THE EFFECTS OF LITTER QUANTITY AND QUALITY ON SOIL NUTRIENTS AND LITTER INVERTEBRATES IN TWO CONTRASTING FORESTS OF SOUTHWESTERN CHINA

by

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ABSTRACT

Yunnan Province in southwestern China is a highly biodiverse region in the world, yet a large part of the forests are cut down every year to accommodate plantations of rubber, tea and eucalyptus. Within the small areas of protected habitats, recent observations indicate that there is a lack of regeneration of seedlings. One possible explanation for this lack of regeneration could be due to litter dynamics. We designed and carried out a short term litter decomposition experiment to test the direct effects of litter manipulations on soil nutrients and litter invertebrates in a lowland and a montane tropical forest.

Our experimental design involved testing both litter quantity and litter quality. To test for the effects of litter quantity, we manipulated different depths of mixed litter, with and without periodic topping up. To test for the effects of litter quality, we compared single species of litter versus mixed litter control plots. The response of soil nutrients to these manipulations were monitored using ion exchange membranes. We show a clear difference in the available soil nutrients between the two sites, and while the litter quantity experiment had an effect on soil nutrients at the lowland site, it had no effect at the montane site. The litter quality treatments produced no overall difference in responses at either site.

The response of litter invertebrates to litter manipulations was estimated using pitfall traps. We show that, litter invertebrate composition between the two forests is different. Invertebrate abundance does increase with increasing biomass at the montane site, although there seems to be a threshold of litter volume beyond which there is an effect. In the lowland site, invertebrate abundance is highest in the litter removal plots, although a general increase with litter biomass was detected. Litter species does not have an effect on either invertebrate abundance or richness at either site.

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CHAPTER 1

Introduction

Southwestern China is a biodiversity hotspot containing about 20,000 species of higher plants (6% of the world's total). The biodiversity of Asian evergreen broad-leaved forests is under threat from loss of habitat due to logging, and the planting of economic plants. Approximately 15-20% of higher plant varieties are endangered, threatening the existence of 40,000 species of organisms related with them (Yang *et al.* 2004). Trees are the dominant species in this system and trees provide a major structural component of these diverse forests, yet seedlings are rarely observed (Su and Zhang 2002). It has been variously argued that the lack of seed germination and seedling regeneration in general may be due to predation by small mammals or birds (Wenny 2000), dispersal limitation (Dalling *et al.* 1998), attack by weevils (Espelta *et al.* 2009), or environmental conditions caused by litter (Facelli 1994). Thus, an understanding of the factors that influence the germination and establishment of seedlings in these forests is critical to their maintenance. In this thesis I will focus on the effects of plant litter on the forest soils.

Plant litter is dead plant material, such as leaves and twigs that have fallen on the ground. This detritus or dead organic material and their nutrients are added to the top layer of soil. This organic layer is then decomposed and released as inorganic soil nutrients. The study of plant litter has received much attention from ecologists because it is an integral factor in ecosystem dynamics, is indicative of productivity, and influences nutrient cycling and soil fertility. Litter decomposition and nutrient mineralization may provide up to 70-90% of the total

nutrient requirements of trees in forests (Waring and Schlesinger 1985), and is the main pathway for the return of nutrients from plants to the soil.

Plant litter is an integral part of the nutrient cycling process and is an indicator of an ecosystem's productivity and stability. Leaf senescence and fall is a major component of litter, and the organic compounds of the litter are physically and chemically broken down by detritivores and decomposers into inorganic nutrients that plants are able to uptake. The rate at which the turnover of nutrients occur, as well as litter production and litterfall, are measures of the primary productivity of an ecosystem. Plant litter also acts as a protective layer for the forest floor, acting as a barrier to minimize soil erosion and nutrient leaching. It also serves as a habitat for communities of organisms such as decomposers, herbivores, and even small mammals, and may be an effective barrier preventing seed germination and seedling establishment.

The depth and persistence of the litter layer varies with tree species and with rates of litter production and decomposition (Facelli and Pickett 1991). A number of studies have demonstrated that the depth of the litter layer determines the differential establishment of some species (Goldberg and Werner 1983, Gross 1984, Beatty and Sholes 1988, Collins and Pickett 1988). Litter decomposition rates are mediated by the decomposer community and the quality of the litter and may vary considerably between different forest types (Burghouts *et al.* 1992). The species of litter can affect and alter decomposition rates and nutrients released, and this has been shown in both temperate (Hobbie *et al.* 2006) and tropical (Loranger *et al.* 2002, Sundarapandian and Swamy 1999) forest environments. Litter depth and quality have also been

shown to affect insect herbivore richness and abundance (Barajas-Guzman and Alvarez-Sanchez 2003, Wardle *et al.* 2006).

Study Site Background

The research described in this thesis was conducted at two highly biodiverse sites located approximately 500 km apart in Yunnan province in southwestern China -Xishuangbanna Tropical Botanical Garden (XTBG) at 550 m elevation, and Ailaoshan Nature Reserve (ANR) at 2200 m above sea level. The two sites have very different abiotic conditions thus the forest at Xishuangbanna is categorized as a tropical lowland forest, while the forest at Ailaoshan is a tropical montane forest.

Nutrient dynamics can vary between forests of different elevation, with net primary productivity (NPP) greater in warmer forests at lower elevation than tropical montane forests (Vitousek 1984, and Tanner *et al.* 1998). The difference in productivity can influence which nutrient may be mostly limiting plant growth. At Xishuangbanna, like most tropical lowland forests, there is rapid turnover of litter and nitrogen is rapidly cycled and available for plant uptake, but the main limiting nutrient for plant growth is phosphorus (Tanner *et al.* 1998). In tropical montane forests such as Ailaoshan, soils may be younger and phosphorus is still being mobilized by weathering, and combined with the low NPP where turnover rates are slower, plant growth is more limited by nitrogen (Tanner *et al.* 1998).

Thesis Overview

The overall objective of my research was to manipulate litter to test if differences in litter biomass and quality would influence soil nutrients and invertebrates in these diverse forests ecosystems. In tropical forests, seeds lying on the soil surface are covered by litter, which alters the microenvironment potentially influencing germination and establishment (Molofsky and Augspurger 1992, Facelli 1994). Litter may act as a physical barrier to the emergence of seedlings. Indeed, Grime (1979) argues that litter production is a method of plant competition whereby an individual, by producing litter, prevents the establishment of seedlings by decreasing habitat space and shading out the seedlings. However, litter may in some cases facilitate seedling establishment by providing the nutrients required for establishment, but there are few experimental tests of this hypothesis (Quested and Erikkson 2006). In general then, there are direct and indirect interactions of litter quality and litter depth on the decomposer community and soil nutrient levels in a way that may impact seed germination and seedling establishment.

The quantity of litter on the forest floor may influence the availability of soil nutrients and the composition of the litter invertebrate community. Increasing litter biomass should result in greater availability of soil nutrients, and should also lead to an increase in invertebrate abundance by providing a more structurally complex habitat for many different trophic levels of invertebrates, both decomposers and detritivores.

The quality of litter refers to the nutrient content and decomposability of litter and can vary between different species of trees. Thus, litter species manipulations should have an effect

on soil nutrients, as well as attracting different invertebrate communities, influencing species richness of litter invertebrates.

Consequently, I tested the following predictions:

- The removal of litter will result in a decline in nutrient availability to plant roots in the soil, and a decrease in soil invertebrates.
- 2. The addition of litter will result in an increase in nutrient availability to plant roots in the soil, and an increase in abundance and diversity of invertebrates
- Litter comprised of a mixture of species will provide more favourable conditions for decomposers, and lead to faster decomposition, and higher levels of soil nutrients compared to single species litter.
- 4. Litter mixtures will attract a more diverse range of invertebrates (decomposers and detritivores) than single species litter, and lead to a high richness of invertebrates.

CHAPTER 2

The Effects of Litter Quantity and Quality on Soil Nutrients in the Understory of Two Forests in SW China

Introduction

Plant litter is an essential part of a healthy forest ecosystem, playing a major role in the recycling of nutrients, providing habitat for soil invertebrates (Arpin *et al.* 1995), and several ecosystem services such as reducing leaching (Mo *et al.* 2003) and erosion (Ross and Dykes 1996). The hot and humid conditions in tropical forests result in rapid litter decomposition and high turnover rates. The nutrients released have a short residence time in the soil and are quickly taken up by plant roots and used by the plants. Current increases in levels of carbon in the atmosphere will likely lead to increases in primary productivity and therefore more litter. Litter addition experiments, therefore, provide an opportunity to test the effects brought on by increasing CO₂ levels in the atmosphere (Sayer 2006). Here we investigate how the rate of nutrient availability to plant roots in the soil is influenced by the quantity of litter, and its quality.

Litter quantity effects on nutrient cycling

Forest floor litter is an important physical component of tropical ecosystems. Litter provides a barrier and thus reduces changes in soil moisture content, and temperature, as well as serving as a competitive mechanism to prevent, or reduce seedling establishment (Grime 1979). Several studies have shown that with long term litter removal, nutrient concentrations in soil are drastically decreased (Fisk and Fahey 2001, and Dzwonko and Gawronski 2002).

Molofsky and Augspurger (1992), and Facelli (1994) argue that deeper litter provides a more heterogeneous cover than shallow litter, and thereby promotes species coexistence and plant diversity.

Litter addition studies are not as common as litter removal experiments. Sayer (2006) suggests that the results of experiments that manipulate litter quantity, both by removal and addition, are exaggerated and confounded because litter manipulation simultaneously changes several uncontrolled variables. Previous litter addition experiments have investigated the effects of litter mass on seedling emergence and survival, however, long term litter addition has the potential to change the physical and chemical structure of the forest floor. Our study will attempt to tease out the effects of litter removal, litter addition, and the effects of topping in the litter addition plots.

Litter quality effects on nutrient cycling

The species of tree from which litter is derived can affect and alter decomposition rates and nutrients released from the litter, and this has been shown in both temperate (Hobbie *et al.* 2006) and tropical (Loranger *et al.* 2002, Sundarapandian and Swamy 1999) environments. Different species of plants have different nutrient requirements and uptake soil nutrients in different concentrations. Thus, the stoichiometry of nutrients in plant litter would also vary from species to species. These unique combinations of nutrients within the litter may also attract different decomposers. It has long been thought that a mixture of different species of litter would provide a more favourable combination of nutrients for decomposers, and attract a wider range of herbivores, and lead to faster breakdown of litter (Hobbie *et al.* 2006).

Litter compounds such as lignin, anti-herbivory chemicals, and leaf cuticles can also affect the decomposability of the litter. These chemical properties vary with season, species of plants, and even within genotypes of the same species (Muller *et al.* 1987). Tropical rain forests have a high species richness and diversity of plants, and this generates a diverse standing litter, which creates a more favourable combination of litter traits (Hättenschwiler *et al.* 2008). In other studies, researchers have argued that a monoculture of litter decomposed faster under the canopy of its own species (Vivanco and Austin 2008, Ayres *et al.* 2009), likely due to adaptation to a specific set of soil macroinvertebrate activity (Negrete-Yankelevich *et al.* 2008).

A series of experiments on litter species decomposition has been done in Yunnan province in southern China, primarily in two areas, Ailao Mountain and Xishuangbanna. Liu *et al.* (2000) compared decomposition of leaf litter between canopy trees, understory bamboo, and mixtures of moss over a period of nine years. The authors measured decomposition rates, turnover time, as well as nutrient concentrations in the decomposing litter, and related the data to forest systems around the world. In a subsequent paper, Liu *et al.* (2002) compared the decomposition of leafy and woody litter and their respective nutrient output, and how these nutrients influenced the soil surface organic layer. Over a nine year period, the authors noticed significant yearly variations in litter production in relation to the masting year of canopy species as well as unique abiotic events such as strong winds and snow. Zheng *et al.* (2006) have also analyzed litter decomposition and nutrient release in the forests near Xishuangbanna. They measured the rate of decay of litter, turnover time, and also nutrient residence

times on the forest floor. Their results show that litter decomposition rates in the tropical rainforest of Xishuangbanna are slower than typical rainforests, but are similar to other broadleaf semi-deciduous forests in Northern Turkey, Southern Australia, and Tasmania (Bailey, 1995). These studies are highly useful to assess the litterfall, standing biomass and nutrient availability in these highly diverse, yet understudied systems.

Predictions

Based on the literature, three general predictions may be made; one general prediction for the overall study, and two specific predictions for the site. Considering the various effects that litter has been reported to have in the functioning of forest ecosystems, and especially in relation to nutrient cycling, I predict that the available and exchangeable nutrients will be different at these two sites – Xishuangbanna lowland forest will be phosphorus limited, and the Ailaoshan montane forest will be nitrogen limited.

In the litter quantity experiment, I predict that by removing litter, the concentrations of nutrients in the soil will decrease in comparison to the natural litter (control). Also, with increasing litter biomass from different treatments of litter depths and topping, I predict that the soil nutrients will increase with increasing litter biomass. The addition of litter will result in an increase in nutrient availability to plant roots in the soil, but the magnitude of this effect will decline as the litter decomposes; in plots where litter depth is maintained through periodic topping-up, there will also be an increase in nutrient availability in the soil, and the magnitude of this effect will not decline through time.

In the litter quality experiment, I predict that litter comprised of a mixture of species will provide more favourable chemistries for decomposers, and lead to faster decomposition, and a higher level of soil nutrients compared to single species of litter.

Methods

Study Sites

The study was conducted in the forest understory at two sites located in Yunnan Province,

China (Figure 2.1).



Figure 2.1. Map of Yunnan Province showing the location of the two study sites at Xishuangbanna Tropical Botanical Garden (XTBG) and Ailaoshan Nature Reserve (FERS). Modified from Young and Herwitz (1995).

The Xishuangbanna Tropical Botanical Garden (XTBG) (21°55' N, 101°15'E) is located within the Xishuangbanna Dai Autonomous Prefecture in southern Yunnan, bordered by Laos and Myanmar (Zheng *et al.* 2006) (Figure 2.2a). It is located at the northern edge of tropical Asia, where tropical and subtropical vegetation coexist in a unique ecosystem (Zheng *et al.* 2006). It has distinct dry and wet seasons, with a tropical climate due to its relatively low elevation (550 m), and is categorized as a tropical lowland forest. The mean annual rainfall is 1221 mm with 85% of this occurring in the rainy season from May to October (Zheng *et al.* 2006). Mean annual temperature is 21.7°C ranging from 14.8°C in the coldest month (January) to 25.3°C in the warmest month (June) (Yang and Chen 2009). The soils are thick, loose and porous having a distinctive red colour, with a pH ranging from 4.5-5.5 (Cao *et al.* 2006), and the surface is usually covered by about 2-3 cm of litter (Zhang, *et al.* 2009).

The second study site was located within the Ailaoshan National Nature Reserve (23°35′-24°44′N, 100°54′- 101°30E) which is the largest area of natural evergreen broad-leaved forest in China (Young and Herwitz 1995, Qiu *et al.* 1998). The study site is within a 504 km² area of pristine old-growth forest, and experimental plots were established close to the Forest Ecosystem Research Station (FERS) (Figure 2.2b) of Kunming Institute of Ecology. Mean annual temperature at the FERS is 11.3°C with July and January being the warmest (mean 16.4°C) and coldest (mean 5.4°C) months respectively. Mean annual rainfall is 1931 mm, with 85% of this occurring in the rainy season (May to October) and mean annual humidity is 86% (Liu *et al.* 2000). The area is located at an elevation of 2200 m above sea level, and is classified as a subtropical montane evergreen broad-leaved forest, dominated by members of the Fagaceae, Euphorbiaceae, Theaceae and Lauraceae families (Hua 2006). It shares the same distinct wet and dry seasons as the forest at Xishuangbanna, but due to elevation, has a more temperate climate. At this site, soils are acidic (pH 4.2-4.9) with a high organic matter and nitrogen content, and litter typically ranges from 3 – 7 cm deep (Liu *et al.* 2000).



Figure 2.2. Photograph of the understory at the study sites at (a) Xishuangbanna Tropical Botanical Garden (XTBG), and (b) Ailaoshan (FERS). Note the extensive litter layer at both sites

Experimental Design

Three replicate experimental blocks, each approximately 6m x 6m, were chosen in open, flat areas to avoid large trees transecting the plot in each of the two study sites –XTBG and FERS (Figure 2.3). Within each block we removed all loose litter with gentle brushing and marked out 30 plots, each 0.5 m x 0.5 m. We then applied 3 replicates each of 10 different experimental treatments (Figure 2.4); all treatments were assigned randomly on the 30 plots.



Figure 2.3. Photograph of the plots set up in the understory at (a) Xishuangbanna Tropical Botanical Garden (XTBG), and (b) Ailaoshan Mountain (FERS).



Figure 2.4. One replicate block of experimental treatments in the understory of two forests in SW China. Treatments were randomly assigned to plots, but for clarity of explanation they have not been randomized in this figure. Plots labeled 1-4 had litter added from four different species of canopy trees. Control plots had 5 cm of the natural mixed litter from the site reapplied to the plot. Plots labeled 10 cm and 25 cm (black print) had natural litter from the sites added to depths of either 10 cm or 25 cm and these depths were maintained throughout the experiment by weekly top-ups. Plots with red print had mixed-species litter added to depths of 10 cm and 25 cm, but these were not topped-up.

To test the effects of litter quality on soil nutrients, we gathered litter from the four most abundant species of trees in the forests and placed the four types of litter in plots labeled 1-4, at the same depth as the natural litter depth, which is 5 cm. At XTBG, these were: *Bambosa vulgaris* Schrad ex J.C. Wendl, *Cinnamomum burmannii* (Nees & Th. Nees) Nees ex Blume, *Dipterocarpus retusus* Blume, and *Parashorea chinensis* Wang Hsie. At Ailaoshan, the four species were: *Schima noronhae* Reinw. Ex Blume, *Castanopsis wattii* (King ex Hook. F.) A. Camus, *Lithocarpus hancei* (Benth.) Rehder, and *Lithocarpus xylocarpus* (Kurz) Markgr. For the plots that tested the effects of litter species, a loose nylon net (mesh size 4-5 cm) was secured at the four corners of the plot and placed over the litter (approximately 5cm deep) to prevent it blowing away. All other plots were left uncovered. The control plots had a natural mixture of litter that was removed while clearing the plots, and reapplied to a depth of 5cm and not topped up during the experiment; this represented the effect of "naturally occurring" litter. To test for the effects of litter quantity, two different depths of litter (10 cm and 25 cm) were placed on plots surrounded by a mesh net to maintain the 10 cm and 25 cm heights. In half (18) of these plots, the litter levels were periodically topped up to the original levels as the leaves decomposed (black plots in Figure 2.4), while in the other 18 plots, the litter levels were allowed to decline, and were not topped up during the experiment (red plots in Figure 2.4). The litter for these plots was the same natural mixture of litter as used for the control plots. For comparison, the final treatment was bare ground with no litter on the plots. All plots were monitored weekly and new litter (fallen leaves) was removed.

Soil Nutrients

To measure soil nutrient availability within each plot throughout the experiment, we used PRS ion-exchange Probes[™] (Plant Root Simulator Probes; Western Ag Innovations, Saskatoon). Each probe contains a cation or anion exchange membrane enclosed in a plastic frame. The probes provide a measure of the availability of 14 different cation and anion elements (NO₃, NH₄, K, Ca, Cl, Na, SO₄, H₂PO₄, Mg, Mn, Cu, Zn, Fe, Al). There were two pairs of anion and cation probes in each 0.5 m x 0.5 m plot, and the data were pooled to account for within-plot heterogeneity. The first set of probes was inserted in the plots 3 weeks after the initial experimental set up and remained in the soil for 3 weeks. After 3 weeks, the probes were removed, and immediately replaced with new probes, and this was repeated after a further 3 weeks; when the three sets of probes were pooled, this gave a measure of total soil nutrient availability over nine weeks. Removed probes were washed with deionized water, stored at room temperature in the lab at XTBG and then sent to Western Ag Innovations for analysis.

Ion exchange membranes are different from the majority of previous litter nutrient studies (Loranger *et al.* 2002, Sayer and Tanner 2010), which used indirect methods such as assessing litter nutrient concentration, and soil cores. These ion exchange probes simulate plant roots and provide an accurate, biologically meaningful snapshot of the nutrients availability in the soils (Qian and Schoenau 2002).

Statistical Analysis

Nutrient data were summed through time to provide the total accumulation of each nutrient throughout the 9 weeks of the experiment. An exploratory Principal Components Analysis (PCA) was done to show the relationship of each plot to the correlation pattern among the 7 nutrient variables (Total N, NO₃, NH₄, Ca, Mg, K, and P) in multivariate space. In addition, the difference between treatments was tested using a permutational multivariate analysis of variance (perMANOVA), using Euclidean distances. All multivariate analyses were performed using PC-ORD statistical software, version 5.0 (McCune and Mefford, 2006).

A one-way repeated measures ANOVA was used to test the effects of Time, and Time x Treatment interactions on both the litter depth treatments and the litter species treatments on unpooled nutrient data, analyzing each time period separately.. To test for differences between the two sites, total nitrogen was analyzed using repeated measures for Xishuangbanna and

Ailaoshan litter depth treatments, as most of the other nutrients did not meet the assumptions of this test.

Using the pooled nutrient accumulation throughout the field season, each nutrient was analyzed independently within each site as follows. Differences between each treatment mean were tested using a one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison. Five soil nutrients were analyzed – Total N, NO₃, Ca, Mg, and K. Phosphorus and NH₄ were initially included, but the levels of these two nutrients were very low, and did not meet either the normality (using the Shapiro-Wilk test) or homogeneity of variances (Levene test) assumptions of ANOVA, and were excluded from further analyses. For graphing purposes, each of the nutrients was presented as a contrast between the treatment and the control, using: $CI_{0.95} = (\vec{X}_1 - \vec{X}_2) \pm t_{0.05} s_{\vec{X}_1 - \vec{X}_2}$

where $(\bar{X}_1 - \bar{X}_2)$ is the difference between the two means, and $(s_{\bar{X}_1 - \bar{X}_2})$ is the standard error of the contrast which is calculated as:

$$S_{\bar{X}_1 - \bar{X}_2} = \sqrt{MS_{error}\left(\frac{2}{n}\right)}$$

The one-way ANOVAs and repeated measures ANOVAs were performed using the SPSS statistical package (SPSS Statistics version 20.0).

Results

The two experimental sites show clear segregation in soil nutrients: Xishuangbanna had higher levels of total nitrogen, nitrate (NO₃) and ammonium (NH₄), while the levels of calcium (Ca), magnesium (Mg), potassium (K) were higher at Ailaoshan. Supple rates of phosphorus were equally low in both sites.



Figure 2.5. PCA ordination of soil nutrients from Xishuangbanna (XTBG) and Ailaoshan plots, (a) in response to litter depth treatments and (b) litter species treatments. Each symbol represents a single 0.5 m x 0.5 m treatment plot. Vectors denote direction and strength of correlations between each nutrient and the PCA axes.

For different litter depths (Figure 2.5a), the first two PCA axes accounted for 69.3% of the variance in the data. The seven nutrients are overlaid to indicate the direction and strength of relationships of the variables on the ordination. The first PC axis shows that Total N, NO₃, and NH₄ have high, negative loading values (<-0.27), while Ca, Mg, K and P show high, positive

loading values (>0.34). For litter species treatments (Figure 2.5b), the first two PCA axes explained 69.1% of the variance in the data. The first PC axis shows that Total N, NO₃, and NH₄ have high, negative loading values (<-0.30), while Ca, Mg, and P shows high, positive loading values (>0.35). On the second axis, K has a strong, negative loading value (-0.77). These results show that in both the litter depth treatments and the litter species treatments, the first axis separates the two experimental sites at Xishuangbanna and Ailaoshan. These are significantly different (litter depth; PerMANOVA: $F_{(38,1)} = 26.70$, p<0.001), (litter species; PerMANOVA: $F_{(38,1)}$ = 42.81, p<0.001) and based primarily on differences in nitrogen, and other elements such as Ca, Mg, K, and P.

Treatment Effects

Table 2.1. Summary of ANOVAs testing for treatment effects of litter depth on soil nutrients in Xishuangbanna. The Sum of Squares and Mean Squares are for between groups. **Bold** values are significant at p<0.05.

Nutrient	d.f	SS	MS	F	Р
Total N	5,18	681056.51	136211.30	3.305	0.027
NO ₃	5,18	726728.46	145345.69	6.198	0.002
Са	5,18	3827753.71	765550.74	5.300	0.004
Mg	5,18	403890.98	80778.20	10.725	<0.001
К	5,18	612087.26	122417.45	1.728	0.179

Nutrient	d.f.	SS	MS	F	Р
Total N	5,18	186869.83	37373.97	1.194	0.351
NO3	5,18	209251.71	41850.34	3.335	0.026
Ca	5,18	2851112.83	570222.57	3.885	0.015
Mg	5,18	127525.71	25505.14	5.171	0.004
К	5,18	731602.33	146320.47	2.623	0.060

Table 2.2. Summary of ANOVAs testing for treatment effects of litter species on soil nutrients in Xishuangbanna. The Sum of Squares and Mean Squares are for between groups. **Bold** values are significant at p<0.05.

Table 2.3. Summary of ANOVAs testing for treatment effects of litter depths on soil nutrients at Ailaoshan. The Sum of Squares and Mean Squares are for between groups. **Bold** values are significant at p<0.05.

Nutrient	d.f	SS	MS	F	Р
Total N	5,18	7959.59	1591.92	1.035	0.427
NO ₃	5,18	7443.97	1488.79	1.220	0.340
Са	5,18	8105383.53	1621076.71	2.670	0.056
Mg	5,18	349897.85	69979.57	4.491	0.008
К	5,18	706777.19	141355.44	0.670	0.651

Table 2.4. Summary of ANOVAs testing for treatment effects of litter species on soil nutrients at Ailaoshan. The Sum of Squares and Mean Squares are for between groups. **Bold** values are significant at p<0.05.

Nutrient	d.f	SS	MS	F	Р
Total N	5,18	16186.15	3237.23	0.746	0.599
NO ₃	5,18	17358.72	3471.74	0.870	0.521
Са	5,18	5691482.88	1138296.58	1.315	0.302
Mg	5,18	179349.33	35869.87	1.582	0.216
К	5,18	291439.33	58287.87	1.062	0.412

Table 2.5. Supply rates of each soil nutrient (± 1SE) in soils after 9 weeks of litter manipulation treatments in the forest at Xishuangbanna. Different letters denote differences between treatment responses where standard errors do not overlap.

XTBG Litter Depth						
Nutrient (mg/9weeks)	No Litter	Control	10cm NT	25cm NT	10cm T	25cm T
Total N	219±62 a	462 ± 86 b	358 ± 95 ab	436 ± 66 b	601 ± 150 bc	746 ± 122 c
NO ₃	149±36 a	362 ± 75 b	259±78 b	380 ± 68 b	387±66 b	717 ± 115 c
Са	534±120 a	1262 ± 219 b	1051 ± 195 b	1329 ± 163 b	1301±51 b	1881 ± 294 c
Mg	102 ± 12 a	289 ± 75 b	295 ± 41 b	379 ± 45 b	333±16 b	540 ± 75 c
К	220±57 a	698±181 b	242 ± 29 a	614 ± 158 b	371±81 c	552 ± 195 bc

XTBG Litter Species						
Nutrient (mg/9weeks)	NL	Control	C. burmannii	B. vulgaris	S. chinensis	D. retuses
Total N	219±62 a	462 ± 86 b	453 ± 62 b	458±126 b	412 ± 36 b	332 ± 121 ab
NO ₃	149±36 a	362 ± 75 b	359 ± 67 b	206 ± 81 ac	334 ± 27 b	153 ± 10 ac
Са	534 ± 120 a	1262 ± 219 b	1649 ± 198 bd	1394 ± 277 b	1085 ± 158 bc	1056 ± 128 bc
Mg	102 ± 12 a	289 ± 75 b	329 ± 44 b	282 ± 46 b	292 ± 25 b	262 ± 31 b
К	220±57 a	698±181 b	700±128 b	448 ± 106 bc	582 ± 123 b	374 ± 70 c

Table 2.6. Supply rates of each soil nutrient (± 1SE) in soils after 9 weeks of litter manipulation treatments in the forests at Ailaoshan. Different letters denote differences between treatment responses where standard errors do not overlap.

Ailaoshan Litter Depth						
Nutrient (mg/9weeks)	No Litter	Control	10cm NT	25cm NT	10cm T	25cm T
Total N	56±13 a	109 ± 26 b	102 ± 13 b	89 ± 25 ab	68 ± 20 ab	89±15 b
NO ₃	38±12 a	85±27 b	70±13 b	38±9 a	46 ± 19 ab	63±19 ab
Са	1728 ± 287 a	3073 ± 221 bc	2515 ± 631 ab	2570 ± 386 ab	2303 ± 352 a	3575±329 c
Mg	388±51 a	629 ± 59 b	501±66 c	570 ± 71 bc	475 ± 66 ac	766 ± 59 d
К	799 ± 190 ac	883±72 a	934 ± 137 ac	1200 ± 294 b	699 ± 101 c	1107 ± 400 abc

Ailaoshan Litter Species						
Nutrient (mg/9weeks)	No Litter	Control	S. noronhae	C. wattii	L. hancei	L. xylocarpus
Total N	56±13 a	109 ± 26 b	100 ± 28 b	131±50 b	130±44 b	85±21 ab
NO ₃	38±12 a	85 ± 27 b	46±17 b	110±53 b	103±41 b	71 ± 20 b
Са	1728 ± 287 a	3073 ± 221 b	2537 ± 445 b	2577 ± 690 ab	3248 ± 594 b	2788 ± 374 b
Mg	388±51 a	629±59 b	541 ± 111 ab	500 ± 90 ab	647±80 b	566±31 b
К	799 ± 190 ab	883 ± 72 a	651±48 b	761 ± 166 ab	589±25 b	591 ± 102 b

In Figure 2.6, the response of each nutrient to litter manipulation is shown in a difference from control. Within each nutrient, the bars are arranged by increasing litter amounts from litter removal (no litter) treatments, to 25 cm deep litter with continuous topping up. For total nitrogen, nitrate, calcium and magnesium, there was a significant decrease in each of these nutrients measured in the litter removal (no litter) treatments compared to the natural litter treatment. The maximum load of litter (25 cm of litter with topping) showed a significant increase in supply rates of each of the four nutrients. However, all other treatments did not significantly affect the supply rates of the four nutrients, although the general trend was an increase in each nutrient with increasing litter amounts. Potassium did not follow the same trend, and decreased in all treatments.



Xishuangbanna Litter Depth

Figure 2.6. Mean (\pm 95% CI) soil nutrients at Xishuangbanna under each litter depth treatment compared to the control represented by the zero line. Error bars that do not overlap the zero line are significantly different from the control. Treatments are shown in the legend, with abbreviations NT = no topping up of litter, and T = topping up of litter.

In Xishuangbanna litter species experiments, most of the nutrients decreased in concentration in comparison to the control, with the only exception being the concentration of calcium in *Cinnamomum burmannii* (Figure 2.7). The variation in datawas also generally larger than the data for litter depth treatments at the same site. Species differences may contribute to this variation in effects in soil nutrients. Xishuangbanna Litter Species



Figure 2.7. Mean (±95% CI) soil nutrients at Xishuangbanna under each litter species treatment compared to the control represented by the zero line. Error bars that do not overlap the zero line are significantly different from the control. The right Y-axis corresponds to the nutrients to the right of the break of the X-axis (Ca, and K), and the left Y-axis corresponds to the nutrients to the left of the break (Total N, NO₃, and Mg). The species are: *Cinnamomum burmannii, Bambosa vulgaris, Parashorea chinensis,* and *Dipterocarpus retusus.*

For the litter depth experiment at Ailaoshan, litter removal and litter addition treatments all yielded lower concentrations of soil nutrients compared to the control (Figure 2.8), however, only a few nutrients measured were significantly different from the control. The magnitude of change in nitrogen is also quite different from the litter depth experiment at Xishuangbanna. In another contrast to Xishuangbanna, the litter removal (no litter) plots did not result in a bigger decrease in soil nutrients as compared to the other treatments.

Ailaoshan Litter Depth



Figure 2.8. Mean (\pm 95% CI) soil nutrients at Ailaoshan under each litter depth treatment compared to the control represented by the zero line. Error bars that do not overlap the zero line are significantly different from the control. Treatments are shown in the legend, with abbreviations NT = no topping up with litter, and T = topping up with litter. The right Y-axis corresponds to the nutrients to the right of the break on the X-axis (Ca, Mg, and K), and the left Y-axis corresponds to the nutrients to the left of the break (Total N, and NO₃).

The litter species experiment at Ailaoshan also had some surprising results, with different soil nutrients showing different changes below ground. Total nitrogen, nitrate, calcium, and potassium generally all showed a decrease with different species of litter, with a few treatments being significantly different from control (Figure 2.9). However, magnesium increased in concentration for all species treatments, even in the litter removal (no litter) treatment. Ailaoshan Litter Species



Figure 2.9. Mean (±95% CI) soil nutrients at Ailaoshan under each litter species treatment compared to the control represented by the zero line. Error bars that do not overlap the zero line are significantly different from the control. The right Y-axis corresponds to the nutrients to the right of the break on the X-axis (Ca, Mg, and K), and the left Y-axis corresponds to the nutrients to the left of the break (Total N, and NO₃). The species are: *Schima noronhae, Castanopsis wattii, Lithocarpus hancei,* and *Lithocarpus xylocarpus.*

Soil Nutrients over Time

Changes in soil nutrients over time were mostly nonsignificant, with many data sets violating the assumption of sphericity, and also nonsignificant interaction between Time x Treatment. Generally, nitrogen levels at Xishuangbanna increased over time, and higher litter input resulted in a greater magnitude of increase (Figure 2.10), however, the only significant difference was between the no litter treatment and the 25 cm litter with topping up. In contrast,

the plots at Ailaoshan showed a decrease in soil nitrogen levels over time, even with litter

addition treatments (Figure 2.11). This conflicting trend was intriguing, as the treatments at

both sites occurred concurrently, for a span of 3 months.

Table 2.6. Summary of one-way repeated measures ANOVA testing for effects of litter depth on changes in Total N in the soil measured once every three weeks between April 18th and June 25th, 2011. P-values of Total N at Xishuangbanna were adjusted using the Greenhouse-Geisser correction to account for violation of the sphericity assumption.

	d.f.	Time		Treatment		Time*Treatment	
		F	Р	F	Р	F	Р
Total N (Xishuangbanna)	5.26, 19.86	5.131	0.027	3.912	0.023	0.89	0.511
Total N (Ailaoshan)	8, 30	5.058	0.013	1.028	0.425	0.555	0.806

Xishuangbanna Litter Depth 500 No Litter Total N supply rate (mg/10cm²/3 weeks) 10cm NT 25cm NT 400 10cm T 25cm T 300 200 100 0 2 3 1 Time

Figure 2.10. Total Nitrogen (\pm 1SE) at time points 1 (April 22 – May 14), 2 (May 14 –June 5) and 3 (June 5-27) in Xishuangbanna litter depth treatments. The time between each time measure was three weeks. Treatments are shown in the legend, with abbreviations NT = no topping up with litter, and T = topping up with litter.


Figure 2.11. Total nitrogen (\pm 1SE) at time points 1 (April 18 - May 10), 2 (May 10 - June 1) and 3 (June 3 – 25) in Ailaoshan litter depth treatments. The time between each time measure was three weeks. Treatments are shown in the legend, with abbreviations NT = no topping up with litter, and T = topping up with litter.

Discussion

The two experimental sites showed clear segregation in soil nutrients, Xishuangbanna had higher levels of total nitrogen, nitrate (NO₃) and ammonium (NH₄), while the levels of calcium (Ca), magnesium (Mg), potassium (K) were higher at Ailaoshan. Phosphorus was equally low in both sites. This result is consistent with previous findings from tropical lowland forests which typically have abundant nitrogen but are limited by phosphorus, while tropical montane forests are more typically limited by nitrogen, similar to temperate forests (Vitousek 1984, Tanner et al. 1998, Sayer et al. 2012). This is likely due to lowland tropical forests having a higher net primary productivity than montane tropical forests, thus nitrogen is cycled quickly, and plant growth is mostly limited by the low levels of phosphorus. Tropical montane forests are more consistent with temperate forests with lower net primary productivity and litterfall, and slower decomposition, and plant growth is limited by the reduced nitrogen (Tanner et al. 1998). While the levels of soil Ca, Mg and K were generally higher in Ailaoshan sites, their levels based on sites and elevations are sometimes unpredictable, and potassium did not associate strongly with either site, indicating that the concentrations of this nutrient were similar in both. This is consistent with numerous studies showing unpredictable variable responses of Ca, Mg, and K in a wide range of litter manipulation studies in tropical areas (reviewed in Tanner et al. 1998), where the authors suggested that the cycling of these cations are more linked to soil type, than vegetation type and ecosystem properties. The soils of our field sites were acidic and porous and resulted from the weathering of limestone (Cao et al. 2006), and could explain why Ca, Mg, and K are not limited in either site. Phosphorus levels of these soils are very low, and this pattern is consistent with previous studies and reviews (Vitousek 1998, Sayer et al. 2012), likely

due to the many years of leaching and erosion of old weathered soils. Furthermore, a significant proportion of inorganic phosphorus is locked in biologically unavailable components of the soil (Vitousek and Sanford 1986).

At Xishuangbanna, added litter resulted in an increase in soil nitrogen, nitrate, calcium, and magnesium, with potassium showing an exception with an overall decrease in each treatment. Potassium cycling follows a similar pattern to nitrogen (Tripler *et al.* 2006), but primarily enters the soil from through-fall rather than litter fall (Vitousek and Sanford 1986). Sayer *et al.* (2012) found that potassium concentrations were greatly reduced in both soil and litter after litter removal manipulations. They suggested that potassium cycling is tightly controlled by the living biomass, and that any available potassium is quickly absorbed via plant roots. In essence they argue that the amount of potassium cycling through the soil is low, but rapid and continuous. Therefore, by using ion exchange membranes we would expect that the addition of litter would have resulted in an increase flow rate of potassium, but the ion exchange membranes did not detect this. Tripler *et al.* (2006) argue that the availability of potassium can fluctuate based on seasonal variations of litterfall and plant uptake. This may explain our result, although other nutrients should have reacted similarly.

At Xishuangbanna, two of the species used are native species in subtropical areas of Asia (*Cinnamomum burmannii* (Indonesia Cinnamon) and *Bambosa vulgaris*, (Golden Bamboo)), but are able to grow as cultivated stands in other areas of the world, thus have a more generalist growth strategy. The other two species (*Parashorea chinensis*, and *Dipterocarpus retusus*) are highly endemic and threatened, and highly specialized to the habitats of southern Asia.

Taxonomically, these two species are also more closely related to the mixed Dipterocarpus leaves used as natural litter for the control. Thus, the significant reduction in nitrate in *D. retusus* treatments compared to the control is surprising. This may be due to the fact that this field site was in a Dipterocarpus stand, and decomposition occurred faster and the change in soil nutrients due to *D. retusus* compared to control is amplified. This is consistent with a review by Ayres *et al.* (2009) where the authors argued that litter placed beneath the canopy of the same species decomposed faster because of specialization in the soil biota due to the canopy species. Our overall result is consistent with our original hypothesis that mixtures of litter species provides a more favourable stoichiometric combination for decomposition, as all of the single species of litter showed a decrease in soil nutrients in comparison to the mixed species control. Recently, however, Hättenschwiler and Jorgensen (2010) showed that the while the effects of litter species used.

At Ailaoshan, the magnitude of change of nutrient levels in the litter depth experiments was considerably less than at Xishuangbanna. The addition of litter also did not seem to have an effect on soil nutrients. These results could be due to the nitrogen-poor forests of Ailaoshan, and any increases in nutrients due to the addition of litter could be either taken up immediately by vegetation, or lost to leaching. The large variation in soil nutrients could be attributed to soil heterogeneity and the patchy nature of tropical soils. Sayer's (2006) review of litter addition experiments concluded that increasing litter artificially on a forest floor will not result in a corresponding increase in soil fauna, fungal or microbial activity, and will simply result in an increased biomass on the forest floor. This may explain why by adding litter, the magnitude of

change in soil nutrients in Ailaoshan did not increase, as it seems that forest systems are more sensitive to litter removal changes. It is interesting that this conclusion does not apply to both sites, as litter decomposed rapidly from litter addition plots at Xishuangbanna, probably because of the combination of higher temperature and rainfall providing more ideal conditions for decomposition.

In a previous experiment conducted at the Ailaoshan site, Liu *et al.* (2002) investigated litterfall and foliar nutrient concentrations of several dominant species. They used three of the four species we used, and this provides a good basis for comparison of the amount of each nutrient in the leaves, prior to decomposition. Liu *et al.* (2002) reported that *Castanopsis wattii* had the highest amount of foliar Ca and second highest amount of foliar N of all the species studied. This was not corroborated by our results. This could be explained by our earlier results, which suggested that Ailaoshan soils are nitrogen-limited, thus the high nutrient content originally measured in the leaves may be locked in by the microbial community, and not reflected in our PRS probes. Liu *et al.* (2002) also reported that above ground biomass at Ailaoshan had moderate quantities of N, P, K, Ca, and Mg, similar to other tropical montane forests in Venezuela, Jamaica and New Guinea. Our contrasting results, however, may be attributed to the different method of sampling nutrient concentrations. The ion-exchange probes pick up nutrient availability to plants, while Liu *et al.* (2002) measured foliar nutrients prior to decomposition, and not what is available to the vegetation.

The difference in nutrient availability from litter manipulation may be attributed to differences between the montane region of Ailaoshan (>2000 m in elevation) and the lowland

region of Xishuangbanna (500 m in elevation). Knops *et al.* (2002) suggested that much litter nitrogen is locked up in the soil organic matter, in a tight microbial nitrogen loop, which is driven by recent plant carbon inputs. At Ailaoshan, decreasing nitrogen levels over the course of our experiments is consistent with the idea that tropical montane forests are limited by nitrogen (Tanner *et al.* 1998), and when any additional nutrient (in the form of litter) is added to the system, it is quickly taken up by the microbial decomposer community. In Xishuangbanna, however, the rise in soil nitrogen over the 9 weeks of our treatment is perhaps due to the fact that this system is not as nitrogen limited, and any addition of nitrogen (in the form of litter) is just contributing to an already sizable pool of nitrogen. The low pH values of both sites (4.2-4.9 at Ailaoshan, and 4.5-5.5 at Xishuangbanna) are notable, because a low pH indicates that there is a high loss of bases due to leaching, and nutrient levels tend to decrease as pH drops (Tanner *et al.* 1998).

Our results show distinct patterns in how litter manipulation, either through removal or addition, and species composition affects the soil nutrients in two tropical forests. Many of these differences may be attributed to the environmental conditions of these two separate sites – one a tropical lowland forest (Xishuangbanna), and one a tropical montane forest (Ailaoshan). Within each site, we also show distinct patterns in soil nutrients caused by manipulations of litter quantity and quality, and the high variation in responses in heterogeneous, nutrient-poor soils. These results are consistent with previous experiments done in similar tropical systems, however, the wide variation in our data suggest that there are complex dynamics in litter-soil interactions in the relatively understudied forests of Southwest China that are not simply due to differences in elevation, litter quantity, or species.

CHAPTER 3

The Effects of Litter Quantity and Quality on Litter Invertebrates in the Understory of Two Forests in SW China

Introduction

Litter invertebrates play a vital role in nutrient cycling, especially in tropical systems where the soils are nutrient poor yet productivity is high. Along with microbes, soil invertebrates are responsible for the physical and chemical break down of litter, so the nutrients can recycle back into the soil and be available for uptake by plant roots. Many studies have shown that the exclusion of herbivores and litter invertebrates leads to a drastic reduction in litter breakdown and decomposition rates (Seastedt and Crossley 1980, Couteaux *et al.* 1991).

Several studies have also shown that litter properties strongly influence the diversity and abundance of litter invertebrates (Bultman and Uetz, 1984, Hättenschwiler and Gasser 2005, Wardle *et al.* 2006, Sayer *et al.* 2010). However, there have also been others that suggest that while litter quality and quantity have some effect on soil invertebrate abundances and diversity, abiotic factors such as temperature and rainfall play a larger role and override the litter effects (Yang *et al.* 2007).

Litter on the forest floor may act as a food source, as well as a physical structure and habitat for a community of litter invertebrates. Furthermore, litter helps maintain a favourable and stable microclimate for invertebrates (David *et al.* 1991, Arpin *et al.* 1995). Experimental studies on the effects of litter on the diversity and abundance of invertebrates have more often involved the removal of litter, and less often on increasing the amount of litter. Sayer (2006)

wrote extensively in her review regarding the detrimental effects of litter removal on arthropod abundance and diversity, citing both the direct and indirect loss of litter as food source in trophic interactions of delicate tropical food web relationships. Specifically, litter removal not only impacts the food source of primary herbivores and decomposers, but also affects secondary consumers and predators of these soil fauna.

In contrast, studies generally show that with litter addition, there is a slight increase in soil invertebrate abundance and diversity (David *et al.* 1991, Arpin 1995), but the effects are minimal compared to the effects with litter removal studies. Indeed, some studies have shown no effect of litter addition on invertebrates (Sayer *et al.* 2006) and others have shown a negative effect (Uetz 1979). Sayer *et al.* (2006) suggests that adding litter adversely affect soil invertebrates due to reduced oxygen in the litter mass, as well as addition of any phytochemicals and predators.

In comparison to the wide range of litter quantity studies and their effects on soil fauna, the influence of litter quality on the decomposer community is not as well understood (Gartner and Cardon 2004), and results are inconsistent. Kaneko and Salamanca (1999) showed that litter mixing resulted in greater habitat heterogeneity and contributed to an increase in litter fauna abundance. A similar conclusion was reported by Hansen and Coleman (1998), where mixing litter species led to an increase in abundances and diversity of mites. Wardle *et al.* (2006) compared litter monocultures to mixed species of litter, and found that litter mixing had little effect on the abundance of mesofauna and macrofauna, but had significant effects on microfauna. In a recent study on litter manipulations and their effects on arthropods in Panama,

Sayer *et al.* (2010) found that there were clear differences in arthropod communities across treatments, most likely due to litter depth as well as phosphorus concentrations. This is consistent with another study by Hättenschwiler and Gasser (2005), where the authors found that mixed species of litter altered decomposition patterns, and that soil fauna was the determining factor in the magnitude and direction of change in litter species effects.

Previous studies at Xishuangbanna have been conducted on the impact of litter composition on invertebrate populations. Yang (2004) investigated how mixed species of litter affected the diversity and abundance of meso-microarthropods during litter decomposition, and showed that diversity of insect herbivores was highest in the middle stages of decomposition. At the same site, Yang and Chen (2009) showed that the diversity and richness of arthropods seemed to play a larger role in litter decomposition of the primary rainforest, in comparison to the secondary or broadleaf evergreen forest.

Predictions

Based on the literature, three general predictions may be made: one general prediction for the overall study, and two specific predictions for the site.

Considering the various effects that litter has been reported to have in the functioning of forest ecosystems, and especially in relation to soil invertebrates, I predict that the composition and diversity of soil invertebrates will be different at these two sites.

In the litter quantity experiment, I predict that by removing litter, soil invertebrate abundance and diversity will decrease, but in litter addition plots there will be an increase in litter invertebrate abundance and diversity, although the magnitude of change will not be as large as in the litter removal treatments. By removing litter, I will be physically removing some of the community of invertebrates living in that biomass, thus reducing the invertebrates in those plots. Furthermore, the removal of litter directly removes a food source and habitat for the invertebrates and will again lead to a reduction in their numbers. By increasing litter depth, and also regular topping-up of litter, I will be providing biomass and a food source, as well as structural complexity for the invertebrate community. If soil invertebrate abundance is a reflection of litter mass and quantity, then our results would show an increased invertebrate abundance with increasing litter biomass. However, if there is a threshold value of litter biomass that provides enough structural complexity for litter invertebrates, then we should see the most soil fauna in the treatments with the most litter (25cm with topping).

In the litter quality experiments, I predict that mixed natural litter plots will have the highest diversity of soil invertebrates, while the single species litter treatments will have lower invertebrate diversity, but a different species composition of invertebrates. Different species of trees will attract different suites of herbivores and decomposers, while the mixed natural litter will provide a favourable chemical combination for a wider range of species.

Methods

Study Sites

The study was conducted in the forest understory of two sites in Yunnan Province, China – within the Xishuangbanna Tropical Botanical Garden (XTBG) (21°55' N, 101°15'E), and in Ailao Mountain National Nature Reserve (23°35′- 24°44′N, 100°54′- 101°30E). The XTBG site has a tropical climate and has a mean annual temperature of 21.7°C (Yang and Chen 2009), and a mean annual rainfall of 1221 mm (Zheng *et al.* 2006). The Ailaoshan site has a more temperate climate, as it is higher in elevation, and has mean annual temperature of 11.3°C, mean annual rainfall is 1931 mm, and mean annual humidity if 86% (Liu *et al.* 2000). The area is dominated by members of the Fagaceae, Euphorbiaceae, Theaceae, and Lauraceae families (Hua 2006). Soils have a pH of 4.2-4.9 and litter typically ranges from 3-7 cm deep (Liu *et al.* 2000). The soils have a distinct red colour, and the surface is usually covered by about 2-3 cm of litter (Zhang *et al.* 2009).More details of the study sites are provided in Chapter 2.

Experimental Design

Litter was removed from three replicate experimental blocks in each of the two study sites. The blocks were chosen so they are spaced apart, on a relatively flat terrain, and to avoid any large trees from transecting the blocks. Within each block I marked out 30 plots, each 0.5 m x 0.5 m, and applied 3 replicates each of 10 different experimental treatments; all treatments were assigned randomly to the 30 plots. To test the effect of litter quality on soil invertebrates, litter from the four most abundant species of trees in the forests was placed on the plots. At XTBG, the four species were: *Bambosa vulgaris* Schrad ex J.C. Wendl, *Cinnamomum burmannii*

(Nees & Th. Nees) Nees ex Blume, Dipterocarpus retusus Blume, and Parashorea chinensis Wang Hsie. At Ailaoshan, these species were: Schima noronhae Reinw. Ex Blume, Castanopsis wattii (King ex Hook. F.) A. Camus, Lithocarpus hancei (Benth.) Rehder, and Lithocarpus xylocarpus (Kurz) Markgr. For plots used to test the effects of litter species, a loose nylon net was secured and placed over the litter to ensure that natural litter fall would not be added to the plot. All other plots were left uncovered. Control plots had a natural mixture of litter that was removed while clearing the plots, and reapplied to a depth of 5cm and not topped-up during the experiment; this represented the effect of "naturally occurring" litter. To test for the effects of litter quantity, two different depths of litter (10 cm and 25 cm) were placed on plots surrounded by a mesh net to maintain the 10 cm and 25 cm depths. Half (18) of these plots had litter periodically added to maintain the treatment depths while the other half did not. The litter for these plots was the same natural mixture of litter used for the control plots; the final treatment was bare ground with no litter on the plots. All plots were monitored weekly and new litter (fallen leaves) was removed, except in control plots. More details of the experimental design are described in Chapter 2.

Invertebrate Trapping

Pitfall traps were used to sample the soil invertebrate communities associated with the different litter treatments. In the centre of each 0.5 m x 0.5 m plot, a 250 ml plastic drinking cup was placed, countersunk in the ground with the rim of the cup at ground level, covered with a 10 cm x 10 cm piece of plywood elevated at 1 cm above the cup to prevent litter falling into the trap. Each cup was filled to 3 cm deep with a solution of 95% ethanol for preservation

and a small amount of liquid detergent to prevent fauna from climbing up the walls of the cup. Traps were placed in the ground for one week at a time at the beginnings of weeks 1, 4, 7, and 10 for a total of 720 pitfall trap samples. After collection, the invertebrates and ethanol solution were transferred to a plastic bottle for storage. Invertebrate identification was done at the Kunming Institute of Zoology using a key of photos identified by an insect taxonomist, and classified to order and/or family.

Statistical Analysis

Invertebrate data were summed over all four sampling periods to reflect their total abundance and taxa richness captured by pitfall traps throughout the experiment. An exploratory Principal Components Analysis (PCA) was performed to show the relationship of each plot to each of the invertebrate orders in multivariate space. Next, the difference between groups was tested using a permutational multivariate analysis of variance (perMANOVA). Euclidean distances were used in the perMANOVA because a zero value of invertebrates in a trap is meaningful, therefore, the relative nature of Bray-Curtis distances do not apply. All multivariate analyses were performed using PC-ORD statistical software, version 5.0 (McCune and Mefford, 2006).

Count data from insect pitfall traps were analyzed by fitting a generalized linear model using the SPSS statistical package (SPSS statistics version 20.0). Invertebrate taxa richness data were fit to a Poisson distribution with a logarithmic link function. To account for overdispersion,

the invertebrate abundance data were fit to a negative binomial distribution with a logarithmic link function. Both sets of analyses were done using the log-likelihood ratio chi-squared test.

Invertebrate diversity was calculated using the Shannon's Diversity Index (H):

$$H = -\sum_{i=1}^{S} p_i * (ln * p_i)$$

where *S* = number of taxa present in a sample (taxa richness), and p_i = proportion of individuals made up of the *i*th taxa.

The equitability of the invertebrate abundance was calculated as Shannon's equitability (E_H) :

$$E_H = H/H_{max} = H/\ln S$$

where $H_{max} = \ln S$. Equitability values range between 0 and 1, with 1 meaning that all species have the same relative abundance.

Results

A total of 9421 individual invertebrates representing 16 orders were captured in pitfall traps during the 2011 field season, from March 31 to June 12: 1850 individuals in Xishuangbanna litter depth treatments; 1438 in Xishuangbanna litter species treatments; 4043 in Ailaoshan litter depth treatments; and 1080 in Ailaoshan litter species treatments. At both field sites, Hymenoptera (bees, wasps and ants), and Diptera (flies) were the most abundant species, with Isoptera (termites) and Araneae (spiders) also common in the Xishuangbanna site.

Orders	Abbreviations	Common Name
Diptera	Dipt	flies
Orthoptera	Orth	crickets
Archaeognatha	Arch	bristletails
Coleoptera	Cole	beetles
Isoptera	lsopt	termites
Hymenoptera	Hyme	bees, ants
Dermaptera	Derm	earwigs
Hemiptera	Hemi	cicadas, aphids
Blattaria	Blatt	cockroaches
Polydesmidae	Poly	millipedes
Lithobiomorpha	Litho	centipedes
Haplotaxida	Haplo	worms
Isopoda	Isopo	woodlice, pillbugs
Stylommatophora	Stylom	snails, slugs
Opilione	Opil	harvestmen spiders
Araneae	Aran	spiders

Table 3.1. Abbreviations for the 16 invertebrate orders trapped in both litter depth and litter species treatments at Xishuangbanna and Ailaoshan.

PCAs were performed using the 16 invertebrate orders. PCA ordinations of the two sites for both the litter species and litter depth experiments show a segregation of plots by site (Figure 3.1). The invertebrate communities captured by the pitfall traps are clearly different between the two sites, segregating on Axis 1 in both experiments. Invertebrate orders such as Polydesmidae (millipedes), Opilione (harvestman spiders), and Stylommatophora (snails and slugs) show a strong association with Ailaoshan plots in the litter depth experiments. Orders such as Hemiptera (cicadas), Archaeognatha (bristletails), and Dermaptera (earwigs) associate strongly with the Xishuangbanna plots on Axis 1. Orders such Hymenoptera (bees and ants) and Isopoda (pillbugs) are equally abundant at both sites.



Figure 3.1. PCA ordination of invertebrate communities from Xishuangbanna and Ailaoshan plots, in response to (a) litter depth and (b) litter species treatments. Each data point represents the contents of one pitfall trap. Vectors denote direction and strength of correlations between invertebrate orders and the PCA axes. For abbreviations of invertebrate orders, see Table 3.1.

While the two sites show clear separation in invertebrate community composition, PCA

ordinations for each litter experiment at each site did not show strong effects of treatments.

Differences between groups of treatments were tested using perMANOVA, using

Euclidean distances (Table 3.2). Invertebrate communities were significantly different (p=0.01)

between the Xishuangbanna litter depth treatments, and marginally significant (p=0.06) at

Ailaoshan. Litter species treatments at both sites showed no significant difference between

invertebrate communities, although at Ailaoshan, L. xylocarpus and L. hancei are marginally

(p<0.08) different from the natural litter control treatments (Table 3.2).

Table 3.2. Summary of one-way perMANOVA testing for treatment effects of litter depth and litter species on the composition, by Orders, of invertebrate communities. **Bold** values are significant at p<0.05. Significant pairwise comparisons for each treatment are reported for the contrasts. Abbreviations are: NL, no litter; 10NT, 10 cm no topping-up mixed litter; 10T, 10 cm topping-up mixed litter; 25NT, 25 cm no topping-up mixed litter; 25T, 25 cm topping-up mixed litter; C, control (natural litter). Symbols: * p<0.05, ** p<0.01, *** p<0.001, † p<0.08.

Xishuangbanna Litter Depth						
	d.f.	SS	MS	F	р	Contrasts
						[NL, 10NT***], [NL, 25NT*], [NL,
Treatment	4	10199	2439.8	1.9118	0.0102	25T**]
Residual	40	53350	1333.8			
Total	44	63549				
Xishuangbanna Li	itter Species					
	d.f.	SS	MS	F	р	Contrasts
Treatment	4	2933.4	733.34	1.6902	0.1314	
Residual	40	17355	433.88			
Total	44	20289				
Ailaoshan Litter Depth						
	d.f.	SS	MS	F	р	Contrasts
						[NL, 25NT*], [10NT, 25NT*],
Treatment	4	289400	72350	2.1871	0.0678	[10T,25NT ⁺]
Residual	40	1323200	33080			
Total	44	1612600				
Ailaoshan Litter Species						
	d.f.	SS	MS	F	р	Contrasts
						[C, Lithocarpus xylocarpus*], [C,
Treatment	4	627.2	156.8	1.6204	0.122	Lithocarpus hancei†]
Residual	40	3870.7	96.767			
Total	44	4497.9				

Invertebrate Abundance

In Xishuangbanna, litter depth treatments did not have a significant effect on total invertebrate abundance (χ^{2}_{5} = 8.016, *p*=0.155), while litter species treatments did (χ^{2}_{5} = 13.773, *p*=0.017), although in both cases, invertebrate abundance in the No Litter plots were significantly different from the natural litter controls (*p*=0.015). Although not significantly different, there was a general trend of higher invertebrate abundance in 10 cm-topping plots and 25 cmtopping plots than the same depths without any topping (Figure 3.2). In Ailaoshan, litter depth treatments had a significant effect on total invertebrate abundance (χ^{2}_{5} = 55.96, *p*<0.001), with both 25 cm (no topping up) and 25 cm (with topping up) having a significantly higher number of invertebrate individuals than any other treatment (Figure 3.3). Litter species treatments in Ailaoshan had no significant effect on invertebrate abundance (χ^{2}_{5} = 8.670, *p*=0.123).

Invertebrate Taxa Richness

In Xishuangbanna, neither litter depth treatments nor litter species treatments had any effect on total invertebrate taxa richness: litter depth ($\chi^{2}_{5} = 4.146$, p=0.529), litter species ($\chi^{2}_{5} = 13.773$, p=0.017). In Ailaoshan, litter depth treatments had a significant effect on total invertebrate taxa richness ($\chi^{2}_{5} = 13.583$, p=0.018), with both 10 cm with topping and 25 cm with topping having significantly lower invertebrate taxa richness than both no litter plots and natural litter control plots (Figure 3.5). Litter species treatments in Ailaoshan had no significant effect on invertebrate abundance ($\chi^{2}_{5} = 5.208$, p=0.391).



a. Total Invertebrate Abundance in Xishuangbanna Litter Depths

Figure 3.2. Mean (± 1SE) number of invertebrate individuals caught in pitfall traps at Xishuangbanna: Litter depth treatments (a) and litter species treatments (b). Mean values with the same letters are not significantly different (*p*>0.05). Abbreviations: NL: no litter, C: control (natural litter), 10NT: 10 cm no topping-up of litter, 25NT: 25 cm no topping-up of litter, 10T: 10 cm with topping-up of litter, 25T: 25 cm with topping-up of litter; 1: *Cinnamomum burmannii*, 2: *Bambosa vulgaris*, 3: *Parashorea chinensis*, 4: *Dipterocarpus retusus*.



Figure 3.3. Mean (± 1SE) number of invertebrate individuals caught in pitfall traps at Ailaoshan: Litter depth treatments (a) and litter species treatments (b). Mean values with the same letters are not significantly different (p>0.05). Abbreviations: NL: no litter, C: control (natural litter), 10NT: 10 cm no topping-up of litter, 25NT: 25 cm no topping-up of litter, 10T: 10 cm with topping-up of litter, 25T: 25 cm with topping-up of litter; 1: *Schima noronhae*, 2: *Castanopsis wattii*, 3: *Lithocarpus hancei*, 4: *Lithocarpus xylocarpus*.



a. Invertebrate Taxonomic Richness in Xishuangbanna Litter Depths

b. Invertebrate Taxonomic Richness in Xishuangbanna Litter Species



Figure 3.4. Mean (\pm 1SE) number of invertebrate taxa caught in pitfall traps at Xishuangbanna: Litter depth treatments (a) and litter species treatments (b). Mean values with the same letters are not significantly different (p>0.05). Abbreviations: NL: no litter, C: control (natural litter), 10NT: 10 cm no topping-up of litter, 25NT: 25 cm no topping-up of litter, 10T: 10 cm with topping-up of litter, 25T: 25 cm with topping-up of litter; 1: *Cinnamomum burmannii*, 2: *Bambosa vulgaris*, 3: *Parashorea chinensis*, 4: *Dipterocarpus retusus*.



a. Invertebrate Taxonomic Richness in Ailaoshan Litter Depths

Figure 3.5. Mean (\pm 1SE) number of invertebrate taxa caught in pitfall traps at Ailaoshan: Litter depth treatments (a) and litter species treatments (b). Mean values with the same letters are not significantly different (p>0.05). Abbreviations: NL: no litter, C: control (natural litter), 10NT: 10 cm no topping-up of litter, 25NT: 25 cm no topping-up of litter, 10T: 10 cm with topping-up of litter; 25T: 25 cm with topping-up of litter; 1: *Schima noronhae*, 2: *Castanopsis wattii*, 3: *Lithocarpus hancei*, 4: *Lithocarpus xylocarpus*.

Invertebrate Diversity

In Xishuangbanna plots, different litter depths had little effect on either invertebrate diversity or equitability (Table 3.3). Litter species also had similar diversity and equitability, except for *C. burmannii* which had a lower diversity (*H*=0.659) and equitability (E_H =0.276) than the other species. In the Ailaoshan litter depth treatments, the two 25 cm plots had very low diversity and equitability compared to other treatments (25 cm without topping-up: *H*=0.301, E_H =0.121, 25 cm with topping-up: *H*=0.462, E_H =0.193). However, litter species at Ailaoshan all had very similar diversity and equitability.

Table 3.3. Shannon's Diversity Index, and Shannon's Equitability Index as measures of invertebrate diversity and evenness, respectively, for each treatment at each site. Shannon's Diversity Index is a relative measure, with a higher value indicating more diversity. Shannon's Equitability is a value between 0 and 1, with 1 meaning that all species are equally abundant.

	H (Shannon's Diversity)	E _H (Shannon's Equitability)
Xishuangbanna Litter Depth		
No Litter	0.883	0.383
Control	1.219	0.491
10cm No Topping-up	1.206	0.549
25cm No Topping-up	0.973	0.468
10cm Topping-up	1.018	0.442
25cm Topping-up	0.996	0.415
Xishuangbanna Litter Species		
No Litter	0.883	0.383
Control	1.219	0.491
Cinnamomum burmannii	0.659	0.275
Bambosa vulgaris	1.029	0.447
Shorea. chinensis	1.324	0.603
Dipterocarpus retuses	1.234	0.514
Ailaoshan Litter Depth		
No Litter	1.657	0.667
Control	1.237	0.482
10cm No Topping-up	1.305	0.544
25cm No Topping-up	0.301	0.121
10cm Topping-up	1.035	0.471
25cm Topping-up	0.462	0.193
Ailaoshan Litter Species		
No Litter	1.657	0.667
Control	1.237	0.482
Schima noronhae	1.187	0.571
Castanopsis wattii	1.258	0.525
Lithocarpus hancei	1.520	0.660
Lithocarpus xylocarpus	1.423	0.593

Discussion

The contents of the insect pitfall traps show that the two field sites – Xishuangbanna and Ailaoshan - differed in soil invertebrate composition. This is not unexpected because of differences in elevation, temperature and rainfall at these two sites. This is consistent with previous studies done on the sampling of invertebrates with elevation gain in tropical forests (Richardson *et al.* 2005, Rodriguez-Castaneda *et al.* 2010).

Some of the differences in invertebrate composition may also be attributed to the soil nutrient content of each site. For example, the availability of phosphorus influences the diversity of arthropods in tropical forests in Panama (Sayer *et al.* 2010). The soils at our two study sites had very different nutrient environments, fitting the trend that tropical montane forests are limited by nitrogen, and tropical lowland forests are limited by phosphorus. These soil nutrient differences likely underlie major differences in the soil invertebrate community.

Invertebrate Abundance Difference by Treatments

At the Xishuangbanna site, neither the litter addition treatments nor litter species treatments had a significant effect on soil invertebrate abundance. Indeed, the only significant difference in invertebrate abundance was the lower abundance of invertebrates in natural mixed litter treatments compared with those plots with litter removed. This partially rejects our original prediction that more litter will attract more decomposers and herbivores. While not statistically significant, the remainder of the prediction trends in the expected direction and there was an increased invertebrate abundance from natural mixed litter to 25 cm of litter to 25 cm of litter with topping up. This result is consistent with our prediction that increased litter

biomass results in higher invertebrate abundance. This general conclusion is also consistent with the findings of Sayer *et al.* (2010), who showed that increased litter depth often provides a larger habitat space for invertebrates. Another explanation that cannot be ruled out is that the application of the treatments, i.e. the addition of increasing litter was simultaneously adding increasing numbers of invertebrates, contributed to the availability of invertebrates to be captured by the traps. However, Sayer *et al.* (2010) also argues that sometimes the abundance of invertebrates captured by pitfall traps are not correlated with litter depth, and that more litter provides structural complexity that reduces the chances of litter fauna moving around and falling into pitfall traps. The increased number of invertebrates captured in plots from which litter was removed is surprising, because litter removal generally result in a decline in the populations of litter fauna (Sayer 2006). This may be due to the nature of pitfall trapping, and that only active invertebrates are moving across the forest floor to seek out areas with litter and structural complexity, and this increases their possibility of falling into a pitfall trap.

At Xishuangbanna, the different litter species did not result in significantly different invertebrate abundances, and this result rejects our prediction that different species of litter will attract different herbivores and decomposers. The invertebrate abundance and richness data also show similar results. This result is consistent with Donoso *et al.* (2010), in the forests of Panama, who showed that litter invertebrates are not specialized to different tree species, and that mixing several species of litter did not result in an increase in invertebrate diversity.

At Ailaoshan, litter depth did have a significant effect on invertebrate abundance, specifically in the two 25 cm litter depth treatments. Both treatments resulted in a significantly

higher invertebrate abundance, regardless of topping up, than the other treatments and the control. The increased invertebrate abundance was largely comprised of ants (order Hymenoptera) and it may be argued that the plots affected were on, or close to, ant mounds. This explanation is unlikely, and can be discarded, given that the plots for both treatments (n=18) were randomly placed in all 3 experimental blocks, and blocks were separated by about 40m. The depth and structural complexity of the litter added may have been an important factor for ants. This is consistent with results from previous studies in Malaysia (Burghouts et al, 1992), Puerto Rico (Yang *et al.* 2007), and Panama (Sayer *et al.* 2010) which suggest that an increase in litter quantity is more important to invertebrate abundance than an increase in litter quality. It may be argued that some of the ants could have been added along with the litter in the litter addition treatments. However, if this was a significant effect it would also have shown up in the 10 cm plots.

At Ailaoshan, different species of litter did not influence invertebrate abundance in general, and only those plots with *Lithocarpus xylocarpus* used as the litter treatments had a significantly reduced number of invertebrates. While we predicted that different species of litter would result in a different composition of invertebrates, this result is not surprising, because the four species are the dominant species of this forest, and other species would be taxonomically similar. However, *Lithocarpus xylocarpus* causing a decline in invertebrate abundance is surprising, because it is taxonomically similar to *Lithocarpus hancei*, and I would have predicted that these two species within the same genus would affect invertebrate decomposers the same way.

Invertebrate Taxa Richness

In the litter depth plots at Ailaoshan, the treatments with 10 cm of litter with topping up, and 25 cm of litter with topping up, resulted in decreased invertebrate richness in comparison to the litter removal and the natural mixed litter plots. I predicted that regular topping up of litter, and the resulting increased mass of litter available would increase invertebrate richness and abundance, but the opposite was detected. This pattern is also inconsistent with the invertebrate abundance data, where both the 25 cm of litter with and without topping up resulted in the highest abundance of invertebrates, mainly ants (Order Hymenoptera). Thus, I expected that the same two treatments would result in the lowest soil invertebrate richness in. Perhaps the regular topping of litter created a disturbance in the structure of the litter plots, and hindered soil fauna movement, thereby reducing the chances of falling into the pitfall traps. Interestingly, the same pattern was not seen in the litter depth experiment at Xishuangbanna, which followed the same litter topping frequency. This difference may be due to the spatial scale of the plots and the active invertebrate decomposers present. Due to the limited size of the field site, our experimental blocks and plots were placed closer together at Xishuangbanna than at Ailaoshan. Informal observations at both sites suggest that there were more active macroinvertebrates present at Xishuangbanna, probably due to the warmer and more humid climate. It is possible that the macroinvertebrates move around over a relatively large area, much larger than the area of my plots, and therefore the litter treatments did not result in any effect on these invertebrate abundances at Xishuangbanna.

At Xishuangbanna, neither litter quantity nor litter quality had an effect on invertebrate richness. While we captured 16 orders of invertebrates overall, the variation of taxa richness within each pitfall trap was very high, with an average of only 6-8 invertebrate orders per treatment. Initially, we classified invertebrates to Family, and sometimes to genus level, but for consistency across treatments, the data were pooled and scaled back to the Order level. This means that the finer level of taxa richness was not reflected in our data.

Sayer *et al.* (2010) found that at the local scale, arthropod abundance is correlated with litter mass and depth, and arthropod diversity is strongly associated with litter quality and nutrient concentrations. Therefore, it was surprising that the different species of litter used for treatments did not have an effect on invertebrate richness. Perhaps the amount of litter used for the litter species treatments were not large enough to show effects of nutritional quality on invertebrates.

Diversity and Evenness Index by Treatments and Site

Based on previous data in invertebrate abundance and richness, it is not surprising that Shannon's Diversity Index and Evenness shows similar results. In Xishuangbanna, the plots with litter removed resulted in a lower diversity and evenness than the other treatments in the litter depth experiment. This confirms our prediction that as the physical and chemical properties of litter and their effects on the forest floor are removed, diversity and evenness of soil fauna is affected. In the litter species experiment, plots with *Cinnamomum burmannii* had a lower diversity and evenness compared to the other treatments. Interestingly, *C. burmannii* is grown as commercial cinnamon in many parts of the world, and perhaps there are some leaf chemical properties that deter herbivores and decomposers, which would explain that only a few specialized invertebrates would be chomping at the leaves and result in lowered diversity and evenness. The phenolic properties of *C. burmannii* are documented in previous studies, and are consistent with our conclusions (Penuelas, *et al.* 2010, Li *et al.* 2012), and these are likely to have deterred herbivores and decomposers from eating and decomposing the leaves.

At Ailaoshan, 25 cm of litter resulted in high abundance and low richness of litter invertebrates, and this trend is reflected in the low Shannon's diversity and equitability values at with the same treatment. The 25 cm plots had high numbers of ants (order Hymenoptera) which decreased the overall diversity and evenness values, because other orders of invertebrates were relatively much less common. In contrast, at Ailaoshan the identity of litter species had no influence on diversity or evenness in any of the treatments. The low invertebrate abundance in pitfall traps under *Lithocarpus xylocarpus* was not reflected in the diversity and evenness values, so while the overall numbers were low, the relative abundance of the constituent invertebrates were quite even.

Sampling Using Pitfall Traps

The dependent variable used throughout this experiment is the number of invertebrates that were captured by pitfall traps. The use of pitfall traps, however, is not without problems. The number of soil invertebrates is likely to be underestimated. Because pitfall traps were buried beneath each litter treatment, they may not accurately capture all the invertebrates within the litter, especially microarthropods that only move very small distances. In addition, the 25 cm litter treatments may harbour many invertebrates that never make it to ground level.

Other common problems associated with pitfall traps include capturing non-target organisms such as small rodents and frogs, added rainfall which dilutes the preservative solution, and the washing in of litter debris such as leaves and twigs. To overcome the above problems, the traps had plywood covers that were raised above the opening using small sticks, and the gaps in the opening of the pitfall traps were only about 5 cm wide, and would exclude some larger-bodied macroarthropods. This also prevented any small rodents and frogs from getting in, and we did not find any non-target organisms in our samples. The plywood cover also helped in preventing lots of litter debris and rainwater from diluting our cups, but sometimes due to the slope of the plot and heavy rainfall, a handful of samples had litter debris and increased water levels.

Our results show interesting comparative patterns of invertebrate abundance, composition and richness between a tropical lowland forest (Xishuangbanna), and a tropical montane forest (Ailaoshan) in southwestern China. Many of the differences may be attributed to the different abiotic and biotic conditions at the sites. At the local scale, differences in invertebrate abundance may be explained by litter mass, and there is a general trend of increasing invertebrate abundance with increasing litter mass, however, this result was not statistically significant, and may reflect a threshold in the amount of litter present in order to have an effect on the abundance of litter invertebrates. Litter species manipulations largely did not have any effect on invertebrate abundance or diversity, which was not consistent with our predictions. Our results mostly agree with previous studies that litter mass and quality influence soil invertebrate activity, but more studies in this region of the world should be done to tease apart the litter and soil invertebrate dynamics.

CHAPTER 4

Conclusions and Future Directions

This research explored the litter and nutrient dynamics of two subtropical forests in Yunnan province in southwestern China. We compared the effects of litter quantity and litter quality on soil nutrients and litter invertebrates for 3 months in a tropical lowland and a tropical montane forest. We found that litter quantity manipulations had effects on both soil nutrients and litter invertebrates, but the results were only significant with the extreme litter quantity manipulations - 25 cm of litter with topping, and removing litter. The two sites had significant differences in both soil nutrients and invertebrate abundance and richness. Litter species treatments did not have any major effects on either soil nutrients or litter invertebrates. Overall, this research provides insight into the litter and nutrient dynamics of two highly diverse, contrasting forests in China.

Litter Treatments

To test the effects of litter biomass in these forests, we only used one response variable – soil nutrients, and any differences in litter decomposition between treatments were indirectly implied with changes in soil nutrients between the treatments and the control. Further research would benefit from analyzing the nutrient content of the brown litter of each species and, rather than comparing soil nutrient levels under different litter treatments, instead compare soil nutrient levels with the nutrient levels in the brown litter. This information, together with our soil nutrient data, would allow us to identify which nutrients were lost through decomposition. Also, we did not calculate rates of decomposition in either the litter quantity or litter quality experiments, because we were measuring soil nutrient responses based on litter depth, and not actual weight. In a future study, it would be useful to weigh the plant litter before and after the field season and calculate a decomposition rate. This way, each litter treatment would have a standardized litter mass, instead of approximately the same litter depth or volume.

The Use of Soil Nutrient Probes

Plant root simulator probes are typically used for agriculture and soils where nutrient concentrations are high, and their use is relatively new in experimental ecology. Probes have an enormous advantage over traditional soil cores and other methods of sampling, because the use of the ion-exchange membrane enables researchers to get a more precise estimate of what levels of various nutrients that are biologically available to the plants over an extended time period, rather than availability at one point in time. The probes are also capable of measuring numerous nutrient levels in the soil simultaneously, and there is no need to take separate readings, which furthers increases accuracy.

Invertebrate Sampling Methods

Using pitfall traps to sample invertebrates may result in some underestimation of the population of invertebrates that are present in the litter. Because the pitfall traps are placed at the bottom of each litter treatment, many of the invertebrates in higher strata of the litter may not actually get close enough to traps so as to be caught. Also, the types of invertebrates that were caught using the pitfall traps may not be all detritivores. My samples also included

different orders of spiders and larger insects that may act as predators of smaller invertebrates. While these larger organisms do not directly influence the breakdown of litter, the pitfall traps allowed us to sample a high diversity of the litter invertebrates, which provides more insight into the trophic interactions within the complex litter habitat.

Future Directions

While this research has distinguished the effects of both litter quantity and quality on both soil nutrients and litter invertebrates, the next step is to connect these treatments to the lack of regeneration of tree seedlings in this region. The original research plan was to manipulate the litter treatments to test if there would be a response in seedling germination and survival. However, the seasonality of our experiment precluded this test. The next step would be to observe the percentage of germination and survival of the natural seedlings in the forest. This could potentially be done by mixing seeds from the natural seedbank into the treatment plots and monitor how they respond to the different soil chemistries and measure the rates of germination and establishment. In addition, seedlings of species that can be germinated in the greenhouse could be transplanted into each plot, and their subsequent survival monitored over several months. These experiments would directly test if the quality and quantity of litter, through their effects on soil nutrients and the invertebrate community, has changed soil conditions to such an extent that seed germination rates will be affected. Overall, we have shown that the litter dynamics are quite different between the montane and lowland sites, and litter manipulations can have varied effects on soil nutrients, litter invertebrates, and potentially regeneration of the next generation of forests.

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