Leaf Litter Quality and Quantity as a Determinant of Invertebrate Diversity, Order Richness, Density, and Abundance

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Abstract

To better understand how leaf litter amount and composition affect the diversity, richness, density and abundance of invertebrate communities, our study compared samples from differing treatment plots within the University of Michigan Biological Station's Detritus Inputs Removals and Transfers (DIRT) experiment. We hypothesized that due to the effects of leaf litter on invertebrates, there would be a positive correlation between the amounts of leaf litter and invertebrate diversity, richness, density and abundance. We also hypothesized there would be a negative correlation between woody litter and invertebrate diversity, richness, density and abundance due to the limited nutrients available. We sampled control, woody litter, double litter and no litter treated plots from the DIRT experiment using three different sampling techniques to target differing invertebrate communities. The samples were processed, classified and tabulated by order. We found that typically, order richness was greater in the O-horizon samples than the other sample techniques. Invertebrate abundances were generally not related to amounts or compositions of litter of the plots in which they were collected. However, we found a positive correlation between litter volume and order diversity in the leaf litter and O-horizon samples. Litter volume was also positively correlated with abundance of macro invertebrates collected in the pitfall traps. While our interpretations were constrained by a small sample size, we saw trends supporting our hypothesis that invertebrate diversity, richness and abundance increase as litter amounts increase.

Introduction

Invertebrate species living within the leaf litter and the organic soil horizon of the forest floor are crucial to the decomposition of organic material and the restoration of nutrients to the soil, a key process which ensures the health of forests and forest ecosystems. The importance of invertebrate communities to the health of forest ecosystems has become increasingly acknowledged within the field of ecology because they are responsible for the breakdown and decomposition of organic matter and the critical in returning nutrients to the soil (Edward, et al, 1969).

Despite the importance of invertebrate biodiversity to the vitality of forest ecosystems, little research has been done on what conditions promote biodiversity within an invertebrate community. There is very little information to be found, for example, on the leaf litter cover conditions that offer the most hospitable environment for invertebrates. In order to better understand the effect of litter cover types and amounts on invertebrate community biodiversity, our experiment capitalizes on forest-floor conditions already created by a long-term experiment at the University of Michigan Biological Station. The “Detritus Input, Removals and Transfers” (DIRT) experiment has manipulated amounts and forms of organic litter inputs in forest floor plots for the past five years. The goal of the experiment is to quantify the relationships between the annual rates of plant litter inputs (leaf, woody, and root material) and soil organic matter formation and nutrient cycling. The project has focused on soil gas exchange and chemistry but the effects of the litter manipulations on invertebrate communities have not yet been researched.

We are interested in uncovering patterns between type and amount of litter cover and its effect on invertebrate abundance and diversity. We specifically seek to understand whether invertebrates have a preference for a certain amount or type of litter over another and why this might be, as well as how litter cover affects invertebrate diversity and abundance. Study of these factors can lead to a better understanding of the processes that influence biodiversity within floor-dwelling invertebrate communities, important communities that ensure organic matter decomposition and nutrient recycling within forest ecosystems. Previous research has shown correlations between invertebrate biodiversity and nutrient availability in soil and forest floor environments. The stoichiometry of detritivore communities shows that they are extremely nutrient limited (Martinson, et al, 2008). Therefore, we hypothesize that invertebrate abundance and biodiversity is positively correlated with the amount of litter available within habitats. We predict that, since they are limited by nutrient availability, invertebrates will show a preference for—and therefore have a higher abundance and biodiversity in— habitats with increased amounts of litter.

We predict that even though the woody litter plots have the same weight in litter relative to the double litter plots, they will harbor less abundance and biodiversity. The difficulty of breaking down wood fibers will lead to a lower concentration of nutrients, especially nitrogen. This would create in a nutrient-poor habitat for detritivores and their predators. We predict that order richness, organism abundance, Shannon-Weiner diversity, and organism density will be lower in the woody litter plot, as compared to the control and double litter plots.

Materials and Methods

Invertebrate community samples were obtained from four litter treatment plots from DIRT experiment. We sampled 5x5 m plots representing the control, no leaf litter, double leaf litter, and leaf and wood litter input treatments.

Each litter treatment plot is surrounded by a plastic gate and covered by plastic mesh to maintain the integrity of the treatments. Each plot is regularly cleared of vegetation and has no trees inside the boundaries of the gate. Each fall, researchers operating the DIRT experiment collect leaf-fall and distribute it on the plots. The leaves that fall on the plastic mesh are weighed and distributed evenly on the control plot. Double this volume of leaves is distributed over the double litter plot. The woody litter plot receives the same volume of litter as the double litter plot, however half that volume is composed of woodchips. The no litter plot has no inputs, and is covered with a shade cloth to keep mosses from colonizing the bare soil.

Three replicate blocks are established by the DIRT experiment and we took invertebrate samples at each of the four treatments in the blocks, totaling twelve sampling plots. Sample locations within the 5x5m plots were chosen randomly for invertebrate sampling, but with care taken to avoid sampling near permanent instruments and sensors. If a randomly chosen location risked interference with the equipment, another randomly chosen site was sampled instead.

We sampled invertebrate communities using three different methods for three levels of the forest floor: pitfall traps for mobile organisms, O-horizon soil blocks, and leaf litter samples. We placed a pitfall trap in each of the three control, no litter, double litter, and woody plots. We took nine leaf litter samples and twelve O-horizon brownies. Leaf litter

samples were taken from one 20 cm x 40 cm quadrant within each of the 3 replicate plots per treatment (except the no litter treatment plots). O-horizon soil samples were taken in 14x14 cm “brownies” of soil depths of 1.5 to 4 cm, allowing for a volumetric invertebrate density calculation.

Pitfall traps were used to assess the mobile and predatory invertebrate community within each of the plots. Traps consisted of plastic cups (11cm diameter), four 45 cm x 6 cm plastic rectangles that served as directional gates, and a lid to prevent leaf build-up within the traps (Figure 1). The plastic cups were buried in the ground so that the lips of the cups were even with the soil, and the four plastic gates were trenched at right angles to the circumference to guide invertebrates into the cups. Propylene glycol (approx 15ml) was poured in the bottom of each cup to capture and preserve invertebrates. Traps were left in the plots for 26 hours.

We used Berlese funnels to separate invertebrates from the soil and leaf litter samples. Samples were placed within the funnels and a lamp was shone directly over the top. Jars filled with propylene glycol were placed underneath the mouth of the funnel. Over the course of 72 hours, invertebrates seeking to escape the increased light and heat traveled further down the sample material, were filtered through mesh screening, and fell through the funnel into the jar of propylene glycol.

The preserved invertebrate samples were filtered into Petri dishes filled with ethanol. The invertebrates were then observed using a dissection microscope, counted and collected with tweezers and classified to order. Individual invertebrates that were too small to count and handle were approximated by taking three random samples of a known area. The species were identified, counted, and the average abundance of the three sub-samples was taken.

Because subsamples were taken from 56.75 cm^2 Petri dishes, we multiplied subsample numbers by 75.25 to estimate total abundances per sample.

To calculate richness among the invertebrate communities sampled we used a modified version of the Shannon Weiner Diversity Index, replacing the typically used species frequency variable with a variable of taxonomic order frequency. We tested mean abundances, order richness, and Shannon-Weiner diversity using ANOVA to test for differences between the four treatments and three layers of the forest floor. Regression analyses were also used to test for correlations between litter densities, amounts of carbon and nitrogen versus the measures of abundance and diversity stated above.

Results

Eighteen different orders of macro invertebrates and eleven orders of micro invertebrates were identified within the samples. For a summary of the organisms and their abundances within each treatment, see Table 1.

Shannon-Weiner diversity indices (Shannon, 1997) were calculated for each of the samples. The diversity indices were compared across treatments as well as forest floor layer (mobile organisms, O-horizon, and litter). There was a significant difference in litter layer diversity between the control and double litter treatments ($p = .05$). The double litter treatment also had the highest mean O-horizon and litter layer diversity (Figure 2). There was no difference in diversity of the O-horizon layer between the control and double litter treatments ($p = .827$). However, both the control and double litter treatments showed significant differences from the no litter treatment in terms of O-horizon layer diversity ($p = .05$, $p = .05$).

Regression analysis showed a positive correlation (r^2=.478, p=.039) between density of leaf litter and invertebrate diversity in the litter layer (Fig. 3). There was also a positive correlation between density of leaf litter and invertebrate diversity in the O-horizon (r^2= .479, p=.013). There was no relationship between the diversity of mobile organisms and the density of litter.

No significant differences in order richness means between treatments and forest floor layers were found. In the litter layer, there was little correlation between macro and micro invertebrate richness and leaf litter density (r^2= .197, p= .232 and r^2= .147, p=.309) for all treatments. However, in the O-horizon, macro invertebrate order richness showed significant positive correlation to density of leaf litter (r^2= .426, p=.021). We found no correlation for micro invertebrates ($r^{2=}$.049, p=.487). There was little correlation between mobile invertebrate order richness and density of leaf litter (r^2= .195).

Organism density was calculated for the leaf litter in terms of organisms per cm^2, and in the O-horizon in terms of organisms per cm^3. There were no statistically significant differences in density of invertebrates amongst treatments and layers. Regression analysis showed no relationship between macro and micro invertebrate densities in leaf litter and density of the litter (r^2=.04, p=.607 and r^2= .082, p=.455). Results for the O-horizon showed a positive correlation between micro invertebrate densities (r^2= .437, p=.019) and litter density, but no similar correlation for macro invertebrates (r^2=.031, p=.585). A strong positive correlation was found between the overall abundance of mobile macro organisms and the density of leaf litter ($r^{2=}$.648, p=.002, see Fig. 4).

Hymenoptera (ants and wingless wasps) had the highest mean abundances of macro invertebrates in the samples. Mean abundances of hymenoptera were compared across

treatments with independent samples t-tests, with a significant difference found between double and woody litter treatments (p= .046). However, there were no significant differences between hymenoptera abundances amongst the rest of treatments. Acari (mites) were the most common micro invertebrate identified in the samples. Mean Acari abundances were sampled across treatments, with no significant differences found.

Discussion

Previous studies (Poser, 1990; Sayer, 2006) have not shown pronounced increases in diversity of soil fauna with litter addition treatments. However, our results did show evidence of a significant increase in diversity in plots with more litter. The double litter plots had the highest mean species richness (Fig. 5) and differed significantly from the control plot in terms of diversity. Regression analysis correlating density of litter and diversity also supports the hypothesis that diversity was greater in a higher-density litter environment. This trend could be because of higher nutrient availability to detritivores and more habitat niche space for different species. The no litter treatment plots had the lowest mean species richness (Fig. 5), and were significantly less diverse than either the control or double litter plots. This may be due to the lack of nutrients and habitat space in an environment with no detritus inputs.

We may not have been able to support this conclusion since nitrogen and carbon abundances were taken in 2005, just one year after the various litter treatments started. Current analysis of carbon and nitrogen would most likely show distinct differences in nutrient levels amongst the various litter treatments. Further analysis could be done in the

plots to discover whether nutrients are higher in the double litter plots and whether this correlates with increased species richness, diversity, or density.

Previous studies have shown that litter additions do not produce a pronounced change in soil fauna abundance (Poser, 1990). Similarly, our study showed density and abundance of organisms did not seem to be affected by the amount of litter. Perhaps increased litter creates more niche space for a more diverse community (Uetz, 1979), but nutrient availability, predation, and competition still limits the amount of organisms that can live in a given area. Further study would be useful to attempt to replicate this pattern of increasing diversity but not abundance, perhaps with more trials and replicates.

Isolating the limiting factors in both population growth and species richness may be an interesting topic of future research. Does richness continue to increase linearly with increased litter or is a certain equilibrium reached at an optimal level of litter? Understanding this relationship could help us to understand the optimal conditions for detritivore community biodiversity and success.

Increased litter did appear to correlate strongly to an increased abundance of predatory (mobile) macro invertebrates. The increased volume of litter may have created better foraging conditions for predatory invertebrates, which could in turn be a limiting factor for abundance of micro invertebrates within the litter layer.

Contrary to our hypothesis, there was little evidence of significant differences in species richness or diversity between the regular and woody litter. The mixed litter could, in fact, provide a more diverse habitat that fosters different organisms—including those that may prefer a woody habitat, such as Isoptera (termites). The significant differences between regular and woody litter in hymenoptera could show that different species have

different preferences for amount of wood in the litter. Also, past research has shown that acidity may be a more salient determinant of invertebrate habitat than wood composition of litter (Price, 1997). Further experimentation should look for trends of pH and invertebrate densities within the plots.

There did appear to differences in diversity between the layers of the forest floor. The O-horizon appeared to have greater diversity of organisms that either the litter or the mobile communities. This could be due to the higher levels of nutrients and decreased predation within the top layer of the soil. A comprehensive study of the O-horizon could compare nutrient levels of the different treatments and look for correlations with richness, density, and diversity.

Overall, our study showed increased diversity with increased litter, while abundance remained constant across treatments. Order richness showed no distinct trends between treatments. Mobile organisms were more abundant with greater litter. Future study should focus on the patterns of individual species preferences or correlations between PH and nutrient content and invertebrate diversity and abundance.

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Tables and Figures

(Table 1) Summary of invertebrate orders and abundances by treatment

Control Treatment					
Litter Sample		O Horizon Sample		Pitfall Trap	
Order	#	Order	#	Order	#
Collembolla	28.08	Annelida	6	Hymenoptera	18
Hymenoptera	190	Hymenoptera	4	Araneida	1
Acari	457.32	Collembolla	48.16	Gastropoda	3
Pseudoscorpion	1	Araneida	48.16	Diptera	2
Coleoptera	3	Acari	72.24	Collembola	2
Dermaptera	5	Archeognatha	50.06		
Opilione	25.08	Pseudoscorpion	25.08		
		Isoptera	24.08		

Woody Litter Treatment					
Litter Sample		O Horizon Sample		Pitfall Trap	
Order	#	Order	#	Order	#
Diptera	6	Annelida	4	Gastropoda	2
Hymenoptera	10	Archeognatha	9	Hymenoptera	20
Araneida	48.16	Araneida	96.32	Araneida	5
Pseudoscorpion	24.08	Pseudoscorpion	24.08	Annelida	1
Coleoptera	2	Collembolla	24.08	Diplopeda	3
Annelida	2	Isoptera	168.56	Diptera	15
Chilopoda	1	Acari	361.2	Hemiptera	3
Archeognatha	1	Dermaptera	48.16	Coleoptera	1
Collembolla	1	Coleoptera	1		
Acari	313.04	Hymenoptera	1		
		Diptera	2		

Double Litter Treatment					
Litter Sample		O Horizon Sample		Pitfall Trap	
Order	#	Order	#	Order	#
Hymenoptera	102	Acari	264.88	Hymenoptera	74
Psuedoscorpion	2	Annelida	190.56	Araneida	10
Araneida	3	Araneida	72.24	Diplopeda	2
Coleoptera	5	Archeognatha	134.4	Coleoptera	7
Hemiptera	27.08	Coleoptera	1	Annelida	1
Annelida	24.08	Collembolla	97.32	Collembola	6
Protura	48.16	Dermaptera	48.16	Chilopoda	6
Acari	122.4	Heptoa	1	Diptera	2

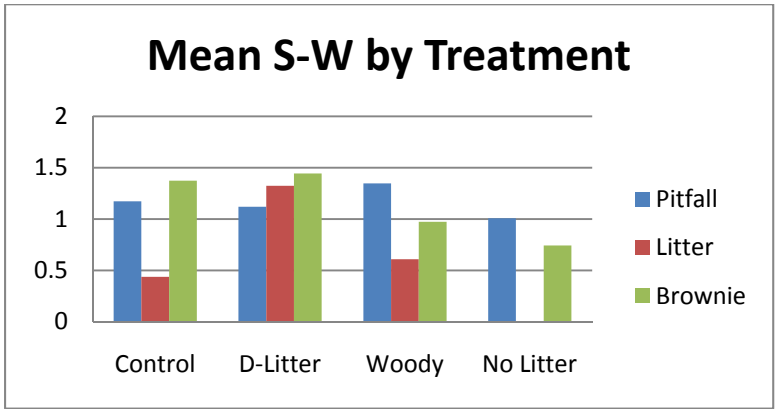
Collembolla	49.16	Hymenoptera	7	Gastropoda	1
Diplopeda	1	Isoptera	1		
Isopoda	1	Pseudoscorpion	51.16		
Archeognatha	1				

No Litter Treatment					
Litter Sample		O Horizon Sample		Pitfall Trap	
Order	#	Order	#	Order	#
		Annelida	7	Araneida	3
		Hymenoptera	4	Diptera	3
		Protura	722.4	Hymenoptera	2
		Opilione	96.32	Isoptera	1
		Acari	963.2	Coleoptera	1
		Archeognatha	75.24	Diplopeda	1
		Diptera	3	Aranelida	1
		Collembolla	24.08	Coleoptera	1
				Protura	6634.958333
				Acari	24.08

(Figure 1)

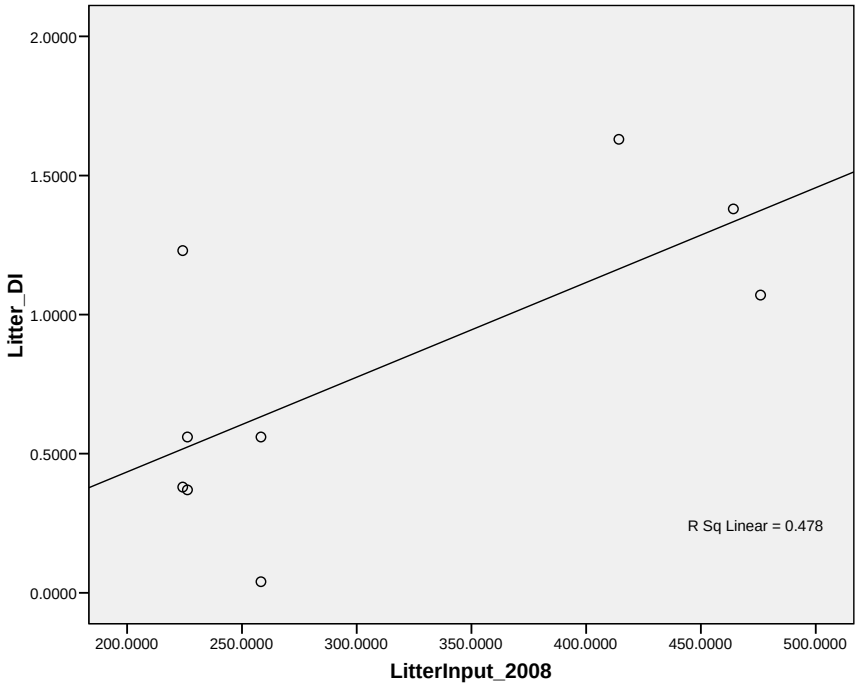
An example of placing the pit fall trap, used to sample mobile predatory invertebrates, within the sample plot.

(Figure 2) Differences in Shannon-Weiner Diversity by Treatment



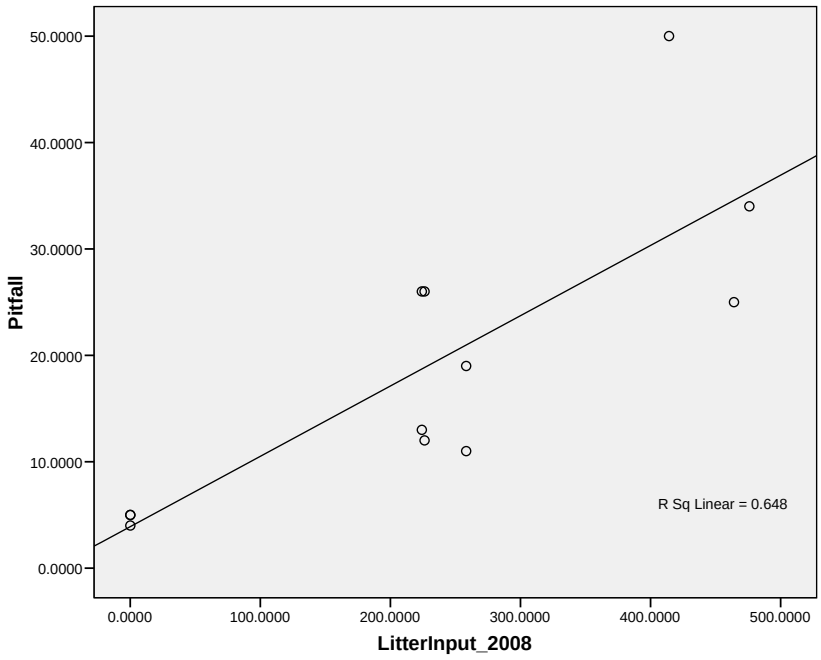
Double litter has highest overall mean diversity.

(Figure 3) Density of Leaf Litter vs. Shannon-Weiner Diversity in Litter



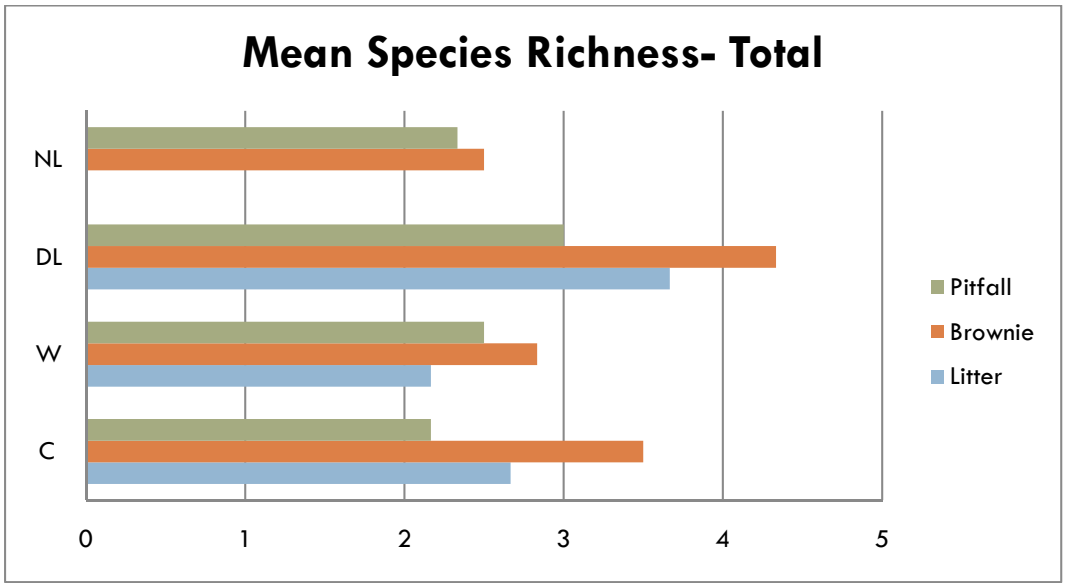
Increased litter inputs correlate to greater diversity within the litter ($r^2 = .478$, $p = .039$)

(Figure 4) Density of Leaf Litter vs. Mobile Macro Invertebrate Abundance



Increased litter inputs correlate with greater abundance of mobile macroinvertebrates (r^2 = .648, p= .002)

(Figure 5) Mean species richness by treatment



Double litter has the highest total mean species richness, no litter has the lowest.