was defined as litter particles with dimensions less than 0.5 mm for adult millipedes of both species, and 0.25 mm for immature millipedes of both species. The larger number (0.5 mm) has been used previously to separate frass from adult *H. haydeniana* (Carcamo *et al.* 2000). The smaller size was used for the immature millipedes in recognition of their smaller size. Although a formal study of the particle size in millipede frass was not made, microscopic examination of frass from adult *H. haydeniana* found occasional particles greater than 0.5 mm in narrowest dimension, but the majority of particles were less than 0.5 mm (N. Baumeister, personal observation).

For each litter, three replicate microcosms with no millipede were treated identically to the millipede microcosms in order to account for weight loss due to microbial decomposition and for weight loss due to fragmentation that occurred during sieving and was not due to millipede feeding. The amount of litter consumed by the millipedes was calculated by subtracting the weight of unconsumed litter in appropriate size class (either >0.5 or >0.25 mm) in the millipede treatment from the weight of litter in that size class in the control. Then the specific feeding rate (g litter g^{-1} millipede d^{-1}) was calculated by dividing the total amount of litter consumed by the weight of the millipede(s) in the microcosm and the number of days in the incubation (14).

Assimilation efficiency and decomposition rate were not calculated in the study because the primary goal was to accurately measure millipede feeding rate. In order to measure the millipede feeding rate, the unconsumed litter had to be completely separated from the millipede frass. Previous experience had indicated that frass from *H. haydeniana* dries to a hard crust which does not disperse well in water even after re-wetting, and there was concern therefore that the litter particles within each frass pellet would not separate freely from each other if the frass was

allowed to dry before the litter and frass mixture was sieved. Therefore, in this experiment, the litter and frass mixture in the microcosms was sieved immediately, without being allowed to dry first. During sieving, the very fine material that passed through the 0.25 mm sieve was lost and therefore couldn't be weighed. Therefore, mass loss to decomposition and mass loss of the very fine particles cannot be tallied separately. Ideally, mass loss due to decomposition would be calculated by drying and weighing the control litter, and mass assimilated by the millipedes would be calculated from the mass lost from the millipede microcosms, after subtracting the decomposition loss. However, calculating decomposition rate and assimilation rate requires first drying the litter, which would have introduced an error of unknown proportion from the loss in ability to separate frass from litter effectively. In this study, therefore, determination of the assimilation efficiency of the millipedes was sacrificed to accuracy in determining the feeding rate.

One-way ANOVA was used for juvenile and adult male *H. haydeniana* to detect differences in feeding rate between litter types (alder, big-leaf maple, Douglas-fir and the litter mixture). Two-way ANOVA (species, maturity) was used to determine if millipede species or life stage affected feeding rate on the litter mixture.

RESULTS

Of the three litters fed to millipedes, alder had the highest N content (2.8% N) and a moderately high Ca content (1.2%). Bigleaf maple litter had moderately high N content (1.6%) and the highest Ca content (1.4%). Douglas-fir litter had the lowest N and Ca contents (1.3% N and 0.9% Ca).

Feeding rates for adult male *H. haydeniana* were determined for alder, maple and Douglas-fir litters and an equal-part mixture of the litters (Table 2.1). Feeding rates for adult female *H. haydeniana* were only measured on the litter mixture. The feeding rate for adult male *H. haydeniana* was highest on alder and bigleaf maple (0.31 ± 0.07 and 0.26± 0.10 g litter g^{-1} live millipede d^{-1} respectively) and lowest on Douglas-fir (0.17 ± 0.06 g litter g^{-1} live millipede d^{-1}). The feeding rate on the litter mixture (0.21 ± 0.06 g litter g^{-1} live millipede d^{-1}) was slightly lower than average of the three individual litters (0.25 ± 0.07 g litter g^{-1} live millipede d^{-1}). ANOVA did not detect a significant effect of litter species on feeding rate ($F = 1.67$, $df = 2,6$, $p = 0.263$) but there was some evidence that feeding rates were higher on the broadleaf litters (bigleaf maple and alder) than on the Douglas-fir litter (two sample t-test, separate variance, $t = 2.2$, $df = 6$, $p = 0.07$).

Feeding rates for adult female *H. haydeniana* were determined only for the litter mixture (Table 2.1). The feeding rate for female *H. haydeniana* was 0.18 ± 0.05 g litter g^{-1} live millipede d^{-1}. Feeding rates for males and females were not significantly different.

For juvenile *H. haydeniana*, the difference in feeding rate between the litter species was smaller (less than 8% difference between the most consumed and least consumed litter) than for adults (Table 2.2). The mean feeding rate (average of all three litters) for juvenile *H. haydeniana* was higher (0.28 ± 0.6 g litter g^{-1} live millipede d^{-1}) than the mean feeding rate for adults ($F = 26.12$, $df = 1, 25$; $p < 0.001$).

Table 2.1. Feeding rates for adult *H. haydeniana* on forest litters. Mass of litter (mean ± SD) retained on the 0.5 mm sieve for the no-millipede control treatment, the millipede (*H. haydeniana*) treatment, difference between the control and millipede treatments (mass loss due to millipede feeding) and feeding rate (g litter g^{-1} live millipede d^{-1}).

Millipede	Litter	Control (g litter)	Millipede (g litter)	Mass loss (g litter)	Millipede feeding rate
Adult male	Alder	8.1 ± 0.4	5.8 ± 0.6	2.3 ± 0.6	0.31 ± 0.07
Adult male	Maple	8.2 ± 0.2	6.3 ± 0.9	2.0 ± 0.9	0.26 ± 0.10
Adult male	Douglas-fir	7.8 ± 0.3	6.6 ± 0.4	1.2 ± 0.4	0.17 ± 0.05
Adult male	Mixture	7.5 ± 0.2	5.8 ± 0.6	1.6 ± 0.6	0.21 ± 0.06
Adult female	Mixture	7.5 ± 0.2	5.5 ± 0.6	1.9 ± 0.6	0.18 ± 0.05

Table 2.2. Feeding rates for juvenile *H. haydeniana* on forest litters. Mass of litter (mean ± SD) retained on the 0.25 mm sieve for the no-millipede control treatment, the millipede (*H. haydeniana)* treatment, difference between the control and millipede treatments (mass loss due to millipede feeding) and specific feeding rate (g litter g^{-1} live millipede d^{-1}).

Millipede	Litter	Control (g litter)	Millipede (g litter)	Mass loss (g litter)	Millipede feeding rate
Juvenile	Alder	8.3 ± 5	6.4 ± 0.3	1.9 ± 0.3	0.27 ± 0.04
Juvenile	Maple	8.3 ± 1	6.5 ± 0.2	1.9 ± 0.2	0.27 ± 0.03
Juvenile	Douglas-fir	8.1 ± 6	6.1 ± 0.6	2.0 ± 0.6	0.29 ± 0.09
Juvenile	Mixture	8.0 ± 3	6.3 ± 0.5	1.8 ± 0.5	0.28 ± 0.08

Feeding rates for adults and juveniles of the two millipede species, *H. haydeniana* and *T. uncigerus*, were compared for the litter mixture only. Two-way ANOVA was used to test the effect of species and maturity. Juveniles of both species consumed more litter than adults consumed (Table 2.3; $F = 40.75$, $df = 1, 11$; $p < 0.001$). The mean feeding rate for adult and juvenile *H. haydeniana* was higher than for adult and juvenile *T. uncigerus*, but the difference was not significant ($F = 1.9$, $df = 1, 11$; $p = 0.20$). The interaction term in the ANOVA was non-significant ($F = 1.1$, $df = 1, 11$; $p = 0.32$).

Because the three litter types were presented to *T. uncigerus* only in a mixture, it is not possible to determine which of the three types *T. uncigerus* consumed or the relative proportion of each litter type consumed. Nevertheless, from examination of the remaining litter, it appears that *T. uncigerus* consumed at least some of all three types.

Table 2.3. Litter comminution rates (g litter g^{-1} live millipede d^{-1}, mean ± SD, n = 3) for adults and juveniles of two millipedes species feeding on a litter mixture (equal-parts alder, bigleaf maple and Douglas-fir).

Species	Age	N	Live weight (mg)	Weight gain (mg)	Feeding rate[a]
H. haydeniana	Adult	6	650	-3 ± 2	0.20 ± 0.05
T. uncigerus	Adult	3	2040	16 ± 42	0.12 ± 0.02
H. haydeniana	Juvenile	3	71	5 ± 1	0.28 ± 0.08
T. uncigerus	Juvenile	3	280	87 ± 40	0.26 ± 0.01

DISCUSSION

Effect of litter species on feeding by *H. haydeniana*

Harpaphe haydeniana consumed all three litters (alder, bigleaf maple and Douglas-fir). Feeding rates varied from 0.17 (Douglas-fir) to 0.31 (alder) g litter g^{-1} live millipede d^{-1}. These rates are quite close to those recorded for *H. haydeniana* by Carcamo *et al.* (2000). He used a similar method for measuring feeding rate, but separated frass from unconsumed litter by gently pushing the dry litter/frass mixture through a 0.5 mm sieve. His feeding rates were from 0.09 (Douglas-fir and western hemlock) to 0.20 (western redcedar) g litter g^{-1} live millipede d^{-1}.

Although *H. haydeniana* expresses preferences for certain litter types, they will consume even non-preferred litters at a significant rate. In this study, the feeding rate of the less-preferred Douglas-fir litter was still 55% of the feeding rate on the most-preferred alder litter. Similarly, of the seven tree litters offered simultaneously to *H. haydeniana* (Carcamo *et al.* 2000), the feeding rate on the least-preferred litter (bigleaf maple) was still 60% of the feeding rate on the most-preferred litter (Douglas-fir).

It is unclear what role litter species plays in determining the feeding preferences or feeding rates for *H. haydeniana*, since contradictory results can be seen both between studies and within a single study. For example, in this study, adult *H. haydeniana* consumed more alder and maple litter than Douglas-fir litter, but in Carcamo *et al.* (2000) more Douglas-fir litter was consumed than alder or bigleaf maple. In this case, the apparent contradiction may be due to the difference in age or stage of decomposition of the litters. In Carcamo *et al.* (2000) the litters were collected in November shortly after litterfall, and the alder and bigleaf maple litters in particular would have been less weathered than the July-collected litter used in my experiment. In the field, bigleaf maple litter loses little weight to

feeding by soil fauna during the first 6 months of exposure (winter and early spring); feeding by soil fauna increases between 6 and 12 months after litterfall (DeCatanzaro and Kimmins 1984). Others have found that the acceptability of litter as food for millipedes varies both with the species of the litter and the stage of decomposition of the litter (Kheirallah 1979). In some cases, a single additional month of aging for the litter doubled the feeding rate of the millipedes on the litter.

The juvenile *H. haydeniana* millipedes did not discriminate between the three litter types- all were consumed at a remarkably similar rate. It is possible that these millipedes were feeding at the maximum rate that is physiologically possible for them. While the feeding rate for juveniles on the deciduous litters was very close (within 13%) of the feeding rate for adults, the feeding rates for juveniles on the Douglas-fir litter were up to 70% higher than the feeding rate for adults. The increased consumption of Douglas-fir litter by juvenile *H. haydeniana* may have been in compensation for the lower nutritional value of Douglas-fir litter. Compensatory feeding by millipedes has not been established, however many foliage-feeding insects compensate for low nutrient content in foliage by increasing consumption rate (Slansky and Rodriguez 1987). Overall, juvenile *H. haydeniana* consumed about 40% more of the litter mixture per bodyweight than adults consumed. The higher feeding rate of juveniles is probably a consequence of higher metabolic demand associated with growth. In insects, relative feeding rates are higher in younger animals (Scriber and Slansky 1981). The assimilation efficiency of the juveniles may have been higher as well, since smaller millipedes shred consumed litter into smaller particles (Kheirallah 1990) and have higher assimilation efficiency (Kohler *et al.* 1991).

Millipede species

The relative feeding rate of *H. haydeniana* adults was higher than the feeding rate of adults of *T. uncigerus*. Since *T. uncigerus* adults were almost 4-fold

larger than *H. haydeniana* adults, the lower feeding rate may be a result of the lower metabolic rate of the larger animal (Reichle 1967).

In contrast, the feeding rate for juveniles of the two species was quite similar, despite an almost 4-fold difference in size between the smaller *H. haydeniana* and the larger *T. uncigerus*. However, *T. uncigerus* grew more than *H. haydeniana* during the 14-day experiment, and the higher metabolic demand for growth may have combined with the lower metabolic rate of the larger species to produce a feeding rate similar to the feeding rate of the slower growing, smaller species.

Ecological implications

The ecological impact of feeding by *H. haydeniana* can be estimated by combining these feeding rates with estimates of the population density of *H. haydeniana*. For most of the year, adults (and 7^{th} stadium juveniles) are dispersed across the landscape at low density. But populations of *H. haydeniana* can reach high densities in two circumstances: first, where adults aggregate to mate and second, in sites where cohorts of juveniles are resident.

Large aggregations of adults occur in late spring (May and June), apparently for mating. Depositions of frass reveal that feeding is occurring during this aggregation, although mating may modify the feeding rate. High densities persist for about 2 months. Density can be as high as 2197 millipedes m^{-2} (actual count, N. Baumeister unpub. data). At this highest density (1186 g live biomass m^{-2}), these millipedes could consume 332 g litter m^{-2} day^{-1}. Although the period of high density is limited in time, such intensive feeding in spring could affect nutrient availability to the plant community.

The population density and biomass of juvenile *H. haydeniana* can also be very high. In a site where juvenile *H. haydeniana* were known to occur, a transect line was established and *H. haydeniana* were collected by hand-sorting of 0.25 m^2

plots located at 3 m intervals along the transect. Of the 575 millipedes collected, 99.5% were 6th stadium juveniles and the remaining 0.5% were 7th stadium juveniles. No adults were present in this early July survey, although many had been present earlier in the year. The biomass of *H. haydeniana* varied from 0 to 32 g live mass per m^2 (N. Baumeister unpub. data; n=18, mean = 8.3 g live mass or 127 millipedes m^{-2}). At the highest density this millipede biomass would consume 1613 g of forest floor m^{-2} y^{-1}. Since forest floor mass under Douglas-fir is typically around 2000 g m^{-2} (Binkley 1995, Fogel and Hunt 1979) a substantial fraction of the forest floor may be processed by juvenile *H. haydeniana*.

After the 6th stadium, *H. haydeniana* disperses, thus the effect of feeding by the 7th and adult stadia is more diffuse. Based on a population size of 2 g live biomass per m^2 (two adults and ten 7th stadium juveniles) Carcamo *et al.* (2000) estimated that *H. haydeniana* consumes 72 g litter per m^2 per y^1 in Douglas-fir forests on Vancouver Island, British Columbia.

For this study, the proportion of bigleaf maple and alder litterfall consumed by *H. haydeniana* would be similar to the proportion of Douglas-fir litterfall consumed, because although feeding rates on these litters were higher, litterfall rates are also higher under these species (Fried *et al.* 1990).

CONCLUSIONS

Harpaphe haydeniana consumes alder, bigleaf maple and Douglas-fir litter in the laboratory and likely at similar rates in the field. However, even though *H. haydeniana* consumes Douglas-fir litter, it is not certain that Douglas-fir litter contains sufficient nutrients to support growth in *H. haydeniana*. A study comparing growth of *H. haydeniana* on alder, maple and Douglas-fir litter would be the next step in understanding the relationship between *H. haydeniana* and Douglas-fir forests.

3. EFFECT OF LITTER CHEMISTRY ON MILLIPEDE GROWTH

ABSTRACT

The effect of litter chemistry on growth and survival of the millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) was investigated in a laboratory experiment. Growth of *H. haydeniana* on five litter types (western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), Douglas-fir in mixed stand with western redcedar (*Thuja plicata* Donn ex D. Don), bigleaf maple (*Acer macrophyllum* Pursh) and alder (*Alnus rubra* Bong.) with contrasting nutrient contents was compared. The relative growth rate (RGR) was highest on the two litters containing Douglas-fir. Within the conifer litters, RGR was related to Ca content. Alder appears to be toxic to *H. haydeniana.* Forest management practices that affect tree species distribution have the potential to affect millipede biomass and therefore litter comminution rates and nutrient cycling.

INTRODUCTION

As detritivores, millipedes play a significant role in the regulation of plant litter decomposition and nutrient cycling (Anderson *et al.* 1985). As they consume, process and egest litter, the particle size of the litter is reduced, pH increases, and N mineralization is enhanced. Detritivores are usually thought to be resource limited, that is, the size of the population is limited by the amount and quality of food available (Swift *et al.* 1979). Many studies have demonstrated that millipede biomass differs under different tree species (e.g. McBrayer *et al.* 1977). Millipedes are most abundant in broadleaf forests on neutral soils (Petersen and Luxton 1982), while conifer forests generally have few millipedes. On the average, broadleaf litters contain more N and Ca, and are lower in lignin and tannins than are conifer litters (Chabot and Hicks 1982), so the greater millipede biomass in broadleaf forests could be a result of the higher food quality of deciduous litter to millipedes.

Few studies have been done which directly link millipede growth to specific aspects of food quality. For herbivorous insects, however, N is an important and frequently limiting nutrient in growth and maturation (Scriber and Slansky 1981) and it is likely to be important for millipedes as well. Among the mineral nutrients, Ca is of particular significance to millipedes because the millipede exoskeleton is reinforced with calcium carbonates. The calcium content of millipedes is 15 to 20 times greater than the calcium content of insects (Reichle *et al.* 1969). Thus, the Ca content of litter is likely to affect millipede growth.

Relatively few studies have compared millipede growth rates on different foods. In one study (Striganova and Prishutova 1990), immature julid millipedes (*Rossiulus kessleri*) were raised from first stadium to adulthood on litter from five tree species (all broadleaf) and a grass. Millipedes fed *Fraxinus* and *Armeniaca* litters grew twice as fast as those fed *Populus*. Millipedes fed on the grass (*Elytrigia*) died before reaching maturity. The C, N and Ca content of the litters

were not reported. In another study, growth of the polydesmid millipede, *Orthomorpha gracilis* on two species of litter was compared (Kheirallah and Shabana 1975). They demonstrated that the millipede grew faster on *Ficus* litter than *Hedera* litter.

In Pacific Northwest forests, litter from the two most common broadleaf tree species, red alder (*Alnus rubra* Bong.) and bigleaf maple (*Acer macrophyllum* Pursh), is higher in both N and Ca than is litter from two common conifer species, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Tarrant *et al.* 1951, as cited in Youngberg 1979). However, litter from another conifer species, western redcedar (*Thuja plicata* Donn ex D. Don), is very high in Ca and the presence of western redcedar in Douglas-fir forests increases the Ca content of the forest floor considerably (Binkley 1995).

The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is an important detritivore in the Pacific Northwest. *Harpaphe haydeniana* is common in many forest types at low to mid-elevations along the Pacific Coast from Alaska to California. *Harpaphe haydeniana* consumes a variety of leaf litters, including alder, bigleaf maple and Douglas-fir litter (Chapter 2 this work, Carcamo *et al.* 2000).

The objective of this study was to measure the growth rate of *H. haydeniana* on common litter types and correlate millipede growth with litter chemistry. Juvenile (4^{th} stadium) *H. haydeniana* were raised on forest floor litter from common tree species: alder, bigleaf maple, western hemlock, Douglas-fir and a mixed conifer (mostly Douglas-fir, with some western redcedar and western hemlock).

MATERIALS AND METHODS

Experimental design and treatments

Millipede growth was measured on five different litters (alder, bigleaf maple, western hemlock, pure Douglas-fir and mixed Douglas-fir). These four tree species are the major canopy species in low-elevation forests (Franklin and Dyrness 1988) and are therefore potentially important food resources for *H. haydeniana.* Since these tree species often grow together in mixed stands, an equal-part mixture of the litters was added as an additional treatment. The experimental design was completely randomized with five replicate microcosms per litter treatment and 3 to 4 millipedes (see below) per replicate.

Each individual millipede was weighed at the start of the experiment, and then at 42 and 99 days. At each date, the stadium and live weight of each individual was recorded. At the end of the experiment, the surviving millipedes were dried, weighed, and analyzed for C and N content by flash combustion (Carlo Erba), and elemental content by atomic emission spectroscopy (Thermo-Jarrell Ash). A minimum of 15 mg of millipede dry weight was needed for the analyses, so up to four millipedes were bulked together if needed to get sufficient mass.

The C, N and elemental content of the litters and soils were also analyzed. The C, N and Ca content of the litters were measured on a single bulk sample of each. The pH of the litter/soil mixture in the microcosms was measured at the end of the experiment. Additionally, net N mineralization was measured in each microcosm. Inorganic N was extracted from the litters in a 1N KCl solution (50 ml solution : 5 g litter) and frozen for later analysis. The ammonium (NH_4^+) and nitrate (NO_3^-) concentration of the extract was analyzed colorimetrically (RFA-Alpkem).

Soil and litter collection

The five litter types used in the experiments were gathered in June and July, 1996, from locations in the Siuslaw National Forest, Benton County, Oregon. For each litter, all forest floor layers were gathered in order to include litter at all existing stages of decomposition. Because the alder and bigleaf maple litter decomposes rapidly, there was very little F layer in the forest floor, so the litter consisted mostly of L layer (litter from the previous fall). In contrast, the forest floor under the conifer species included a well developed L, F and H layers were gathered and mixed. Western hemlock litter was gathered from a mature stand. Two sources of Douglas-fir litter were used. The mixed Douglas-fir litter (predominantly Douglas-fir, with some western redcedar and western hemlock) was gathered from the same mature mixed stand from which *H. haydeniana* millipedes were collected. The pure Douglas-fir litter was gathered from an approximately 80 year-old second growth stand about 30 meters upslope from the mixed stand. Soil (0 to 10 cm) was collected from the same sites, dried and sieved (2 mm) and used with litter to make microcosms. The litters were de-faunated by Berlese extraction and sieved through a 5 mm mesh to remove large woody material, reduce the particle size and aid in mixing and homogenization of the litter. The litters were air-dried before use in the experiment.

Microcosm construction

Microcosms for raising millipedes were made from 233 ml plastic containers. A sharp point was used to put about 10 airholes into the lid of each microcosm. Differences in the density of the litters made it impossible to use the same weight of litter in all treatments but the amount of litter used was in all cases in excess of litter consumed. All microcosms received 20 g of the appropriate soil. The microcosms were filled with litter as follows: alder and bigleaf maple (10 g

litter); mixed Douglas-fir, Douglas-fir and equal-part mixture (15 g litter); and western hemlock (20 g litter).

Millipede collections and cultures

The 4th stadium *H. haydeniana* millipedes used in this experiment were extracted by Berlese funnels from litter collected in July, 1996 from a mature mixed conifer stand at about 750 m elevation in the Siuslaw National Forest, Benton County, Oregon. The stand is dominated by Douglas-fir, but also contains western hemlock and western redcedar.

Because the juveniles were heat-extracted in Berlese funnels from the litter, they emerged over a two week period and were added to the microcosms in two groups, one week apart. The first group to emerge from the extractor (48 millipedes) was randomly assigned to replicates 1 and 2 (4 millipedes per replicate microcosm) of all 6 litter treatments. One week later, more millipedes had been extracted. This group was randomly assigned to replicates 3, 4 and 5 (at 3 millipedes per replicate) for all 6 treatments.

Cultural conditions and maintenance

The microcosms were maintained at 100 ± 15% gwc (g moisture g^{-1} dry litter) at room temperature (20 to 22 °C) under variable photoperiod.

Data analyses

One-way ANOVA was used to test for the effect of litter type on millipede performance: survival, live weight, dry weight and Ca content. Mean millipede survival was calculated for each replicate microcosm. Millipede live weight, dry weight and Ca content were calculated for individuals and also averaged for each microcosm. The block effect (first (earlier) and second (later) group of millipedes

extracted from the Berlese extractors) was tested and if not significant, was not included in the analyses. Fisher's F-protected least significant difference (LSD) was used to separate and compare treatment means. In order to satisfy the assumption of homogeneity of variance in ANOVA, variables were transformed to ln (Y) or ln (Y + 1) as needed. Kruskal-Wallis non-parametric test was used when homogeneity of variance could not be attained. Means and standard deviations or standard errors of untransformed data are presented as specified. Additionally, multivariate regression was used to examine the effect of litter chemistry (pH, %Ca, C/N ratio, %N and net N mineralized) and type (broadleaf, mixed or conifer) on millipede performance on the litters. All individual millipedes were used as input to the model.

RESULTS

Litter type significantly affected both millipede survival and millipede growth. After 99 days of feeding on the litters, millipede survival varied significantly ($F = 6.86$, $df = 5, 19$, $p < 0.001$), from a low of 20 % on alder litter to 75 to 88% on the conifer litters (Table 3.1;Figure 3.1). Survival was higher in the conifer litters (mean 80%) than in the broadleaf litters (mean 26%). In all but the alder litter, the surviving millipedes were uniformly distributed among the microcosms, In the alder litter, all the millipedes were dead in four of five microcosms, and in the fifth microcosm, all three individuals were alive (but very small).

In the two broadleaf litters, early patterns of growth and survival changed over time. For example, at 45 days, millipedes in bigleaf maple litter had gained significantly less weight ($F = 8.39$, $df = 5, 22$; $p < 0.001$) than millipedes in the other treatments and survival was also lower (45% versus an average of 79% for the other treatments). However, between 45 and 99 days, there was little additional mortality in the bigleaf maple litter (survival at 99 days was 32%) and the surviving millipedes were above average weight (Table 3.1). Millipedes in alder litter, in contrast, showed average growth and survival at 45 days, but there was considerable mortality between day 45 and day 99 and the surviving millipedes were significantly smaller than average (Table 3.1).

There was no consistent relationship between mean survival in the litter microcosms and mean weight of the millipedes. For example, survival was low (20%) in the alder litter and the surviving millipedes were small (mean dry weight 6.4 mg), but in the bigleaf maple litter, survival was also low (32%), but the surviving millipedes were large (mean dry weight 12.4 mg). In the mixed conifer litter, survival was high (77%) and millipedes were large (mean dry weight 16.0 mg). If the response of the millipedes for both survival and growth is the same in

the field as in this laboratory experiment, then the total population weight (total weight of the millipedes in each litter type) would be a more accurate estimate of the capacity of the litters to support *H. haydeniana* than the mean weight of individual millipedes (average weight of the millipedes in each litter type). However, the reasons for poor survival in the alder, bigleaf maple and litter mixture in this experiment are unknown and therefore I don't know if millipedes living in and consuming these litters in the field would be similarly affected. The conservative assumption is that the poor survival is an artifact of the method. For that reason, I analyzed average values for millipede live and dry weights, millipede %Ca and millipede Ca content, rather than the total production.

Millipede growth was significantly affected by which litter they consumed. The mean live weight of the surviving millipedes varied 5.5-fold, from a low of 17 mg on alder litter to a high of 94 mg on the mixed Douglas-fir litter (Table 3.1; Figure 3.2). Millipedes were smallest in the alder, litter mixture and western hemlock litters (Kruskal-Wallis test statistic = 9.49, df = 5, 19; p = 0.09). Among the conifer litters, millipedes in western hemlock litter were significantly smaller than millipedes in the Douglas-fir or mixed Douglas-fir litter. Dry weights followed a similar pattern. Millipedes were largest in the mixed Douglas-fir, Douglas-fir or bigleaf maple litters (F = 3.58, df = 5, 19; p = 0.02). Again, millipedes in western hemlock litter were significantly smaller than millipedes in the Douglas-fir or mixed Douglas-fir litter.

The litters also affected the elemental content of the millipedes. The average Ca content of the millipedes varied significantly between litters (F = 14.76, df = 5, 19; $p < 0.001$). The average Ca concentration was lowest (11.9%) in millipedes in the litter mixture and highest (16.6%) on the bigleaf maple litter. Millipede Ca concentration varied over a fairly limited range. Within the treatments, the largest span from lowest %Ca to highest %Ca was 3.7%. Among millipedes in all treatments, the lowest %Ca seen for any individual was 10.8% (in the litter

mixture) and the highest value was 17.5% (in the Douglas-fir litter). Total body Ca content was also affected by which litter the millipedes consumed. In the three litters that produced millipedes with the highest %Ca (mixed Douglas-fir, Douglas-fir and bigleaf maple), the millipedes were also largest. Because high %Ca was associated with greatest growth, the millipedes separated into two discrete groups with respect to total body Ca content (Kruskal-Wallis test statistic = 14.76, df = 5, 19, $p < 0.001$). Millipedes in the mixed Douglas-fir, Douglas-fir and bigleaf maple litters contained 2.3 to 2.0 mg Ca per individual, while millipedes in the western hemlock, alder and litter mixture contained 1.1 to 0.9 mg Ca.

Table 3.1. Survival, growth and Ca content (mean ± SE) for juvenile *Harpaphe haydeniana* millipedes raised on litter from five PNW forest canopy species and an equal-part mixture of the litter species.

Litter	Survival	Live weight[1] (mg)	Dry weight[1] (mg)	Ca[2] (%)	Total Ca[3] (mg)
Mixed Douglas-fir	0.77 ± 0.06 a	94 ± 15 ab	16.0 ± 2.3 a	14.8 ± 0.5 b	2.3 ± 0.4a
Douglas-fir	0.75 ± 0.06 a	83 ± 14 ab	12.5 ± 1.6 ab	15.8 ± 0.5 ab	2.0 ± 0.3a
Western hemlock	0.88 ± 0.07 a	56 ± 2 c	8.7 ± 0.5 bc	12.6 ± 0.5 c	1.1 ± 0.2b
Bigleaf maple	0.32 ± 0.10 b	82 ± 27 abc	12.4 ± 2.1 ab	16.6 ± 0.1 a	2.1 ± 0.4a
Red alder	0.20 ± 0.20 b	17 ± 2 d	6.4 ± 0.4 bc	13.8 bc	0.9c
Mixture	0.53 ± 0.06 b	49 ± 6 c	6.7 ± 0.6 c	11.9 ± 1.1c	0.9 ± 0.3c

Numbers within a column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$).

[1] Mean live or dry weight of individual millipedes (n = 3 to 15).

[2] Mean %Ca of individual millipedes (n = 1 to 7, small individuals were bulked together in order to get enough mass for the ICP analysis).

[3] Mean total Ca per individual millipede (n = 1 to 7).

Table 3.2. Litter variables used in models predicting millipede growth on litter from five PNW forest canopy species and an equal-part mixture of the litter species. Means (± SE, n = 5) for microcosm pH and N_{min}

Litter	Type	%Ca[1]	%N	C/N ratio[1]	Final pH[2]	N_{min}[2,3]
Mixed Douglas-fir	Conifer	1.42	1.11	40.3	4.9 ± 0.06 b	901 ± 74 d
Douglas-fir	Conifer	0.88	1.33	33.6	4.7 ± 0.04 c	1613 ± 107 b
Western hemlock	Conifer	0.36	1.17	34.1	4.7 ± 0.02 c	617 ± 69 e
Bigleaf maple	Decid.	1.40	1.63	27.2	5.2 ± 0.03 a	1135 ± 120 cd
Red alder	Decid.	1.18	2.83	15.6	4.9 ± 0.08 b	3124 ± 267 a
Litter mixture	Mixed	1.05	1.61	30.2	4.6 ± 0.02 c	1187 ± 55 c

[1] Measured on a single bulk sample of litter.

[2]Measured in microcosms (litter + soil) (n = 5) at the end of the experiment.

[3] N_{min} = sum of NO_3^- and NH_4^+ mineralized from litter (mg N kg^{-1} litter).

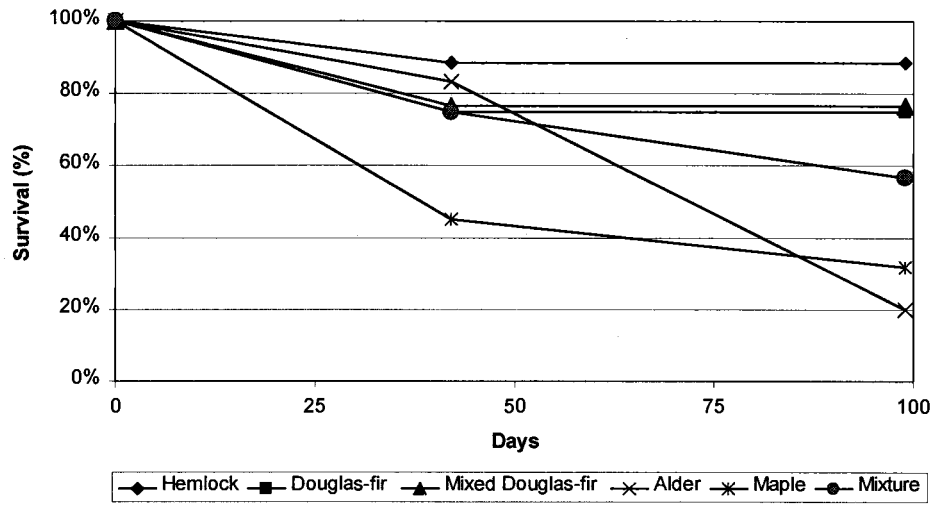

Figure 3.1 Survival for juvenile *Harpaphe haydeniana* millipedes raised on litter from five PNW forest canopy tree species and an equal-part litter mixture.

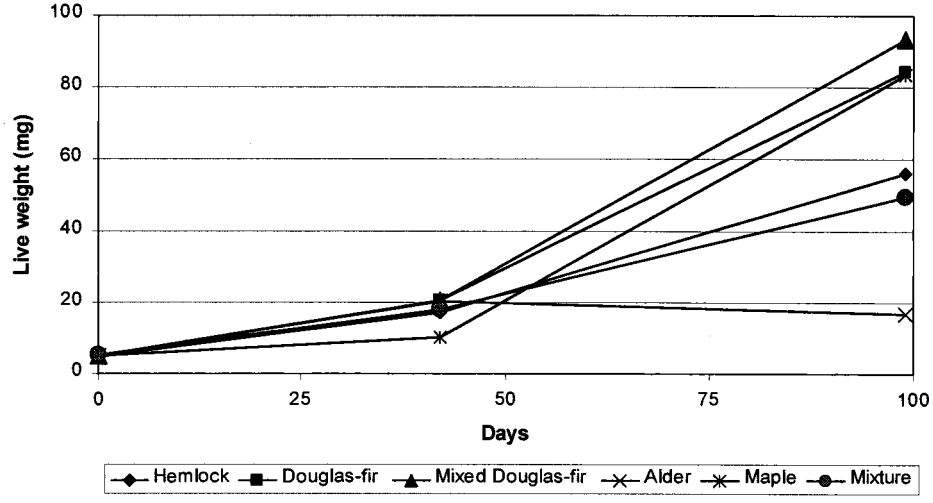

Figure 3.2. Mean live weight of juvenile *Harpaphe haydeniana* fed litter from five PNW forest canopy tree species and an equal-part litter mixture.

The litters used to feed millipedes varied considerably in their %Ca, pH, %N and C/N ratios (Table 3.2). Since the dry weight, %Ca and Ca content (mg Ca per millipede) of the millipedes raised on the different litter types also varied significantly among the litter types, multiple regression was used to describe relationships between the litter variables and the millipede variables.

Correlations between the explanatory variables are presented in Table 3.3. Litter %Ca and pH were significantly correlated with each other so %Ca, rather than pH was used in the model because it is a more commonly measured variable. Similarly, litter %N and C/N ratio were correlated so litter %N was used in the models. The high N content of the alder litter gave those data points a leverage that was more than 5 times the average leverage, so the models using %N were run with and without the alder litter.

Table 3.3. Pearsons correlation coefficients for components of litter chemistry (n = 30)

	Type[1]	C/N ratio	LiN[2]	NMin[3]	LiCa[4]	Li pH
Type[1]	1.0					
C/N ratio	0.85*	1.0				
LiN	-0.79*	-0.96*	1.0			
Nmin	-0.55*	-0.80*	0.88*	1.0		
LiCa	-0.50*	-0.15	0.26	0.24	1.0	
Li pH	-0.53*	-0.27	0.25	0.11	0.59*	1.0

* $P < 0.01$.

[1] Type = 1 for broadleaf litters (alder and bigleaf maple), 1.5 for litter mixture and 2 for the conifer litters.

[2] LiN = % N in litter

[3] Nmin = sum of NO_3^- and NH_4^+ mineralized from litter (mg N kg^{-1} litter).

[4] LiCa = % Ca in litter

Growth of *H. haydeniana* increased with increasing litter %Ca and millipedes were also larger in the conifer litters than in the broadleaf litters (Table 3.4). The model using only litter %Ca explained 9.8% (alder included) or 12% (without alder) of the variation in millipede growth. When the litter %Ca model was restricted to the conifer litters only, the explanatory power increased to 22%. Over all litter types, adding a variable for litter type (conifer, mixed and broadleaf) increased the R^2 value to 22%. The model combining litter type and litter %Ca predicted the mean dry weights of millipedes in the conifer and alder litters well; the predicted mean values were within 10% of the actual mean values. The model did not fit the bigleaf maple litter or litter mixture as well; growth was

underestimated in the bigleaf maple litter by 28%, and overestimated in the litter mixture by 46%.

In contrast to the positive relationship between litter Ca and millipede growth, millipedes grew less well in the litters with higher %N (Table 3.4). Adding litter type to the model did not improve the fit, probably since the broadleaf litters had consistently higher N contents than the conifer litters. Since the data points associated with alder litter had high leverage over the regression line, the model was also run without alder litter. Neither model explained more than 8.6% of the variability in millipede dry weight. When the model was restricted to the conifer litters, the regression was not significant ($F = 0.55$, $df = 1, 39$; $p = 0.46$). The models underestimated mean weights in the mixed Douglas-fir and bigleaf maple litters by 18 to 30% and overestimated mean weights in the western hemlock and litter mixture by 40 to 50%.

Because more N was mineralized from the litters with higher % N, it seemed possible that the higher concentration of NO_3^- or NH_4^+ could negatively affect millipede growth. The variable N_{min} (sum of NO_3^- and NH_4^+) alone did not have a significant relationship with millipede weight ($F = 0.47$, $df = 1, 56$; $p = 0.49$).

Table 3.4. Models for predicting millipede growth on litter from five PNW forest canopy species and an equal-part mixture of the litter species. Y: Average millipede dry weight.

Model	F (df)	p	R^2
Y = 6.4 + 4.9 (Litter %Ca[1]) alder included	6.12 (1, 56)	0.021	9.8%
Y = 6.2 + 5.4 (Litter %Ca) alder removed	7.34 (1,53)	0.009	12%
Y = 5.7 + 7.5 (Litter %Ca) conifer litters only	12.04 (1, 39)	0.0013	22%
Y = - 9.22 + 7.8 (Litter %Ca) + 7.2 (LiType[2])	9.27 (2, 55)	0.0003	22%
Y = 17.5 - 4.6 (Litter %N[3]) alder included	5.29 (1, 56)	0.025	8.6%
Y = 22.4 - 8.4 (Litter %N) alder removed	4.25 (1, 53)	0.044	7.4%

[1] Litter %Ca = %Ca in litter.

[2] LiType = 1 for broadleaf litter (alder and bigleaf maple), 1.5 for litter mixture and 2 for conifer litters.

[3] Litter %N = %N in litter

DISCUSSION

The objectives of this experiment were to:

1) compare growth of *H. haydeniana* on Douglas-fir litter with growth on other conifer and broadleaf forest litters common in the Pacific Northwest; and
2) correlate millipede growth with litter chemistry in order to find out how litter nutrient content affects growth

Litter type and species

Of all litters tested, *H. haydeniana* grew best on the two Douglas-fir litters. This result is significant because Douglas-fir dominates much of the low-elevation forest land in the Pacific Northwest. There is evidence in the field that *H. haydeniana* has an affinity for Douglas-fir. Carcamo *et al.* (2000) reports that *H. haydeniana* is more abundant in old-growth Douglas-fir stands than in regenerating forests dominated by alder, and that *H. haydeniana* is more common in monospecific plantations of Douglas-fir than monospecific plantations of western redcedar or western hemlock.

Of the two Douglas-fir litters tested in my experiments, growth was better on the higher Ca litter from the old-growth mixed Douglas-fir forest, than on the lower Ca litter from a nearby second-growth stand of 100% Douglas-fir. The old-growth Douglas-fir stand is located along a moist riparian corridor. The second-growth Douglas-fir stand is about 10m in elevation above the riparian old-growth stand, yet the Ca content of the old-growth litter was 61% higher than the Ca content of the second-growth stand. The higher Ca content of the litter in the old-growth stand is probably partly due to the presence of western redcedar in the stand which adds Ca-rich litter to the forest floor, although there may possibly be differences in soil type as well. Replacement of mixed forests containing western

redcedar with Douglas-fir monocultures may therefore affect the growth of millipedes and possibly other detritivores as well.

Western hemlock litter supports growth of *H. haydeniana*, but growth was significantly slower than growth in the Douglas-fir litters. The low Ca content of the western hemlock litter may be limiting growth since in this study there was a significant correlation between litter Ca content and millipede growth. Although correlation does not necessarily imply a cause and effect relationship, I think there is some logical evidence for a cause and effect relationship, which could be tested, in a future study. I suggest that Ca availability is limiting growth (dry mass accumulation), rather than growth limiting Ca assimilation. My argument is as follows. If the Ca assimilation rate were limiting dry mass accumulation, then I would expect the millipedes to maintain the minimum %Ca possible. If some other nutritional factor were limiting growth, then I would expect the millipedes to have low dry weight, but higher %Ca. Since the millipedes in the western hemlock litter had significantly lower %Ca *and* were smaller than millipedes in the other conifer litters, that suggests that litter Ca availability was limiting dry mass accumulation (growth). This observation does not constitute proof that litter Ca limits millipede growth, since there was only one litter with such a low Ca concentration. It is possible that some other aspect of the nutritional content western hemlock litter is not ideal for millipede growth, and that the low %Ca in the millipedes is the result of the interaction of other factors, and not solely due to the Ca concentration of the litter.

Millipede growth in bigleaf maple litter presents a more complex story. Millipedes feeding in bigleaf maple litter grew almost as large as those in Douglas-fir litter by the end of the experiment (day 99), but at the midpoint (day 42) of the experiment mortality was significantly higher than average, and live weight was lower than average. Since the bulk of the mortality was early in the experiment, intra-specific competition is not a likely cause of the excess mortality, as the mass

of leaf litter supplied was far in excess of the calculated food requirement at the mid-point of the experiment, and in fact there was unconsumed leaf litter left in all microcosms at the end of the experiment. There was some inter-specific competition for food from fly larvae (Diptera: Sciaridae), which hatched from eggs that were not killed during the preparation of the litter. Air-drying litter at room temperature is not sufficient to kill these eggs and oven-drying at low heat is recommended for future experiments. However, since there was litter left unconsumed in all the microcosms at the end of the experiment, it seems unlikely that feeding by these other detritivorous fauna caused the reduced growth and higher mortality that was seen in *H. haydeniana* at the midpoint of the experiment.

A possible explanation for the increased mortality and reduction in growth in the bigleaf maple litter may have been due to the presence of fungal species with toxic or anti-feedant chemicals early in the experiment. Many fungi, especially early succession species such as *Aspergillus* are known to produce toxins that either kill arthropod grazers or prevent feeding (Shaw 1992). Either starvation or a fatal toxin could have killed some millipedes, while others were able to survive either by innate resistance or by avoiding the toxin by feeding on different parts of the litter. If survival were due to innate resistance to the toxin, then surviving millipedes would be randomly distributed though the microcosms. The uniform, rather than random distribution of the surviving millipedes in the microcosms suggests that avoidance of the toxin is more likely. Millipede mortality due to toxic fungal species may not be common in nature, since *H. haydeniana* are mobile and would be able to select palatable leaves, or even leave the area entirely and seek out a different litter type.

Alder litter proved to be an unsuitable food for *H. haydeniana.* By the end of the experiment, 80% of the millipedes were dead, and the surviving millipedes were small. Additional anecdotal evidence for the unsuitability of alder litter for *H. haydeniana* exists. In the feeding experiments reported in Chapter 2 (this

document), the only millipede which died during the two week experiment was 1 of the 21 juveniles in alder litter. All 21 juvenile millipedes in the bigleaf maple litter and Douglas-fir litter survived. Carcamo *et al.* (2000) reports that alder litter killed a mass culture of adult *H. haydeniana* in less than two weeks. This evidence is anecdotal, since the trial was unreplicated. In contrast to the growth pattern in the bigleaf maple litter, the millipedes in alder litter were unremarkable (average size and mortality) at the midpoint of the experiment. As with bigleaf maple litter, there are many possible explanations for the high mortality and lack of growth, which I can only suggest at this time. There were sciarid larvae present in the alder litter, so inter-specific competition for food is a possibility. Toxic fungal species occurring later in the fungal succession (between day 42 and day 99) could have killed and/or stunted the millipedes.

Another possible explanation for the poor food quality of alder litter is its high phenolic content. Condensed tannins cause death to susceptible species of insects that feed on them by binding irreversibly to the lining of the gut. The phenolic content of litter was reduced by up to 50% after passage through the millipede gut (Neuhauser and Hartenstein 1978). The authors attribute the reduction in phenolics to microbial breakdown, but it could also have been due to binding of phenolics in the gut. Hydrolysable tannins are able to bind metal ions like Ca^{2+} and thereby reduce their availability, possibly inducing a deficiency (Harbourne 1993, Mattson and Scriber 1987). Reduction in Ca availability may have been part of how alder litter affects millipede growth since the millipedes in alder litter had significantly lower total Ca content than millipedes in all the other litters, except the litter mixture.

Alder litter affects millipede growth, even at low concentrations. Alder litter was only 20% by weight of the litter in the litter mixture, yet the dry weight and total Ca content of millipedes in the litter mixture was very close to those values for millipedes in the pure alder litter. Consumption of alder evidently reduced the

digestive efficiency and Ca availability. There were two differences between the groups. First, survival was higher in the litter mixture, perhaps because of the other litters that were present. Second, most (8/9) millipedes in the litter mixture had molted one stadium more than the alder millipedes had. That is why the live weight of the millipedes in the litter mixture was 2.9-fold higher than the live weight of the millipedes in alder, while the dry weight of the millipedes in the litter mixture was only 1.05-fold higher than the live weight of the millipedes in alder. This suggests that the millipedes in the litter mixture were managing to grow, albeit slowly, and might have continued to grow, while it is probable that the millipedes in alder litter would have died if the experiment had been continued.

Litter Ca and millipede growth

Across all litter types, millipede growth was positively correlated with litter Ca content. Although correlation does not require a cause and effect relationship, I think that there may be such a relationship for two reasons: 1) the consistency of the relationship across both deciduous and conifer litter types, and 2) the high Ca requirement of millipedes.

Ca is an essential nutrient for millipedes because their exoskeleton is reinforced with calcium carbonates. The polydesmid millipedes in this study ranged from 10.8 to 17.5% Ca (as % of millipede dry weight) which agrees with the general statement that millipedes (of several orders) are between 9% and 20% Ca by dry weight (Reichle *et al.* 1969, Hopkin and Read 1992).

The Ca content of the tree litters that are potential food resources for *H. haydeniana* in Pacific Northwest forests varies both within a species and between species. Among the conifer species, western hemlock litter is lowest in Ca, Douglas-fir is intermediate and western redcedar is highest. Among the broadleaf deciduous species, alder is generally lower in Ca than bigleaf maple. Ca content can vary considerably within a species as well. For example, the Ca content of

Douglas-fir litter can be as low as 0.3% (Binkley 1995) or as high as 1.7% (Fried *et al.* 1990). The higher values are seen in Douglas-fir trees growing on young soils weathered from basic parent material. Values between 0.7 - 1.0% Ca are typical for Douglas-fir. The Ca content of bigleaf maple litter varies as well; typical values are around 1.2%, but can be as high as 2.6% (Fried *et al.* 1990).

Since most detritivores consume litter only after a period of microbial conditioning, the Ca concentration of the forest floor as a whole may be more relevant than the Ca concentration of new litterfall. The Ca concentration of the forest floor is often slightly lower than the Ca concentration of fresh litterfall (e.g. Fried *et al.* 1990, Fogel and Hunt 1983), but may be higher if the Ca concentration of the litter is extremely low (Binkley 1995).

The Ca content of the forest floor litters used in this study ranged from 0.36% to 1.42% which covers the range of values typical in the Pacific Northwest. Millipedes grew best at Ca concentrations above 0.88%. Litter Ca concentration as low as 0.36% may be adequate for the growth of *H. haydeniana.* However, since less than half as much Ca was assimilated into millipede biomass from the western hemlock litter over the 99 day experiment, maturation of the millipedes growing on low-Ca litter is presumably slowed accordingly.

The N concentrations of the litters in this study were all fairly high (1.11 to 2.83 %N). At these N concentrations, N did not appear to be limiting to millipede growth. In fact, millipede growth decreased with increasing N content, but this effect was mostly driven by the poor growth in the high-N deciduous litters. When only the conifer litters were considered, %N had no effect on millipede growth. For this reason, I believe that the negative relationship between millipede growth and litter N concentration is not a cause and effect relationship, but rather reflects other processes in the litter.

For a given litter Ca and N concentration, growth in the conifer litters was superior to growth in the deciduous litters. This result holds even for the bigleaf

maple litter, which does not have complication of high tannin content that alder litter has. Bigleaf maple litter is probably not a typical diet for juvenile *H. haydeniana*. The juvenile used in this study were extracted from the forest floor under a mixed forest of Douglas-fir, western redcedar and western hemlock. Although the bigleaf maple litter (collected in July) had already undergone a winter and spring of leaching and microbial activity, it may have required more conditioning before being suitable for *H. haydeniana*.

The nature of the microbial community in leaf litter appears to be very important to detritivores. Microbes are important not just as a highly digestable food, but also serve as a source of specific nutrients such as lipids (Anderson and Cargill 1987). The aquatic detritivore *Tipula* requires both fungi and bacteria in its diet, possibly because of the effect of the mixed community in improving the digestibility of the leaf litter or possibly because essential nutrients are supplied by both (Anderson and Cargill 1987). The bigleaf maple litter probably supports a very different microbial community than the conifer litters and it is possible that *H. haydeniana* has become adapted to and dependent on the microbial community in conifer litter, and possibly even specifically to the microbial community in Douglas-fir litter.

In conclusion, *Harpaphe haydeniana* appears to be well adapted to a diet of coniferous litter, particularly if the Ca content is moderate. Growth was reduced at Ca content below about 0.9% (9000 ppm Ca), which means that much of the litter in the Douglas-fir region would support growth in *H. haydeniana*. The positive response to litter Ca is probably direct and can be generalized to other conifer litters, since Ca is a critical nutrient for millipedes. Within the conifer litters, *H. haydeniana* responded strongly to litter Ca content, and not at all to litter N content. This suggests that N is not limiting to *H. haydeniana* in forest floor litter with N contents above 1.1% (C/N ratios around 34 to 40). Overall, the high-N deciduous

litters gave mixed results. The negative response to litter N is mostly due to the poor performance in the deciduous litters and is probably not generalizable.

Since these results were obtained from a study that used several different litter types, it is possible that the relationship between the Ca and N concentration of the litter and millipede growth is circumstantial. This relationship should be tested in a controlled experiment using a single litter source.

4. EFFECT OF MODIFYING THE NUTRIENT CONTENT OF DOUGLAS-FIR LITTER ON MILLIPEDE GROWTH

ABSTRACT

The effect of manipulating the C/N ratio and Ca content of leaf litter on growth of juvenile *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) was investigated by amending Douglas-fir litter with exogenous C, N, and Ca. Increasing either C or Ca doubled millipede growth compared to unamended litter. In contrast, adding N decreased growth. Because *H. haydeniana* is a major detritivore in the Douglas-fir dominated forests of the Pacific Northwest, forest management practices which affect litter chemistry may have the potential to affect the biomass of *H. haydeniana* and therefore litter comminution and nutrient cycling rates.

INTRODUCTION

Millipedes play a significant role in the regulation of plant litter decomposition and nutrient cycling (Anderson *et al.* 1985). As they consume, process and egest litter, the particle size of the litter is reduced, pH increases, and N mineralization is enhanced. The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is common in low- and mid-elevation Pacific Northwest conifer forests from the Pacific Coast to the Cascade Crest, and from southern Oregon to southwest Alaska (Buckett and Gardner 1968).

Detritivores are usually thought to be resource limited; that is, the size of the population is limited by the amount and quality of food available (Swift *et al.* 1979). The lower quality (low N and Ca content, and high lignin and tannin content) of conifer litter relative to broadleaf deciduous litter (Chabot and Hicks 1982) may explain the generally lower biomass of millipedes in conifer forests relative to deciduous forests. However, in a comprehensive survey of the soil fauna of terrestrial ecosystems presented by Petersen and Luxton (1982), the conifer-dominated forests of the Pacific Northwest support a millipede biomass that is higher than the **average** millipede biomass for temperate deciduous forests. The Pacific Northwest forests also had the highest millipede biomass of all the temperate conifer forest sites.

Few studies have been done directly linking millipede growth to specific nutrients. For herbivorous insects, however, N is an important and frequently limiting nutrient in growth and maturation (Scriber and Slansky 1981) and it is likely to be important for millipedes as well. Additionally, Ca is of particular significance to millipedes, because the millipede exoskeleton is reinforced with calcium carbonates. Calcium constitutes 12-20% of millipede dry weight but less than 1.0% of the dry weight of insects (Reichle *et al.* 1969). Finally, high fiber

content in leaves reduces digestibility and decreases protein and mineral availability for herbivorous insects (Mattson and Scriber 1987).

Previous work (Chapter 3, this volume) has shown that *H. haydeniana* grew well on litters with high Ca concentrations (bigleaf maple (*Acer macrophyllum* Pursh) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), with the exception of alder (*Alnus rubra* Bong.). Growth was moderate on the low Ca western hemlock litter (*Tsuga heterophylla* (Raf.) Sarg.). Millipede growth increased with increasing litter Ca concentration and decreased with increasing N concentration. However, correlation does not necessarily require a cause and effect relationship, since litter from these tree species varies in other ways that might affect millipede growth (i.e. lignin/cellulose ratio and phenolic content). The purpose of this experiment was to isolate the effect of the nutrients from other aspects of litter chemistry by manipulating Ca and C/N ratio within a single litter type.

Since Douglas-fir is the dominant tree species in the Pacific Northwest, Douglas-fir litter is an important food source for millipedes. The N and Ca content of Douglas-fir litter varies naturally with soil type and site fertility (Binkley 1995). In addition, Douglas-fir often grows in mixed stands with western redcedar, which increases the Ca concentration of the forest floor, and with alder, which increases the N content of the forest floor (Binkley 1995). Thus, populations of *H. haydeniana* feeding on Douglas-fir litter naturally encounter a range of nutrient contents across the region.

The objective of this study was to examine the effect of litter nutrient content on survival, growth and mineral nutrition of the millipede *H. haydeniana.* In laboratory experiments, juvenile *H. haydeniana* were raised on Douglas-fir litter supplemented with cellulose as a carbon source, protein as an N source, and calcium oxalate as a Ca source in order to determine how nutrient supplementation affected survival, growth and mineral nutrition of the millipedes. If higher litter nutrient content can increase millipede growth in the laboratory, then forests with

more nutrient-rich litter might be able to support larger millipede populations, with implications for nutrient cycling and soil fertility.

MATERIALS AND METHODS

Soil and litter collection

Douglas-fir litter (L, F and H layers) was collected from low elevation (200 m) mature Douglas-fir stands in Benton County, Oregon. Soil (Jory silty clay loam, A horizon: 0 to 10 cm) and forest floor litter were collected from a site in McDonald Forest (managed by the Oregon State University College of Forestry) in November, 1996. When more litter was needed to extend the first experiment and start a second experiment, litter was collected from a second, more accessible site in January, 1997. The second site was about 9 km NE of the first litter collection site and was also in the McDonald Forest and on the same soil type. In each location, the forest floor (L, F and H layers) was gathered, dried (65° C) and sieved through a 2 mm screen to separate needle litter from twigs and other woody debris. The litter was well mixed and stored at room temperature until needed. Because the finer, more decomposed litter tended to separate from the larger needles, the dried and sieved forest floor was further sieved through a 1 mm screen and separated into a 1-2 mm component (mostly L and F layer material) and a < 1mm fraction (H layer). The soil was also oven-dried, mixed, sieved through a 2 mm screen and stored.

Millipede collections and cultures

All *H. haydeniana* millipedes used in the experiments were collected from a mature mixed conifer stand at about 750 m elevation in the Siuslaw National Forest, Benton County, Oregon. The stand is dominated by Douglas-fir, but

contains some western hemlock and western redcedar trees. Millipedes for Trial 1 were collected in October, 1996 and millipedes for Trial 2 were collected in April, 1997. Millipedes were kept at room temperature (ca. 20-22° C) under variable photoperiod in a mass culture in forest floor litter that was gathered at the same location as the millipedes. It was not considered necessary to regulate photoperiod since *H. haydeniana,* like other polydesmid millipedes, have no ocelli and do not appear to respond to light.

Experimental protocols for Trial 1

Experimental design and treatments

In this experiment, the C (available energy), protein and Ca contents of Douglas-fir litter were individually increased in order to examine the effect of these nutrients on growth of juvenile *H. haydeniana*. The form and amount of the nutrient to be added were carefully chosen in order to imitate as closely as possible the biochemical profile of Douglas-fir litter that is naturally high in the nutrient. Some degree of artificiality was unavoidable because it was not possible to incorporate the nutrient into the plant cells, as it would be if the litter was naturally higher in the nutrient. Cellulose (in the form of microcrystalline cellulose) was chosen as the form of energy to add because it is a major form of available C in decomposing leaf litter. This C was expected to be available to the millipedes mostly indirectly through millipede consumption of saprotrophic microflora colonizing the cellulose. It is also possible that some cellulose would be directly assimilated. Calcium oxalate was used as a Ca source because it is a form of Ca found naturally in plant cell walls, and also in the forest floor (Cromack *et al.* 1977; Graustein *et al.* 1977). Finally, casein was chosen as the N source because it is often used as a protein source in artificial diets for insects (Davis 1972).

The rate of nutrient to be added was intended to increase the nutrient content of the Douglas-fir litter up to the nutrient content typical of the deciduous litters which *H. haydeniana* is known to consume and grow well on (Chapters 2 and 3). The following rates were used: C (0.11 g cellulose g^{-1} litter), Ca (as calcium oxalate, 14 mg Ca g^{-1} litter) and N (as casein, at 7.1 mg N g^{-1} litter). Unamended litter was used as a control. A second N treatment using a lower N rate (casein at 0.89 mg N g^{-1} litter) was substituted three weeks after the experiment started because there was very high millipede mortality at the higher N rate (see Table 4.1).

Table 4.1. C/N ratio, nitrogen and calcium content of Douglas-fir litter after amendment with nutritional supplements.

	C/N ratio	N (mg N g^{-1})	Ca (mg Ca g^{-1})
Control (unamended)	29.0	14.6	13.0
Cellulose (45 mg C g^{-1} litter)	33.3	13.4	11.9
Calcium (14 mg Ca g^{-1} litter)	30.6	14.0	25.8
Protein (0.9 mg N g^{-1} litter)	27.6	15.4	12.9
Protein (7.1 mg N g^{-1} litter)	27.1	20.7	12.3

These values were calculated from the average values for litter from site 1 (C/N ratio 31, N 12.9 mg g^{-1}, Ca 14.1 mg g^{-1}) and litter from site 2 (C/N ratio 28, N 16.2 mg g^{-1}, Ca 11.7 mg g^{-1}).

The experimental design was randomized with millipede gender nested within treatment. Twenty replicate microcosms were used for each treatment, except for the lower N treatment (0.89 mg N g^{-1} litter), which had 16 replicates.

Each microcosm had two millipedes: one male and one female. This allowed individual millipedes to be identified and followed through their growth and development.

Millipede selection

Millipedes used in the experiment were selected for uniformity of weight, which was assumed to be an index of condition. First, a large group of field-collected millipedes was sorted by stadium, gender and weight. The 6^{th} stadium millipedes were then sorted into weight classes by 10 mg increment from 30 to 89 mg. Since 80% of the 6^{th} stadium millipedes weighed between 50 and 69 mg, millipedes from those two weight classes were selected for the experiment. From this group of 311 individuals, the 160 needed for the experiment were randomly assigned to the microcosms.

Microcosm construction

Millipedes were raised in microcosms that were intended to be representative of the forest floor environment. Microcosms were made using 15 g forest soil and 15 g forest floor litter in a 233 ml plastic cup with lid (Figure 4.1). Soil was supplied along with litter because *H. haydeniana* naturally consumes soil (N Baumeister personal observation). The forest floor L + F (1-2 mm) and H (< 1 mm) fractions were recombined in the original proportions (10 g L + F and 5 g H) for the first 90 days and 13.5 g L + F layer material and 1.5 g H layer for the second 90 days. A paper punch was used to put two 7 mm diameter airholes, covered with fine mesh (opening < 0.25 mm), in the side of the microcosm.

Nutrient amendments were added to the dry litter/soil in each microcosm and mixed by shaking. The microcosms were inoculated with a filtrate of fresh forest litter to establish a normal microflora and microfauna and allowed to equilibrate for 9 days before the millipedes were added.

Figure 4.1. 6th stadium *H. haydeniana* in a microcosm. Juveniles are light colored through the 7th stadium. The characteristic black and yellow (or orange) coloration is seen only in adults.

Cultural conditions and maintenance

The microcosms were kept at room temperature (approximately 20-22° C) under variable photoperiod. It was not considered necessary to regulate photoperiod since *H. haydeniana,* like other polydesmid millipedes, have no ocelli. Moisture was maintained at 70 ± 15 % gravimetric water content (g moisture g^{-1} dry litter) by weekly watering with distilled water.

After 105 days, the millipedes were moved to fresh litter in a new set of microcosms, along with 5 g of litter from the original microcosm as an inoculum. Millipedes that were molting at that time were moved after they completed the molt (usually 14 days later). Douglas-fir litter collected from the second site (described above) was used. The microcosms were made and treatments applied to that litter in the same way as previously described.

Developmental variables

Because the two millipedes in each microcosm were different genders, data could be recorded for each individual. Survival was first evaluated at day 5. After that, survival, live weight (to the nearest 0.1 mg), molting status and stadium were recorded for each individual at 14 day intervals for a total of 182 days. Dead millipedes were removed from the microcosm and discarded. Millipedes that were in the process of molting were not weighed because previous experience had shown that interrupting the molting process by removing the millipedes from their molting chambers could cause injury or death. After 182 days, surviving millipedes were freeze-dried, weighed and analyzed for C, N and mineral content.

Chemical analyses

The C and N content of the Douglas-fir litter, nutritional amendments and millipedes was analyzed by flash combustion (Carlo-Erba EA 1108). Atomic emission spectroscopy (Thermo Jarrell Ash ICP-AES) was used to analyze the elemental content of the litter, nutrient amendments and millipedes. Since the amendments could modify pH, pH (in water, 5 g litter: 20 ml RO water) was measured on unamended litter and on the microcosms at the end of the experiment.

Data analyses

One-way ANOVA was used to test for the effect of nutrient amendment on millipede performance variables: initial live weight for each stadium, and live and dry weight, relative growth rate, and nitrogen and calcium content at the end of the experiment. Fisher's F-protected least significant difference (LSD) was used to separate and compare treatment means. In order to satisfy the assumption of homogeneity of variance in ANOVA, variables were transformed to ln (Y) or ln (Y + 1) as needed. A Kruskal-Wallis non-parametric test was used when homogeneity of variance could not be attained by transformation. Means and standard deviations or standard errors of untransformed data are presented as specified.

Experimental protocols for Trial 2

Experimental design and treatments

The second experiment was intended to confirm the results of the first experiment. In the second experiment (Experiment 2), the protein effect was further explored by including a second N source (albumen). Casein was added at the same rates as the first experiment (0.9 and 7.1 mg N g^{-1} litter) and albumen was added at four rates (1.1, 2.2, 4.5 and 8.9 mg N g^{-1} litter). An unamended litter treatment was

included as in the first experiment, and a C treatment (0.10 g cellulose g-1 litter) was included as a positive control. The calcium treatment was omitted from this experiment because the calcium treatment in the first experiment did not look interesting at the time this second experiment was designed. The experimental design is a completely randomized design with 2 factors: millipede gender and nutrient treatment. Twenty replicate microcosms were used for each nutrient treatment (8 treatments) and gender (2 genders) combination so the total number of experimental units was 320.

Millipede selection and handling

Millipedes used in the experiment were selected for uniformity of weight, which was assumed to be an index of condition. First, a large group of field-collected millipedes was sorted by stadium, gender and weight. The 6^{th} stadium millipedes were then sorted into weight classes by 10 mg increment. In order to get the 320 millipedes needed for the experiment, millipedes weighing between 50 and 89 mg were selected and randomly assigned to treatments.

Microcosm construction

Microcosms were made using 15 g soil and 15 g forest floor litter in a 175 ml plastic cup with lid (Figure 4.1). The L + F and H fractions were recombined in the appropriate proportions (13.5 g L + F and 1.5 g H). A paper punch was used to put two 7 mm diameter airholes in the side of the microcosm. The airholes were covered with either air-permeable first aid tape or a double layer of fine netting.

Cultural conditions and maintenance

The microcosms were kept at room temperature (approximately 20-22° C) under variable photoperiod. It was not considered necessary to regulate photoperiod

since *H. haydeniana,* like other polydesmid millipedes, have no ocelli and do not appear to respond to light. Moisture was maintained at 70 ±15 % gravimetric water content (g moisture g^{-1} litter) by weekly watering with distilled water.

Developmental variables

Millipedes were first examined at 21 days, and then at 14 day intervals after that. Live weight (to the nearest 0.1 mg), molting status and stadium were recorded for each individual for a total of 180 days. Because mortality was high in this experiment and few millipedes were alive at 180 days, no further analyses (e.g. dry mass and elemental content) were undertaken.

Chemical analyses

Analysis of litter chemistry was performed at the Environmental Protection Agency Environmental Research Laboratory in Corvallis, Oregon. The C and N content of the litters was analyzed by flash combustion (Carlo-Erba EA 1108). Elemental analysis of litters was done by atomic emission spectroscopy (Thermo Jarrell Ash IRIS ICP-AES).

Data analyses

ANOVA (SAS 6.12, SAS Institute, Cary, North Carolina and SYSTAT 9.01, SPSS Inc.) was used to test for the effect of nutrient amendment on millipede initial live weight for each stadium. Fisher's F-protected least significant difference (LSD) was used to separate and compare treatment means. In order to satisfy the assumption of homogeneity of variance in ANOVA, variables were transformed to ln (Y) or ln (Y + 1) as needed. A Kruskal-Wallis non-parametric test was used when homogeneity of variance could not be attained by transformation. Means and

standard deviations or standard errors of untransformed data are presented as specified.

RESULTS

Trial 1

During the 182 day experiment, all millipedes were examined and weighed at 14-day intervals. Thus, live weight, stadium and the duration of the feeding and molting periods during each stadium could be determined. This data was analyzed to gain information about the growth pattern of *H. haydeniana* and the effect of the nutritional supplements on growth and survival.

General growth pattern

Measurements on *H. haydeniana* started during their 6th stadium. Over the course of the experiment, the millipedes developed to the 7th and 8th stadia. Each stadium is divided into a feeding period and a molting period. Initial analysis of the growth pattern of *H. haydeniana* showed that live weight does not increase smoothly during a stadium. Rather, most (about 90%) of the increase in weight occurred while molting (Table 4.2, Figure 4.2).

Thus the initial weight for each stadium was used to compare the effects of nutrient supplements on millipede growth during development, and dry weights were used to compare all individuals at the end of the experiment.

Effect of nutrition on growth and survival

Prior to the beginning of the experiment, the 6th stadium H. haydeniana had been kept in a mass culture. In order to determine if the millipedes had become successfully established in the microcosms, survival was first assessed soon after the millipedes were transferred to the microcosms. At 5 days, millipede mortality in the control, cellulose- and calcium-supplemented litter was 10, 5 and 10% respectively, while mortality in the protein-supplemented litter was 75%. Because of the high mortality, the treatment was not continued. Instead, an additional protein treatment at a lower N rate (0.89 mg N, 16% of the original rate) was added. Early survival in millipedes at the lower protein-supplementation rate was similar to survival in the remaining treatments (Figure 4.3, Table 4.3).

Mortality during the 6th stadium was lowest (13%) in the cellulose-supplemented litter and ranged from 25 to 31% in the other litter treatments. Mortality during the 7th stadium was noticeably higher in millipedes feeding on protein-supplemented litter and 8th instar mortality in the protein-supplemented litter was 100%. Millipedes in the protein-supplemented litter were significantly smaller during the 7th stadium than 7th stadium millipedes in the other diets ($F = 10.53$; $df = 3, 65$; $p < 0.0001$). This trend continued in the 8th stadium. In the protein-supplemented litter, the only two millipedes which developed to the 8th stadium weighed 25% less than 8th stadium millipedes in the cellulose- or calcium-supplemented litter ($F = 2.86$; $df = 3, 43$; $p = 0.038$) and died soon after emerging.

Mortality was also relatively high for 8th stadium millipedes feeding in the unamended litter. Higher mortality may be related to smaller size since in the unamended litter, 8th stadium millipedes which died prematurely were smaller (387 $\pm$ 8 mg, $n = 5$) than millipedes which were still alive at the end of the experiment (438 $\pm$ 14 mg, $n = 8$). At the end of the experiment, all surviving millipedes in the protein-supplemented litter were 7th stadium. In contrast, 42% and 94% of the

millipedes feeding in the unamended litter and cellulose- and calcium-supplemented litter were 8th stadium adults. ($z = 3.41$, $p < 0.0001$).

Dry weight and nutrient content

After 182 days of growth on the nutritionally-supplemented Douglas-fir litter, all surviving millipedes were sacrificed in order to determine the dry weight and mineral content of the millipedes (Table 4.4).

There were no significant differences between male and female millipedes except live weight. Adult (8th stadium) female millipedes in the cellulose- and calcium-supplemented litters weighed 551 ± 19 mg, while adult males weighed 467 ± 11 mg. Although the live weight of adult female millipedes was 18% higher than the live weight for adult males, dry weights for females were only 1.4% higher.

Live and dry weights were highest in millipedes feeding on the cellulose- and calcium-supplemented litters, intermediate in the unamended litter and lowest in the protein-supplemented litter. The average dry weight of millipedes in the cellulose- and calcium-supplemented litters was almost 2-fold higher than in the control litter and 3-fold higher than in the protein-supplemented litter ($F = 37.1$, $df = 3, 55$; $p < 0.0001$). Relative growth rates (RGR) were calculated from the final dry weight and estimated initial dry weight (live weight * 0.203). The relative growth rate (RGR) was highest in the cellulose- and calcium-supplemented litters and lowest in the protein-supplemented litter ($F = 59.58$, $df = 3, 55$; $p < 0.0001$).

Adding additional nitrogen to the litter did not increase the amount of nitrogen assimilated by the millipedes. The nitrogen accumulation rate (mg N accumulated per g millipede dry mass per day) was lowest for millipedes in the protein-supplemented litter and highest in millipedes in the cellulose- or calcium-supplemented litter (Table 4.4; $F = 4.69$, $df = 3, 55$; $p = 0.0055$).

The higher N accumulation rate in the cellulose- and calcium-supplemented litters combined with a higher growth rate in those litters to produce a total body N content (mg/individual) significantly higher in the cellulose-and calcium-supplemented litters than in the unamended litter or the protein-supplemented litter ($F = 27.20$, $df = 3, 55$; $p < 0.0001$).

The N concentration (as percent of dry mass) of the millipedes was relatively unaffected by diet. Although the %N of the millipedes was slightly higher in the protein-supplemented litter (4.92%) and lower in the cellulose-supplemented litter (4.37%), the difference was not significant ($F = 2.11$, $df = 3, 55$; $p = 0.1088$). The C content (as percent of dry mass) of the millipedes showed a reciprocal pattern; C content was lowest in millipedes in the protein-supplemented litter and highest in millipedes in the cellulose-supplemented litter, although the differences were again not statistically significant ($F = 1.08$, $df = 3, 55$; $p = 0.365$). However, because of the reciprocal relationship between millipede C and N content, the C/N ratio of the millipedes was significantly higher in the cellulose-supplemented litter and lower in the control and protein-supplemented litters ($F = 6.80$, $df = 3, 55$; $p < 0.001$).

Adding Ca to the litter increased the availability of Ca to the millipedes (Table 4.4). The Ca accumulation rate (mg Ca accumulated per g millipede dry mass per day) was lowest in the unamended or protein-supplemented litter and highest in the cellulose- or calcium-supplemented litter ($F = 14.57$, $df = 3, 55$; $p < 0.0001$). The high Ca accumulation rate and high growth rate combine to produce a significantly higher total Ca content (25 mg Ca per millipede) in millipedes in the cellulose- and calcium-supplemented litters ($F = 38.77$, $df = 3, 55$; $p < 0.0001$).

Soil and litter chemistry

Although both of the stands from which litter was collected were of similar age and on the same soil type, litter from the second site was higher in N and lower

in Ca). It also had proportionately more needles (L and F layers) and less fine humus than the first litter collection. At the first site, 33% of the collected forest floor was fine enough to pass through a 1 mm sieve. At the second site, only 8% of the forest floor litter collected passed through the 1 mm sieve.

Table 4.2. Trial 1: Weight gain and duration of feeding and molting periods during development of *H. haydeniana* (mean ± SE, n = 52).

Stadium	Initial weight[1] (mg)	End weight[2] (mg)	Feeding (days)	Molting (days)	Change in live weight during feeding period[3]	Change in live weight during molting (%)
6th stadium	59 ± 1[tr]	64 ± 1	27 ± 1[tr]	20 ± 1	5 mg [tr] 0.67 mg d^{-1} 4%	129 mg 6.45 mg d^{-1} 96%
7th stadium	193 ± 4	221 ± 3	42 ± 3	23 ± 1	28 mg 0.67 mg d^{-1} 11%	238 mg 10.35 mg d^{-1} 89%
8th stadium (adult)	459 ± 10	487 ± 12[1]	67 ± 4[tr]	-	28 mg 0.42 mg d^{-1}	-

Values are the average of all millipedes (n = 52) that developed to the 8th stadium and were alive at day 182.
[1] Weight after emergence from molting chambers.

[2] Weight before molting to next stadium.
[3] Change in live weight during feeding period.
[4] Change in live weight during molting period.
[tr] Indicates measures that are truncated because they do not include the beginning of the stadium (for the 6th stadium) or the end of the stadium (for the 8th stadium).

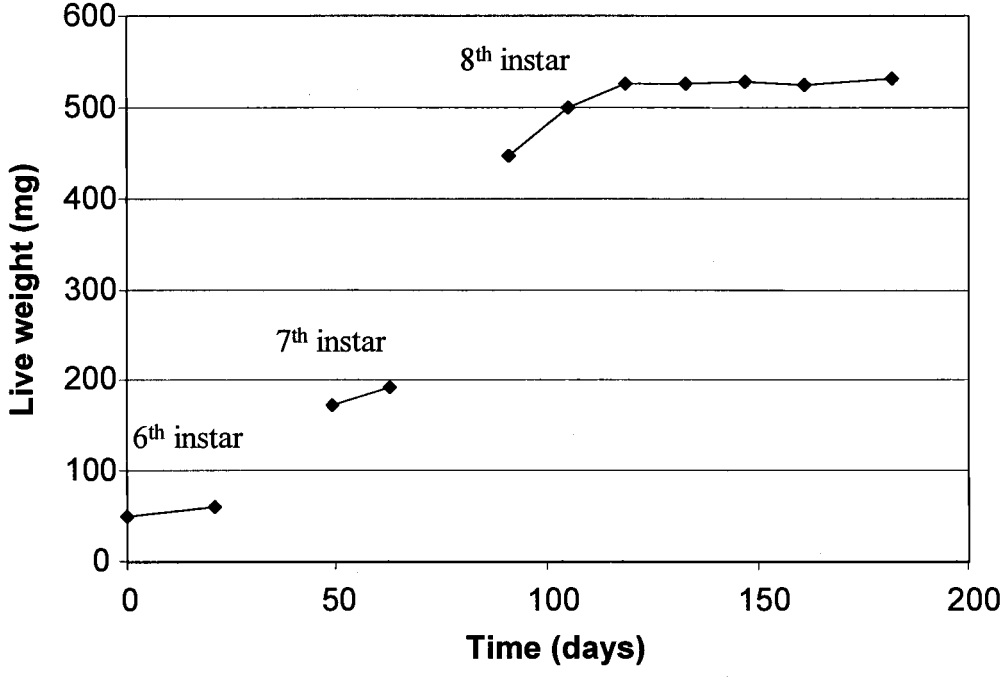

Figure 4.2. Trial 1: Increase in live weight during development of *H. haydeniana*

Table 4.3. Trial 1: Live weight (mean ± SE) and survival during development of *H. haydeniana* raised on nutritionally-supplemented Douglas-fir litter. Numbers within a column followed by the same letter are not significantly different ($\alpha = 0.05$).

	6th stadium[1]		7th stadium			8th stadium	
	Mortality[2]	Completed[3]	Weight[4] (mg)	Mortality[2]	Completed[3]	Weight[4] (mg)	Mortality[2]
Control	0.25	0.75	186 ± 4 a	0.20	0.43	423 ± 11ab	0.39
Cellulose	0.13	0.87	200 ± 5 a	0.18	0.78	467 ± 17 a	0.12
Calcium	0.28	0.72	189 ± 4 a	0.21	0.72	466 ± 16 a	0.00
Protein (0.9 mg N)	0.31	0.69	163 ± 4 b	0.36	0.09	351 ± 8 b	1.00
Protein [5] (7.1 mg N)		-	-		-	-	

[1] At the beginning of the experiment, the 6th stadium millipedes weighed 60±1 mg.

[2] Stadium-specific mortality (fraction of 6th, 7th or 8th stadium millipedes which died during the 6th, 7th or 8th stadium) [3] Fraction of 6th or 7th stadium millipedes which completed the stadium. [4] Initial weight after emergence from molting chambers.

[5] Because of high mortality, this treatment was ended at day 5.

Table 4.4. Trial 1: Growth and nutrient utilization (mean ± SE) of *H. haydeniana* raised on nutritionally-supplemented Douglas-fir litter. Numbers within a column followed by the same letter are not significantly different ($\alpha = 0.05$).

	N	Live weight (mg)	Dry weight (mg)	RGR[1]	NAR[2]	N (%)	C/N ratio	CAR[3]	Ca (%)
Control	19	306 ± 30b	76 ± 6b	7.4 ± 0.2b	0.374 ± 0.011ab	4.63 ± 0.59a	5.2 ± 0.1b	1.33 ± 0.07b	17.3 ± 1.9a
Cellulose	24	497 ± 21a	138 ± 7a	8.8 ± 0.1a	0.402 ± 0.016 a	4.31 ± 0.86a	5.8 ± 0.1a	1.70 ± 0.05a	18.0 ± 1.8ab
Calcium	23	481 ± 23a	135 ± 7a	8.8 ± 0.1a	0.409 ± 0.014 a	4.41 ± 0.48a	5.5 ± 0.1ab	1.71 ± 0.05a	18.7 ± 2.1b
Protein (0.9 mg)	12	193 ± 7c	52 ± 2c	6.0 ± 0.2c	0.333 ± 0.014 b	4.85 ± 0.91a	4.8 ± 0.1b	1.28 ± 0.03b	19.4 ± 1.2c

[1] Relative growth rate (mg gained g^{-1} millipede dry mass d^{-1}).

[2]Nitrogen accumulation rate or Ca accumulation rate (mg N or Ca gained g^{-1} millipede dry mass d^{-1}).

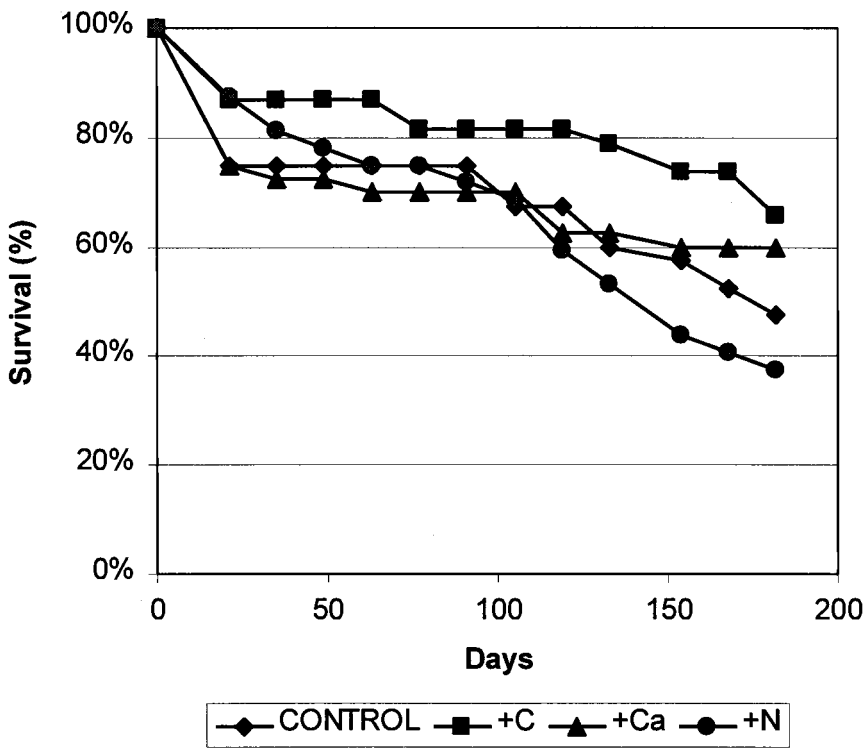

Figure 4.3. Trial 1: Survival of *H. haydeniana* millipedes raised on nutritionally-supplemented Douglas-fir litter.

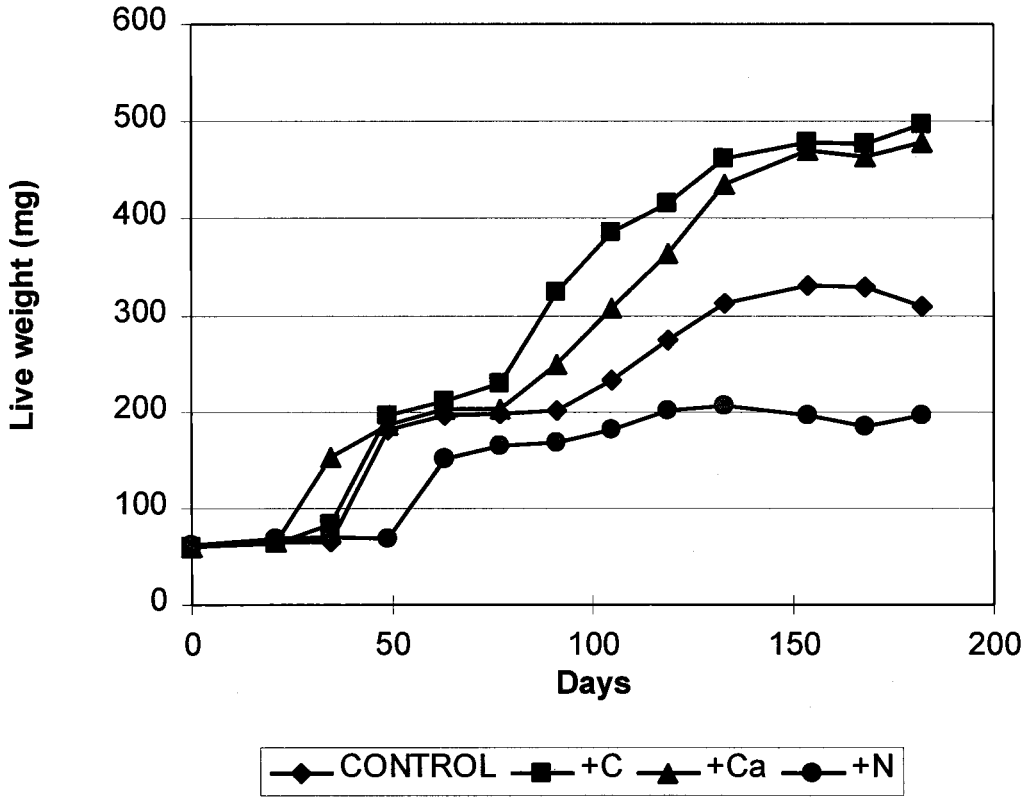

Figure 4.4. Trial 1: Growth of *H. haydeniana* millipedes raised on nutritionally-supplemented Douglas-fir litter.

Trial 2

This experiment was intended to test the repeatability of the negative effect of nitrogen supplementation on millipede growth. The nitrogen treatments from the first experiment were repeated, using the same N supplementation rates. In order to test the possibility that the nitrogen source used in the first experiment was directly toxic, a second N source was added. Also, albumen-N was used in a series of four increasing rates to see if a dose response could be detected. Because the focus of this experiment was nitrogen, the calcium treatment was omitted.

Results were remarkably similar to the first experiment (Table 4.5, Figure 4.5). Growth was best in the cellulose-supplemented litter, intermediate in the unamended litter and decreased with increasing N addition rate. At the highest N rates, no millipedes were able to develop to the next stadium. At the lower N rates, some millipedes were able to molt to the 7^{th} stadium, but were small and died prematurely. The two nitrogen sources had similar effects. Overall mortality was higher in this experiment. After 120 days, only the millipedes in the cellulose-supplemented litter were still alive. Because only millipedes in the cellulose-supplemented litter survived to 180 days, no further chemical analyses were made.

Table 4.5. Trial 2: Live weight (mean ± SE) and survival during development of *H. haydeniana* raised on nutritionally-supplemented Douglas-fir litter. Numbers within a column followed by the same letter are not significantly different ($\alpha = 0.05$). Initial n = 40.

Nutritional supplement	Weight of 6th stadium (mg)	6th stadium mortality[1]	Completed 6th stadium[1]	Weight of 7th stadium[1] (mg)	7th stadium mortality
Control	67 ± 1	0.55	0.45	163 ± 7b	1.00
Cellulose	69 ± 1	0.38	0.62	218 ± 7a	0.44
Casein (0.9 mg N)	70 ± 1	0.68	0.38	153 ± 5b	1.00
Casein (7.1 mg N)	69 ± 1	1.00	0.00	-	-
Albumen (1.1 mg N)	69 ± 1	0.38	0.62	154 ± 5b	1.00
Albumen (2.2 mg N)	69 ± 1	0.85	0.15	128 ± 7c	1.00
Albumen (4.5 mg N)	69 ± 1	1.00	0.00	-	-
Albumen (8.9 mg N)	69 ± 1	1.00	0.00	-	-

[1] Initial weight of 6th stadium millipedes. [2] Stadium-specific mortality (fraction of 6th or 7th stadium millipedes which died during the 6th or 7th stadium). [3] Fraction of 6th stadium millipedes which completed the stadium. [4] Initial weight after emergence.

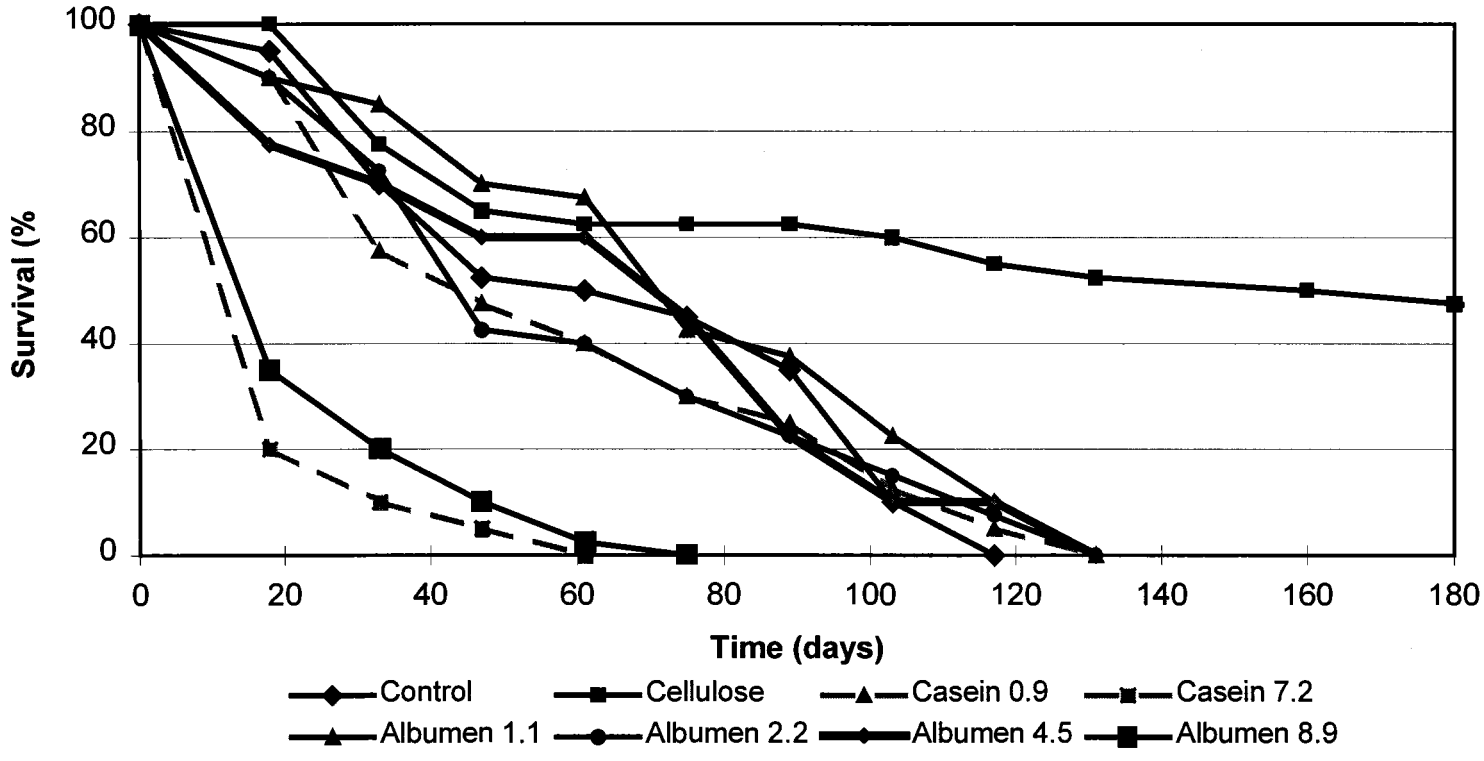

Figure 4.5. Trial 2: Survival of *H. haydeniana* millipedes raised on nutritionally-supplemented Douglas-fir litter.

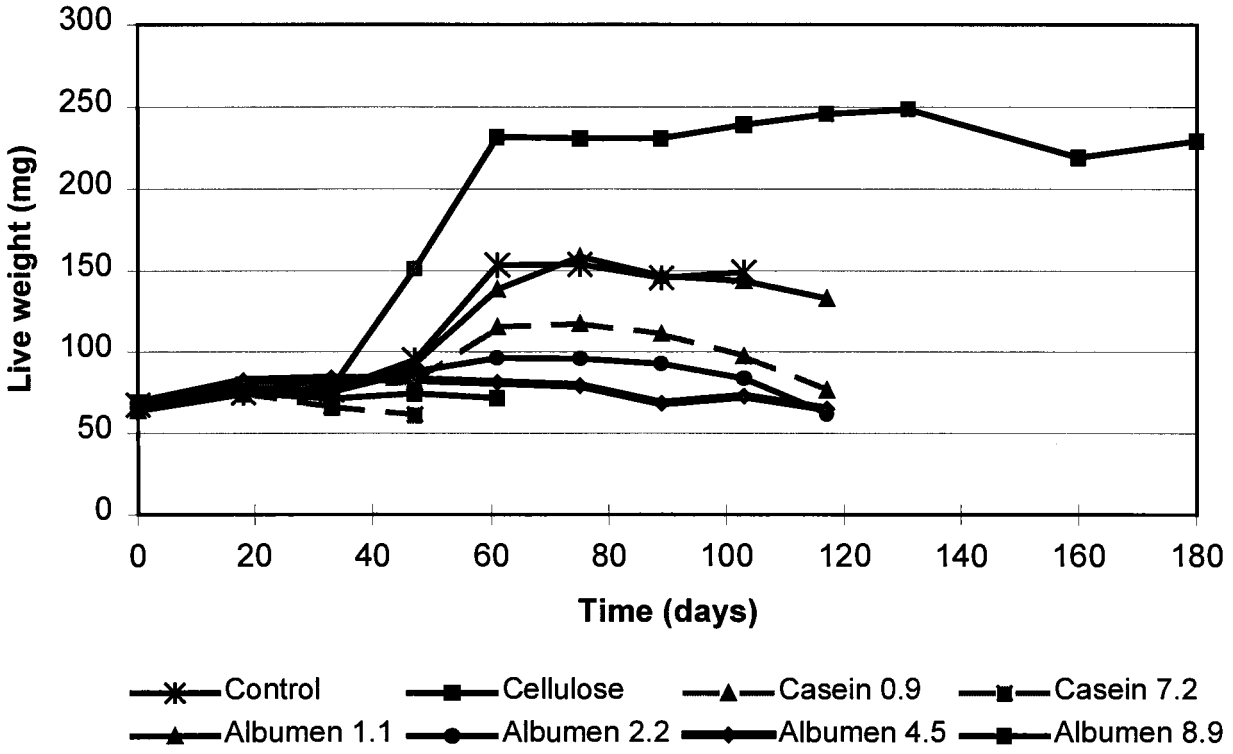

Figure 4.6. Trial 2: Growth of *H. haydeniana* millipedes raised on nutritionally-supplemented Douglas-fir litter.

DISCUSSION

The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is common in low to mid-elevation forests in the Pacific Northwest. In these forests, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees are often the dominant canopy species, and the two common deciduous canopy species bigleaf maple (*Acer macrophyllum* Pursh) and alder (*Alnus rubra* Bong.) exist as isolated individuals or in riparian corridors. Douglas-fir litter has a higher C/N ratio and lower Ca content than maple and alder litter.

Previous work had shown (Chapter 2, this volume) that *H. haydeniana* consumes all three litters. Surprisingly, long-term growth and survival of *H. haydeniana* were higher (Chapter 3, this volume) on the relatively low-nutrient (N and Ca content) Douglas-fir litter than on the higher-nutrient alder or maple litter. Since factors other than nutrients (such as toxic or digestibility-reducing allelochemics) could be affecting millipede growth in the alder and maple litters, I planned in this experiment to separate the effect of nutrient content from other aspects of plant chemistry by working with a single litter type and modifying the potentially important nutrients individually. Douglas-fir litter was the primary focus because as a dominant canopy tree in the Pacific Northwest, Douglas-fir litter is an important potential food source for *H. haydeniana*. Also, the N and Ca content of Douglas-fir litter varies naturally with site fertility and soil type (Binkley 1995), so different populations of *H. haydeniana* could be subject to different nutritional constraints.

Nutritional constraints on detritivores

Surprisingly little is known about the nutritional constraints on millipedes and other detritivores. The microbial biomass in litter is believed to be nutritionally important (Martin 1987, Werner and Dindal 1987) however quantitative evidence is scarce. The millipede *Orthoporus ornatus* (Spirostreptida: Spirostreptidae) produces enzymes capable of breaking down compounds found in both plants and fungi (Nunez and Crawford 1976). The aquatic detritivore *Tipula* gets more than 80% of their C and N directly from litter, and the remainder from microbial biomass, but do not grow well without both the fungal and bacterial components of the microbial biomass. Similarly, leaf litter and fungi alone support growth but not reproduction in caddis flies, because of a lack of carbohydrates required by the animals for lipid synthesis (Anderson and Cargill 1987).

The amount of microbial biomass in litter is controlled by the relative availability of C and N. If either is limiting, adding more of the limiting nutrient will increase microbial biomass. An increase in protein-rich microbial biomass ought to supply more nutrients to *H. haydeniana*, resulting in faster growth.

In this experiment, I modified the availability of C by adding cellulose, a form of carbon which is moderately available to the microbial biomass and is naturally available in quantity in the forest floor. I expected the added C to be immobilized into microbial biomass, based on the availability of N. Since millipede feeding mineralizes N (Anderson *et al.* 1985) the added cellulose would be expected to increase fungal biomass (Entry and Backman 1995).

I added N to the system in an organic form, thus adding both C and N, but I expected the organic N to be rapidly mineralized and then re-immobilized into microbial biomass, based on the availability of C to the microbial biomass and consequent need for N. Casein was chosen as the N source because it is often used in artificial diets for insects (Davis 1972).

Because the millipede exoskeleton is reinforced with calcium carbonate, calcium constitutes 12 to 20% of millipede dry weight (this study, Reichle *et al.* 1969). Since the calcium content is 10 to 20 times higher than the calcium content of insect folivores (Mattson and Scriber 1987), calcium requirements are correspondingly high and there is therefore reason to hypothesize that Ca might be limiting in low-calcium conifer litters such as Douglas-fir.

Response of *H. haydeniana* to changes in nutrient content of litter

In general, *H. haydeniana* responded to differences in the nutrient content of its diet through changes in survival and growth rates.

Addition of N to Douglas-fir litter caused a dose-related response in increased mortality and reduced growth. This result was confirmed by repeating the experiment with a second group of millipedes. The rapid mortality seen at high N rates is most likely due to direct toxicity. Cytopathological changes were seen in the hindgut of millipedes forced to consume an atypical (carnivorous) diet (Schluter 1980). In earlier experiments (Chapter 3, this volume), there was high mortality and poor growth in *H. haydeniana* raised on alder, a high N litter. Alder affected millipede growth even in a mixed litter of only 20% alder. Carcamo *et al.* (2000) reported that a mass culture of adult *H. haydeniana* raised on alder died within two weeks, while other cultures raised on Douglas-fir litter survived. Alder may also contain alkaloids (Harbourne 1993), which could be directly toxic to *H. haydeniana*. The nature of the response to high dietary N is unknown, but I speculate that it could be a result of changes to the gut microflora, since high N content in the diet is also toxic to termites, a group which depends on gut microflora to assist digestion (Waller and La Fage 1987).

RGR (relative growth rate), NAR (nitrogen accumulation rate) and CAR (calcium accumulation rate) were lower for millipedes feeding on the protein-

supplemented litter than in the cellulose- or calcium-supplemented litter. The polyphagous insect *Spodoptera eridania* adjusts its consumption rate and digestive and assimilatory efficiencies to maintain RGR and NAR on diets which vary in protein content, but within a constant protein content, substitution of a lower quality protein reduces RGR and NAR, due to a limiting amino acid (Karowe and Martin 1989).

Could the nutritional additions change the structure of the microbial community in the Douglas-fir litter in a way that affects the amino acid balance? This mechanism for explaining the difference in growth seems unlikely, since RGR and NAR were equally high on two diets (+C and +Ca) where the structure of the microbial community must be quite different.

The four millipede diets separated clearly into two groups with respect to calcium uptake. CAR was high for millipedes feeding on the cellulose- and calcium-supplemented litters and low in millipedes feeding on unamended and protein-supplemented litters. The cellulose used to supplement the litter contained only 52 mg Ca / kg, and so was not a significant source of calcium to the millipedes. Even if all the Ca in the added cellulose was available to the millipede, it would amount to only 0.08 mg of Ca per microcosm. Increased CAR in millipedes feeding in the cellulose-supplemented litter could be due to interaction between the microbial biomass and millipedes. Many fungi accumulate Ca (Cromack *et al.* 1975, 1977) and thus provide a concentrated and highly available source of Ca to soil animals feeding on litter containing fungal biomass.

This experiment illustrates that the components of millipede nutrition will be difficult to separate, primarily because of their interaction with the size and structure of the microbial biomass in litter. For example, when I added C, nitrogen and calcium uptake into millipede biomass increased along with C uptake. Without considering the microbial biomass (i.e. as if *H. haydeniana* were a folivore), the

interpretation would be that the digestable energy content in the litter was limiting growth, with no obvious explanation of why adding Ca would have exactly the same result.

Millipede growth is clearly affected by the nutrient content of their diet. In this study, increasing the N content of the millipede diet reduced growth. For David and Celerier (1997), adding yeast to leaf litter increased growth. Yeast is a high N food (Martin 1987), but also supplies Ca and other nutrients. Kheirallah (1978) explained better growth in terms of protein content and suggested that protein quality may have been a factor as well. In other studies (Striganova and Prishutova 1990, Snider 1984) differences in growth were noted but the nutrient content of the diets was not determined.

There is at this time not enough published information on the response of millipedes to specific nutrients to begin to formulate theories about general patterns, as has been done by Mattson (1980) and Mattson and Scriber (1987) for insect folivores of woody plants. More research is needed to develop this information.

5. STABLE ISOTOPE RATIOS IN DETRITIVORES

ABSTRACT

Carbon and nitrogen stable isotope ratios in animals reflect the stable isotope ratio of their diet with some enrichment, particularly for ^{15}N. Because of this, stable isotope ratios can be used as indicators of trophic level, including fractional changes due to shifts in the assimilation of microbial and plant biomass. In a laboratory study on the nutritional requirements of millipedes (*Harpaphe haydeniana* (Polydesmida: Xystodesmidae), modifying the nutrient content (C, N and Ca) of conifer litter affected millipede growth. Stable isotope ratios suggested that high Ca availability could increase the assimilation efficiency of millipedes for C and N of plant origin. Although the low N content of leaf litter is generally cited as the reason for slow growth rates in detritivores, adding exogenous N did not increase millipede growth, nor were $\delta^{15}N$ values in millipedes consistent with N-limitation.

Additional stable isotope data on millipedes and other soil fauna were gathered in a field study. Three age classes of millipede (*Nearctodesmus insulanus* (Polydesmida: Nearctodesmidae) were found feeding in litter. Stable isotope ratios were significantly different for adults, suggesting that they may have been feeding on a different resource than the juveniles, or that their N balance may differ from the juveniles. Additionally, a correction factor for the bias due to the high $\delta^{13}C$ of calcium carbonate in the millipede exoskeleton was calculated.

INTRODUCTION

Carbon and nitrogen stable isotope ratios can be used to study trophic relationships within ecosystems. Because of the fractionation which occurs during many metabolic reactions, consumers usually become enriched in the heavier isotope relative to their food.

For carbon, the difference between the $\delta^{13}C$ of consumers and the $\delta^{13}C$ of their food (Δ_C) is generally small (Gearing *et al.* 1984). Therefore, where there are large differences between the $\delta^{13}C$ signatures of possible foods, the $\delta^{13}C$ of the consumer can be used to track which food they are eating. For example, the large difference in the $\delta^{13}C$ ratios for C_3 and C_4 plants has been used to determine the relative contribution of those plant groups to herbivore diets (Tieszen and Boutton 1989) and to detect changes in the proportion of corn and alfalfa pollen in ladybird beetle diets (Ostrom *et al.*, 1997). The difference in $\delta^{13}C$ between carbon of terrestrial and marine origin has been used to determine the importance of salmon to bear diets (Jacoby *et al.* 1999).

In soil, the difference in $\delta^{13}C$ signatures between less decomposed plant material (wood and litter) and soil organic matter has been used to rank termites species along a "humification gradient", and also to distinguish between termites which directly consume wood, and those which consume fungi growing on wood (Tayasu *et al.* 1997).

Nitrogen stable isotope ratios have proven to be particularly useful in studying trophic relationships in soil. The magnitude of the enrichment at each trophic level is usually greater for N than for C and $\delta^{15}N$ thus offers more resolution for discerning trophic relationships. Soil food webs are difficult to study directly because of the large number of species, high degree of omnivory, and opacity of soil. Published studies applying stable isotope methods to soil food webs

have produced results which are in general agreement with each other and with a study using direct observation (Gunn and Cherrett 1993). The average Δ_N of 3.4 ‰ (range 1.3 to 5.3 ‰) per trophic level calculated by Minagawa and Wada (1984) has proven to be remarkably robust in soils, at least in studies which included a large number of species (Ponsard and Arditi 2000; Scheu and Falca 2000).

However, while the average Δ_N for detritivores in these two studies was close to 3.4 ‰, Δ_N for individual detritivore species was much more variable, ranging from as low as -1.3 ‰ to a high of 8 ‰. These values could reflect differences in trophic level, but $\delta^{15}N$ is known to increase in starving animals (Gannes *et al.* 1997), or in animals feeding on a low nitrogen diet (Adams and Sterner 2000). For example, Scheu and Falca (2000) separated the detritivores into two trophic levels (primary and secondary saprophage) on the assumption that a $\delta^{15}N$ of 3.4 ‰ separates sequential trophic levels. However, Adams and Sterner (2000) found that within one predator/prey pair, varying the C/N ratio of the prey produced Δ_N values which varied from almost 0 to 6 ‰, values which would be interpreted as representing from 0 to 2 trophic levels. This result is consistent with the observation that the $\delta^{15}N$ of starving (vertebrate) animals often increases (Gannes *et al.* 1997). Earthworms may present an exception as Schmidt *et al.* (1999) found no increase in the $\delta^{15}N$ of fasting earthworms. These results emphasize the need for experimentation on the factors affecting stable isotope ratios at a species level or at least at lower taxonomic levels, as called for by Gannes *et al.* (1997).

Two related studies involving millipedes are reported in this paper. The first is a laboratory study in which *H. haydeniana* millipedes were raised on litter in which the C/N ratio and Ca content had been modified. Stable isotope ratios in the millipedes were analyzed to examine possible mechanisms driving the growth response of millipedes. In the second study, stable isotope ratios in soil fauna

(millipedes, isopods and earthworms) collected from a natural ecosystem were compared.

The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is common in low to mid-elevation forests in the Pacific Northwest. In these forests, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees are often the dominant canopy species, and the two common deciduous canopy species bigleaf maple (*Acer macrophyllum* Pursh) and alder (*Alnus rubra* Bong.) exist as isolated individuals or in riparian corridors. Douglas-fir litter has a higher C/N ratio and lower Ca content than the maple and alder litter. Previous work had shown (Chapter 2, this volume) that *H. haydeniana* consumed all three litters and that long-term growth and survival were highest on the relatively low-nutrient Douglas-fir litter (Chapter 3, this volume). In order to investigate the nutritional requirements of *H. haydeniana* further, the C/N ratio and Ca content of Douglas-fir litter was manipulated in order to examine the effect of these nutrients on growth of juvenile *H. haydeniana*. Increasing the C/N ratio of litter increased millipede growth and survival, while decreasing the C/N ratio resulted in greater mortality and decreased growth (Chapter 4, this volume). Unexpectedly, adding Ca to litter increased millipede growth as much as adding C did. Stable isotope ratios in the millipedes were analyzed to examine possible mechanisms driving the response of millipedes to modification of the nutritional content of their diet.

Riparian stream corridors in the Pacific Northwest have a different plant community than the surrounding coniferous forest. Alder, big-leaf maple and (at lower elevations) oak are common canopy species. Because of the different plant community, they also have a different millipede fauna. While *H. haydeniana* is the dominant millipede in the conifer forested uplands, *Nearctodesmus insulanus* (Chamberlin) is common in riparian forests. As with *H. haydeniana*, adults and juveniles are often present together in litter. One goal of the field study was to

compare the stable isotope ratios of adult and juvenile *N. insulanus* in order to test the idea proposed by Wallwork (1970) that younger millipedes are humus feeders, while adults are more detritivorous (litter feeders). A more general objective was to sample the litter fauna and compare their stable isotope ratios.

MATERIALS AND METHODS

Millipedes fed on nutritionally modified diets

The methods used in the study will be summarized here; for more detail see Chapter 4.

The millipedes were 6th stadium juvenile *H. haydeniana*, collected from a Douglas-fir forest in the Oregon Coast Range. The average initial live weight was 60 mg (dry weight 15.1 mg). Douglas-fir litter used to raise the millipedes was gathered, sieved through a 2-mm screen to separate the needle litter from other forest floor material such as twigs and cones, and re-sieved to separate the finer H layer material (defined as < 1 mm) from the L/F layer material. When the microcosms were made, the H and L/F layers were weighed separately to ensure that each microcosm contained equal amounts of each material. See Table 5.1 for the characteristics of the litter. The stable isotope signature of the soil was not analyzed on the assumption that the soil N and C would not contribute to millipede nutrition.

To increase the C/N ratio of the litter, cellulose was added at a rate of 0.1 g cellulose g^{-1} litter which increased the C/N ratio from 29.0 to 33.3. To decrease the C/N ratio, nitrogen was added at a rate of 0.89 mg N g^{-1} litter (as bovine casein) at which decreased the C/N ratio from 29.0 to 27.6. The Ca content of the litter was increased by the addition of 14 mg Ca g^{-1} litter. For each of the three nutritional

treatments, and an unamended control treatment, twenty replicate microcosms were made with 15 g of forest soil and 15 g of litter.

Millipedes were examined and weighed every two weeks for a period of 6 months. At the end of the experiment, the dry weight, C, N, mineral content, $\delta^{13}C$ and $\delta^{15}N$ of the millipedes was analyzed. Since the $\delta^{13}C$ and $\delta^{15}N$ of the litter amendments differed from the litter $\delta^{13}C$ and $\delta^{15}N$ (Table 5.1), Δ_C and Δ_N was calculated after adjusting the litter δ values for the added amendment δ. Among the three dietary supplements, only the protein supplement contained detectable amounts of N, thus the $\delta^{15}N$ of only the protein-supplemented litter needed to be adjusted in order to calculate Δ_N .

Table 5.1. C, N, C/N ratios, and C and N stable isotope ratios (δ, ‰) in the H and L/F layers of Douglas-fir litter used to raise *H. haydeniana* millipedes.

	C (%)	N (%)	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Litter					
H layer	40.07	1.58	25.4	-27.28	-2.10
L/F layers	52.92	1.55	34.1	-27.50	-2.39
Amendments					
Cellulose	44.55	-	-	-22.98	-
Calcium oxalate	16.67	-	-	-9.58	-
Protein	48.71	14.13	3.45	-24.30	5.06

Trophic structure of litter fauna in riparian litter

In November, 1997, macro-fauna (millipedes, isopods, earthworms, slugs and beetles) were collected by hand-sorting the litter layer in a riparian area along the banks of the Marys River in Corvallis, Benton County, Oregon. The dominant tree species at the site were bigleaf maple (*Acer macrophyllum* Pursh) and white oak (*Quercus garryana*), with an understory of *Rubus* sp.

Litter was collected in a single bulk sample. The litter was approximately 75% bigleaf maple and 25% oak. Bigleaf maple and oak leaves were analyzed separately for C, N, $\delta^{13}C$ and $\delta^{15}N$.

Earthworms, slugs (*Deroceras sp.*), isopods (*Porcellio sp*) and *N. insulanus* millipedes were collected at the site. Most earthworms were a small species tentatively identified as *Lumbricus rubellus* and were found on leaf litter surfaces, rather than on the soil surface. Isopods were not abundant- only 2 *Porcellio* were found. Last instar larvae of *Ellychnia*, a lampyrid beetle which is reported to feed on slugs, were fairly abundant (6 individuals) and were collected and analyzed. *N. insulanus* were present in three life stages: adults (stadium VIII), subadults (stadium VII) and immature (stadium VI), thus of eight stadia, the three oldest were feeding in litter. Stadia in polydesmid millipedes can be definitively identified from the number of segments (Enghoff *et al.*, 1993), since the number of segments added at each molt and the number of segments at sexual maturity is fixed. In the other millipede orders, different numbers of segments may be added at each molt, especially in the later stadia, so other, less definitive methods must be used to determine the stadium.

Isotopic analysis

Individual animals were freeze-dried and ground to a fine powder with a mortar and pestle. Subsamples of 1 mg were weighed into 6 x 4 tin cups for continuous-flow isotope ratio mass spectrometry analysis (CF-IRMS) using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyzer with an ANCA-NT Solid/ Liquid Preparation module. This was operated in the dual-isotope mode, allowing $\delta^{13}C$ and $\delta^{15}N$ to be measured simultaneously on the same sample.

Isotope ratios are expressed in the standard delta (d) notation in parts per thousand: $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/ R_{standard})/ R_{standard} X 1000‰$ where R_{sample} and $R_{standard}$ refer to the 15N/14N or 13C/12C ratio for samples and standards respectively. Atmospheric N was used as the standard for N, and PDB-belemnite was used as the standard for C. Acetanilide was used as an internal standard. An acetanilide sample was run after every 10 experimental samples and a correction for spectrometer drift was made if needed. The standard deviation for the acetanilide standards was 0.12 and 0.07 ‰ (n = 6) for $\delta^{13}C$ and $\delta^{15}N$ respectively.

In order to assess the analytical error involved in subsampling and weighing, 5 repeated subsamples of approximately 0.5 to 1.0 mg were taken from a single individual of two taxa: the isopod *Armadillidium vulgare* and the millipede *N. insulanus*. For the isopod, the average weight of material analyzed was 0.79 mg and the range was 0.53 to 1.2 mg. For the millipede, the average weight of material analyzed was 1.1 mg and the range was 0.76 to 1.8 mg. Thus about 20% of the total weight of each animal was analyzed.

Analytical error (SD) was lower for all measures except $\delta^{13}C$ for the millipede than for the isopod (Table 5.2).

Table 5.2. Analytical error (mean ± SD) for soil fauna.

	C (%)	N (%)	C/N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Millipede	33.1 ± 0.7	5.5 ± 0.1	6.0 ± 0.2	-25.2 ± 0.8	3.1 ± 0.2
Isopod	32.1 ± 4.0	5.1 ± 0.9	6.4 ± 0.6	-26.8 ± 0.8	-2.6 ± 0.6

RESULTS

Stable isotope ratios in millipedes raised on nutritionally modified diets

Millipedes feeding on the cellulose-supplemented litter had significantly higher $\delta^{13}C$ values than millipedes in the other litter treatments (Table 5.3, $F = 11.57$, $df = 3,52$; $p < 0.001$). Since the $\delta^{13}C$ of the litter amendments differed from the litter $\delta^{13}C$, Δ_C was calculated after adjusting the litter $\delta^{13}C$ by amendment $\delta^{13}C$ on the assumption that the added C would mix with the litter C to form a homogeneous pool. How the validity of this assumption affects the interpretation of results will be addressed in the discussion section. Δ_C was highest for the cellulose treatment ($F = 17.57$, $df = 3, 52$; $p < 0.001$) and lowest in millipedes in the calcium-supplemented litter.

Millipedes in the calcium-supplemented litter had the lowest $\delta^{15}N$ values (Table 5.3, $df =$
$3, 52$, $F = 61.88$, $p < 0.0001$). $\delta^{15}N$ for millipedes feeding in the cellulose-supplemented litter was significantly higher than $\delta^{15}N$ for millipedes in the calcium-supplemented litter and the highest $\delta^{15}N$ values were in the protein-supplemented litter and control (unamended) litter. Among the three dietary supplements, only the protein supplement contained detectable amounts of N, thus

the $\delta^{15}N$ of only the protein-supplemented litter needed to be adjusted in order to calculate Δ_N. Results for Δ_N were the same as for $\delta^{15}N$; Δ_N was lowest in millipedes feeding in the calcium-supplemented litter and highest in millipedes feeding on unamended litter.

Δ_C and Δ_N were not correlated with millipede growth. Good growth was associated with low Δ_C and Δ_N in the case of millipedes feeding on calcium-supplemented litter, but with high Δ_C and intermediate Δ_N for millipedes feeding on cellulose-supplemented litter.

Table 5.3. Effects of nutritional supplements on the isotopic content of Douglas-fir litter and the isotopic content and average dry mass of *Harpaphe haydeniana* millipedes raised on the nutritionally-supplemented litter. Numbers within a column followed by the same letter are not significantly different ($\alpha = 0.05$).

	Litter $\delta^{13}C$ (‰)	Litter $\delta^{15}N$ (‰)	Millipede $\delta^{13}C$ (‰)	Millipede $\delta^{15}N$ (‰)	Δ_C (‰)	Δ_N (‰)	Millipede weight (mg)
Control	-27.5	-2.3	-22.2a	0.9a	5.3b	3.2a	80b
Cellulose	-27.1	-2.3	-21.2b	0.3b	5.9a	2.6b	141a
Calcium	-27.2	-2.3	-22.1a	-1.0c	5.0c	1.3c	142a
Protein	-27.4	-1.9	-22.0a	1.1a	5.4ab	3.0a	52c

Riparian soil fauna

The carbon and nitrogen content of the animal taxa varied significantly (Table 5.4, $F = 102.74$ and 156.29 respectively, $df = 3, 31$; $p < 0.0001$). Larvae of

the lampyrid beetle *Ellychnia* had both the highest C (49%) and N (10.2%) content. Earthworms contained more N than the millipedes or isopods. The %C of millipedes, isopods and earthworms ranged from 32 to 36 %C and the means were not significantly different. The C/N ratio of taxa also varied significantly ($F = 25.06$, $df = 3, 31$; $p < 0.0001$). C/N ratio of *Ellychnia* and the earthworm *Lumbricus rubellus* was 4.7 and 4.4 respectively. The isopod *Porcellio* and the millipede *N. insulanus* had higher C/N ratio ratios (6.1 and 6.2).

Table 5.4. Description of trophic level, taxonomic classification, name, number collected, %C, %N and C/N ratios of soil animals.

Trophic level	Classification	Taxon	#	%C	%N	C/N
Predator	Beetle	*Ellychnia*	6	49.0 a	10.2 a	4.7 a
Detritivore	Earthworm	*L. rubellus*	4	34.2 b	7.7 b	4.4 a
Detritivore	Isopod	*Porcellio*	2	34.4 b	5.6 c	6.1 b
Detritivore	Millipede	*N. insulanus*	22	32.6 b	5.3 c	6.2 b

Litter

Bigleaf maple and white oak litter had very similar $\delta^{13}C$ and $\delta^{15}N$ values. $\delta^{13}C$ for bigleaf maple and oak respectively was –27.9 and –28.1 ‰ and $\delta^{15}N$ values were –0.11 and 0.70 ‰, respectively. The estimated relative contribution to annual litterfall (75% bigleaf maple and 25% oak) were used to calculate an average litter $\delta^{13}C$ (–28.0) and $\delta^{15}N$ (0.10).

Trophic structure

$\delta^{13}C$ values for the predator *Ellychnia* were significantly lower than $\delta^{13}C$ of the detritivores, leading to a negative Δ_C, but the $\delta^{15}N$ of *Ellychnia* was 2.5 ‰ higher than the $\delta^{15}N$ of the detritivores (Table 5.5, $F = 17.52$ and 14.96, respectively, $df = 3, 31$; $p < 0.0001$).

Table 5.5. C and N stable isotope ratios for successive trophic levels in a soil food web.

Trophic level	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Δ_C (‰)	Δ_N (‰)
Litter	-28.0	0.1		
Detritivore	-25.4 b	1.2 b	2.6	1.1
Predator	-26.6 a	3.7 a	-1.2	2.5

Detritivores

Fractionation values between detritivores and litter were calculated using a weighted average of the two litter types. Δ_C averaged 2.5 ‰ and the taxa were not significantly different. Δ_N was significantly greater for *N. insulanus* than for the other detritivores.

Table 5.6. Δ_N and Δ_C values for detritivores. Δ values were calculated using an weighted average for leaf litter ($\delta^{13}C$ -28.0 and $\delta^{15}N$ 0.1).

Taxa	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Δ_C (‰)	Δ_N (‰)
Earthworm	-25.4 a	0.8 a	2.6 a	0.7 a
Isopod	-25.5 a	0.8 a	2.5 a	0.7 a
Millipede	-25.3 a	1.9 b	2.7 a	1.8 b
Slug	-24.2	-0.4	3.8	0.5

N. insulanus age groups

The three age groups of *N. insulanus* millipedes were analyzed separately. The %C, %N and C/N ratio of the age groups did not differ significantly (Table 5.7). The $\delta^{15}N$ of the adult millipedes was significantly higher ($F = 4.76$, $df = 2, 19$; $p = 0.02$) than the $\delta^{15}N$ of the younger millipedes. $\delta^{13}C$ of the three age groups were not significantly different ($F = 0.47$, $df = 2, 19$; $p = 0.69$).

Table 5.7. Carbon, nitrogen, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ values for different age *N. insulanus* millipedes. Δ values were calculated using an weighted average for leaf litter ($\delta^{13}C$ -28.0 and $\delta^{15}N$ 0.1).

Stadium	#	%C	%N	C/N	$\delta^{13}C$	$\delta^{15}N$	Δ_C	Δ_N
6^{th}	8	32.4 a	5.3 a	6.2 a	-25.3 a	1.7 b	2.7	1.4
7^{th}	8	32.8 a	5.0 a	6.5 a	-25.4 a	1.6 b	2.6	1.3
8^{th}	6	32.7 a	5.6 a	5.9 a	-25.1 a	2.5 a	2.9	2.2

DISCUSSION

$\delta^{13}C$

In field studies, Δ_C for detritivores is often 5 to 10 times higher than the value which would be expected for a single trophic transfer. Published Δ_C values for the trophic step from plant litter to detritivore are much higher than the expected value of 0.4 ‰ (n = 76, SD = 1.4; Gearing *et al.* 1984) for a single trophic step. These values cover a range from 3.8 ‰ (average of all detritivores sampled at 3 sites on 3 dates, Ponsard and Arditi 2000), to 3.0 ‰ (soil-feeding termites; Tayasu *et al.* 1997), and 2.0 ‰ (earthworms and slugs; Neilson *et al.* 1998). Δ_C is calculated against an average $\delta^{13}C$ value for litter, which is usually sampled at the same time that the fauna samples are taken. However, the spatial and temporal heterogeneity of $\delta^{13}C$ of potential food resources for detritivores is high. Herbaceous litter and herbivore feces are usually depleted relative to canopy tree litter, and fungal structures such as fruiting bodies and rhizomorphs are enriched (Ponsard and Arditi 2000; Tieszen and Boutton 1989). So the $\delta^{13}C$ of the diet of detritivores which are free to seek out a variety of resources may differ from the $\delta^{13}C$ of the leaf litter.

In the laboratory study reported here, *H. haydeniana* millipedes were raised on a restricted diet which ensured that the bulk $\delta^{13}C$ value of the litter does represent the $\delta^{13}C$ of the millipedes diet. Additionally, the length of the experiment and the fact that the millipedes were actively growing (millipede dry weight increased an average of 600% over the 6 month experiment) ensures that the $\delta^{13}C$ of the millipedes reflects their current diet. Under these conditions, Δ_C for *H. haydeniana* millipedes averaged 5.4 ‰, close to the 5.3 ‰ which Ponsard and Arditi (2000) found for a whole detritivore community. However, note that the Δ_C

for individual detritivore taxa in that study ranged from 0.5 to 8 ‰. Do the high and low values represent species feeding on and assimilating C which is depleted or enriched, or do they represent differences in C metabolism between taxa which result in higher or lower Δ_C for that taxa? Both of these effects could be occurring. If the high and low values are due to taxon-specific systematic differences in C metabolism (e.g. methanogenic termites, Tayasu *et al.* 1997) or C storage pools (e.g. calcium carbonate content in avian eggshells, Tieszen and Boutton 1989)), then the Δ_C for each taxon should be relatively consistent over time and space. Variation in Δ_C also may be due to consumption of food resources with $\delta^{13}C$ which differ from the $\delta^{13}C$ of the litter. Ponsard and Arditi (2000), sampling three sites at three dates, found that the $\delta^{13}C$ of taxa was less consistent than $\delta^{15}N$ between sites or dates.

Enrichment of the C stored in calcium carbonate causes a systematic bias. By my calculation from the Ca content (18.3% n = 79, minus 1% to allow for non-carbonate forms of calcium) and C content (24.4% n = 79) of *H. haydeniana* millipedes, 21% of millipede C is stored in the exoskeleton in the form of carbonate. The calcium carbonate in bird eggs is enriched 14.1 ‰ relative to the organic C in eggs (Tieszen and Boutton 1989). If the enrichment factor for millipedes is similar, the inorganic C stored in the millipede exoskeleton would increase the $\delta^{13}C$ of the millipede by 3.0 ‰. While these calculations are based on values derived from adult and sub-adult *H. haydeniana*, they are probably applicable to millipedes in general. Reichle *et al.* (1969) reports that 7 species of millipedes averaged 15% (range 12 to 20%) Ca, while isopods (1 species) and insects (25 species) averaged only 0.8% Ca. It should therefore, be reasonable to adjust millipede $\delta^{13}C$ values downwards to remove this bias. Ponsard and Arditi (2000) noted that the $\delta^{13}C$ of the non-insect detritivores (slugs, isopods and millipedes) was 1.5 ‰ higher than the $\delta^{13}C$ of the insect detritivores. They

hypothesized that the systematic difference was due to the higher inorganic carbon content in the non-insect detritivores. I agree that this hypothesis is plausible, at least for millipedes.

In the three detritivore taxa (millipedes, isopods and earthworms) collected from forest litter, the average Δ_C was 2.6 ‰. There was no difference between the taxa. This is surprising, since the $\delta^{13}C$ of the millipedes should be biased upwards by their inorganic C content. Similarly, there was no difference in the $\delta^{13}C$ of the three age classes of *N. insulanus*. If the carbonate effect proves to be reliable and replicable, then it would be reasonable to adjust millipede $\delta^{13}C$ values. If the Ca content of *N. insulanus* follows the same pattern as *H. haydeniana*, younger millipedes would have lower %Ca than older millipedes. For *H. haydeniana*; adults 18.4 %Ca, n = 54; stadium VI juveniles 13.0 %Ca, n = 56). Based on these numbers, the bias due to inorganic C content would be 1.2 ‰ greater for adults than for juveniles. This suggests that adults could be feeding on a lighter C pool than juveniles.

The single predator collected from this system, *Ellychnia*, was 1.2 ‰ lighter than the average of the detritivores, thus a Δ_C of –1.2. This result is not unexpected. Ponsard and Arditi (2000) also found that the detritivores were slightly heavier than the predators, leading to a negative Δ_C . As noted above, the non-insect detritivores were 1.5 ‰ heavier than the insect detritivores. Removing the non-insect detritivores from their dataset gave a Δ_C for predators of 0.5 ‰. *Ellychnia* is reported to prey on slugs and snails (J. La Bonte, pers. comm.). In this case, the single slug analyzed was surprisingly heavy, about 2.4 ‰ heavier than *Ellychnia*. However, the reason that only one slug was analyzed (of 6 collected) is that they did not grind well in the mortar and pestle. The rest of the slugs were set aside until better methods for grinding could be developed. Therefore, this $\delta^{13}C$ value for slugs may not be very accurate.

$\delta^{15}N$

While the $\delta^{13}C$ of decomposing plant litter and animals feeding on it is controlled more by variation in the relative availability of different biochemical pools of plant and microbial C with differing $\delta^{13}C$ values, the $\delta^{15}N$ of litter and detritivores is controlled by discrimination which occurs during N cycling within the microbial biomass or animal N metabolism. The biochemical N pools within litter have initially similar $\delta^{15}N$ values (Nadelhoffer and Fry 1988). There is little discrimination during mineralization of organic N (Hogberg 1997), but over successive mineralization/ immobilization cycles, the lighter isotope is lost to leaching or plant uptake. Thus, in contrast to $\delta^{13}C$, the $\delta^{15}N$ of the litter, (or more precisely, of the microbial biomass in the litter) clearly increases as it decomposes (Hogberg 1997; Ponsard and Arditi 2000; Scheu and Falca 2000).

The $\delta^{15}N$ of an animal is initially derived from the $\delta^{15}N$ of its diet, but is later modified by fractionation which occurs during internal N metabolism, especially amino acid synthesis. Discrimination against the heavier amine groups by the enzymes responsible for deamination and transamination makes excreted N lighter than retained N (Gannes *et al.* 1997). Therefore, in animals which have sufficient dietary protein with an amino acid profile which fits their needs, consumed amino acids are directly made into body proteins and the $\delta^{15}N$ of the animal will be closer to the $\delta^{15}N$ of the protein in the diet. Protein-starved animals, in contrast, meet more of their amino acid needs by catabolizing body proteins and then synthesizing required amino acids. This effect can have a very large effect on $\delta^{15}N$. Differences in the N content of their food caused Δ_N to vary by 6 ‰ in daphnids feeding on green algae. At the highest N content the $\delta^{15}N$ of the daphnids and the algae were almost identical (small Δ_N) and at the lowest N content Δ_N was almost 6 ‰. A Δ_N of 0 ‰ between two species would normally be interpreted as indicating that they are on the same trophic level, while a difference of 6 ‰ would

be interpreted as indicating that two trophic levels separated them. This experiment points out the need for more species level knowledge of how the N content of the diet affects the $\delta^{15}N$ of the consumer. We don't yet know how generalizable these results are, for example, how they relate to detritivores.

The average value for Δ_N of 3.4 ‰ per trophic level (Minagawa and Wada 1984) has been widely applied to delineate trophic levels in soils (Ponsard and Arditi 2000; Scheu and Falca 2000; Schmidt *et al.* 1999), with results which are broadly consistent with current knowledge of soil fauna.

Δ_N for *H. haydeniana* averaged 2.5 ‰ which is well within range of the expected value for a detritivore. However, modifications of the C/N ratio and Ca content of the litter caused the $\delta^{15}N$ of the millipedes to vary by 1-2 ‰. Small differences in $\delta^{13}C$ or $\delta^{15}N$ may not be meaningful indicators of diet between species or even within a species in a natural environment where there is high spatial heterogeneity. The implications of these differences for millipede nutritional balance will be considered below.

The Δ_N of species broadly classified as detritivores varies over 8 ‰ And variability within taxonomic groups is large. Differences in the $\delta^{15}N$ of 1 to 3 ‰ were interpreted by Scheu and Falca (2000) as indicating fractional shifts in trophic level (i.e., from primary to secondary saprophage; changes in the proportions of plant and microbial N assimilated), but they could also reflect differences in the N sufficiency of the diets (Adams and Sterner 2000), or some taxa could be feeding on resources with $\delta^{15}N$ values different from the $\delta^{15}N$ of the bulk litter. For example, the Δ_N of one of the millipede species in Scheu and Face's study (2000) was –1.3 (*Glomeris marginata)*, while the Δ_N of the other two species was 2.7 and 3.2. In another study involving manipulation of soil food resources, *Glomeris marginata* was the only millipede species not affected by nutrient additions to litter or soil. These two results together suggest that *Glomeris marginata* may have be

feeding on litter from the herb layer (the spring ephemerals would be senescent by the June sampling date).

In the three detritivore taxa (millipedes, isopods and earthworms) collected from forest litter, the average Δ_N of detritivores was 1.1 ‰. In contrast to the $\delta^{13}C$ signature, the $\delta^{15}N$ of the taxa varied significantly. *N. insulanus* millipedes were 1.1 ‰ heavier than earthworms and isopods. Within *N. insulanus*, adults were 0.8 ‰ heavier than juvenile millipedes. Thus, the relative rankings of the taxa from low to high were: earthworms = isopods < juvenile millipedes < adult millipedes. These taxa rank in the same order as those of Scheu and Falca (2000). As in this study, Scheu and Falca (2000) also found that within a species, adult animals (of several earthworm species) were more enriched than younger ones. Ponsard and Arditi (2000) do not present their data by taxon so the relative rankings cannot be compared, but they do report that species rankings were generally consistent over the three sites and 3 sampling dates.

Adult *N. insulanus* had slightly higher $\delta^{15}N$ values than juveniles. This suggests that they may be using different food resources than juveniles, or that their diet is more N deficient. In the Marys River site, the $\delta^{15}N$ of the only predator collected, *Ellychnia* (a lampyrid beetle) was 2.5 ‰ higher than the average $\delta^{15}N$ of the detritivores. *Ellychnia* is reported to prey on slugs and snails (J. La Bonte, pers. comm.), so the millipede, isopod and earthworm detritivores would not be likely prey for Ellychnia. The $\delta^{15}N$ of *Ellychnia* was 4.1 ‰ higher than the $\delta^{15}N$ of the single slug analyzed.

Implications of stable isotope ratios in *H. haydeniana*

The average Δ_C and Δ_N values for *H. haydeniana* were within range of the expected values for a detritivore. However, modifications of the C/N ratio and Ca content of the litter caused the $\delta^{13}C$ and $\delta^{15}N$ of the millipedes to vary by 1-2 ‰.

Small differences in $\delta^{13}C$ or $\delta^{15}N$ may not be meaningful indicators of diet between species or even within a species in a natural environment where there is high spatial heterogeneity. But since these millipedes were raised under identical conditions, it seems valid to consider possible causes for these differences in order to generate hypotheses for future research.

The C and N supplements were expected to become available to the millipedes indirectly, through the microbial biomass, while the Ca supplement was expected to directly increase Ca availability. Cellulose is known to increase fungal biomass in soils (Entry and Backman 1995). And in N-limited soils, adding N increases microbial biomass.

The C and N additions to the Douglas-fir litter created an N availability gradient, with the unamended litter intermediate. Daphnids feeding on low N (high C/N ratio) food grow more slowly and have elevated $\delta^{15}N$, relative to daphnids feeding on higher N food (Adams and Sterner 2000). It is reasonable to suppose that *H. haydeniana* would respond the same way to N limitation. Yet *H. haydeniana* responded in the opposite way; increasing the C/N ratio of the litter greatly increased millipede growth and slightly decreased $\delta^{15}N$.

This result reflects the complexity of the factors affecting the availability of energy and nutrients to millipedes and other detritivores. Litter chemistry, microbial biomass and digestive strategies all interact in determining the availability of nutrients to detritivores. The low N content of leaf litter has been cited as the reason for slow growth in detritivores (Mattson 1980). But the N supply to detritivores may be more determined by the amount of microbial biomass available to them than to a gross measure of the total N content of the litter. David and Celerier (1997) were able to increase the growth rate in *Polydesmus angustus* by adding yeast (microbial biomass) to leaf litter but in a field study, adding

inorganic N to litter increased the microbial biomass but reduced millipede populations (Scheu and Schaefer 1998).

Millipedes grew much better in the calcium-supplemented litter than in the unamended litter. Calcium was added in the form of calcium oxalate. The oxalate would have been decomposed by oxalate-degraders (Cromack *et al.* 1977), releasing the excess Ca^{2+} into the soil solution and increasing pH. Thus, the increased millipede growth in the calcium-supplemented litter could be due to the increased availability of Ca, or to the higher pH. Both $\delta^{13}C$ and $\delta^{15}N$ were lower in millipedes feeding on calcium-supplemented litter than in millipedes feeding on unamended litter. Lower $\delta^{13}C$ and $\delta^{15}N$ are usually interpreted as indicating that the animal assimilated relatively more plant C and N than microbial C and N. Thus, the efficiency with which *H. haydeniana* assimilates plant C and N appears to be affected by Ca availability and/or litter pH.

There are still only a few published studies of stable isotope ratios in soil fauna. These studies have described the trophic structure in forests (Ponsard and Arditi 2000, Scheu and Falca 2000) and agricultural fields (Neilson *et al.* 1998). To date, these have mainly confirmed previously held ideas about the structure of the soil fauna, rather than changing those views. As more studies are published, this empirical data can be used to develop testable hypotheses. For example, the consistency of the species rankings can be evaluated. If the relative rankings prove to be consistent in different ecosystems or at different sampling dates, that would suggest that stable isotope ratios are accurately reflecting trophic level, since the diet of most animals, integrated over time, probably does not vary very much from year to year. On the other hand, if species rankings do vary, then the need for species level research on the physiological factors affecting $\delta^{15}N$ and other stable isotope ratios becomes even more important.

The older literature on soil fauna should not be neglected. There is a large literature from the 1970s and 1980s on the trophic structure of soil fauna, especially the mesofauna. Many studies produced detailed, species level information which could be usefully integrated and compared with data derived from stable isotope methods. For example, how does trophic level of species as determined by stable isotope analysis compare with results of gut or fecal analysis of the proportions of plant or fungal matter consumed by soil animals (e.g. Anderson 1975).

The dual isotope approach is more useful than a single isotope because of the complex diets of soil animals. ^{13}C analysis may have been omitted from some studies because the results for soil fauna have been difficult to interpret. There is a need to know more about the factors which control $\delta^{13}C$ in various animals and in different tissues and why Δ_C is so large for detritivores. Dual isotope analysis may help separate the N sources and regulation of N supply for detritivores.

6. CONCLUSIONS

SUMMARY

The dominant view is that the low N and Ca content of conifer litter limit the biomass of millipedes in temperate conifer forests (Wallwork 1970, Petersen and Luxton 1982, Werner and Dindal 1987). The millipede *Harpaphe haydeniana* Wood (Polydesmida: Xystodesmidae) is common in low to mid-elevation forests in the Pacific Northwest. In these forests, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees are often the dominant canopy species, and the two common deciduous canopy species bigleaf maple (*Acer macrophyllum* Pursh) and alder (*Alnus rubra* Bong.) exist as isolated individuals or in riparian corridors. Douglas-fir litter has a higher C/N ratio and lower Ca content than maple and alder litter. Previous work has shown (Chapter 2, this volume) that *H. haydeniana* consumes all three litters. Surprisingly, long-term growth and survival of *H. haydeniana* were higher (Chapter 3, this volume) on the relatively low-nutrient (N and Ca content) Douglas-fir litter than on the higher-nutrient alder or maple litter. Millipede growth on the very low-nutrient western hemlock litter was slower than on Douglas-fir. While *H. haydeniana* consumes alder, it is apparently toxic to them (Carcamo *et al.* 2000, Chapter 3 this volume). There was high early mortality on maple litter, but good growth in the surviving millipedes.

Since factors other than nutrients (such as toxic or digestibility-reducing allelochemicals) could be affecting millipede growth in the alder and maple litters, I planned in the next experiment to separate the effect of nutrient content from other aspects of plant chemistry by working with a single litter type and modifying the potentially important nutrients individually.

Douglas-fir litter was the primary focus because, as a dominant canopy tree in the Pacific Northwest, Douglas-fir litter is an important potential food source for *H. haydeniana*. Also, the N and Ca content of Douglas-fir litter varies naturally with site fertility and soil type (Binkley 1995), so different populations of *H. haydeniana* could be subject to different nutritional constraints.

Growth in *H. haydeniana* responded positively to additional C and additional Ca and negatively to addition of N. This result suggests that N was not limiting, possibly because *H. haydeniana* may assimilate much of its N from microbial biomass more than from the litter directly. The response to Ca was surprising. Stable isotope analysis suggests that higher Ca availability or higher pH might affect the efficiency with which *H. haydeniana* digests C and/or N from litter.

A few comments on millipede development may be useful for future researchers. Observations specific to *H. haydeniana* are based on my own research and most of the other information is derived from the very useful work by Enghoff *et al.* (1993). Millipedes grow by anamorphosis. In juvenile millipedes, a fixed or variable number of segments are added at every molt. In older millipedes (adult or close to adult), molting may occur without the addition of segments. Immature millipedes are called juveniles, rather than larvae, because there are no special characters (other than the lack of sexual organs) that characterize the younger stadia. Stadia are defined by molts, with or without the addition of segments. The first mobile stage, which has 3 or 4 pair of legs, is designated the first stadium (or stadium I). The convention in the diplopodological literature is to refer to the animal by stadium rather than instar, as e.g. a first stadium juvenile. Roman numerals are often used, but I find them awkward, so I will use a standard numbering e.g. stadium 3, 3rd stadium.

Harpaphe haydeniana, like all polydesmids, has 8 stadia from egg to adulthood. The number of segments added at each molt is fixed and thus an individual animal's stadium can be definitively determined by counting segments. Each segment (= diplosegment) is connected to the next by connective tissue which allows the animal to bend. The decision to molt is probably determined by stretch receptors between the segments (Hopkin and Read 1992). *Harpaphe haydeniana* uses rectal secretions to build a completely sealed protective chamber in which to molt. While molting, the cuticle is softened and expanded and the old exoskeleton is shed, including the calcified external portions. Before the millipede emerges from molting chamber, it eats most of the shed exoskeleton. Eating the shed exoskeleton is a mechanism which conserves Ca.

Molting is a slow process for *H. haydeniana*. This species molts inside a hollow chamber, which the millipede builds from its own frass. This chamber building process is possible because of the semi-liquid nature of *H. haydeniana* frass. As it is deposited in discrete droplets, the moisture in the droplets is wicked away, leaving the solid material behind. Some of the moisture appears to be re-absorbed by the millipede and some by the material that the frass is deposited on. The chambers were sometimes attached the wall of the microcosm, and sometimes free in the litter, usually under the surface. The building process could be observed in the chambers that were built on the surface. First, a pad of frass was deposited on the litter, then the walls were built up, initially forming a bowl and then narrowing so that finally, a round hole just big enough to allow the millipede to enter was left. Finally, the millipede entered the chamber and sealed it from the inside. While inside the molting chamber, the millipede expands, splitting and shedding the old exoskeleton. New segments are added at this time. The old exoskeleton is consumed. After the new exoskeleton hardens, the millipede makes a hole in the chamber wall and emerges from the chamber. Thus, the molting period can be

defined as the length of time that the millipede is sealed inside the molting chamber.

RECOMMENDATIONS FOR FUTURE RESEARCH

There is a pressing need to determine habitat associations (e.g. elevation, temperature, moisture requirements, litter characteristics, soil characteristics) for *H. haydeniana* and to measure population parameters: size, density and age structure. Current knowledge of habitat association is derived from pitfall data. Interpretation of this data is limited because the majority of individuals caught in pitfall traps are adults. Adults are surface-mobile and aggregate seasonally in large numbers to mate and then disperse (N. Baumeister pers. obs). Thus, adults captured in pitfall traps may be only briefly present in a location. Systematic sampling of forest ecosystems for juvenile *H. haydeniana* would be useful to determine the requirements for successful rearing of juveniles.

As forest fertilization and liming may become common practices, it will be important to know how *H. haydeniana* is affected by these practices. The effects may be both direct and short-term (changes in the pH of the forest floor), and long-term through general effects on the litter chemistry and then on the availability of litter C and N and on the microbial biomass.

And finally, in intensively managed systems, native species are often replaced with non-native species which play a similar ecological role. The introduced millipedes and earthworms which are currently found in agro-ecosystems in the PNW may be able to invade managed forests and replace *H. haydeniana*. They would probably have different effects on forest nutrient cycling. There may be an opportunity here to record changes in ecosystem function as they happen.

I hope that this work has excited some interest in *Harpaphe haydeniana* and in the millipede fauna of the Pacific Northwest. The field of millipede ecology is still in its infancy compared to the large literature on earthworm ecology and there are many opportunities to contribute.

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