The Effects of Millipedes (*Harpaphe haydeniana*) on Microbial Decomposition of Leaf Litter

by

Yoriko Suzuki

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Abstract

Most estimates of litter decomposition rates do not account well for the effects of soil macrofauna, and so are suspect in ecosystems in which litter-transforming soil fauna are abundant. In coastal rainforests, millipedes consume substantial amounts of leaf litter, most of which is egested as faecal pellets. Little is known about the fate of this material, which hinders estimation of realistic rates of litter decomposition in these ecosystems.

In this study, I assess the influence of feeding by the millipede (*Harpaphe haydeniana*) on decomposition of leaf litter by comparing rates of CO_2 release during laboratory incubation from leaf litter which has been ingested by millipedes and transformed into faecal pellets with that from litter which has not been ingested by millipedes. Changes in litter microbial communities as a consequence of millipede ingestion are assessed by comparing the PLFA profiles of faeces and uningested litter during incubation.

Rates of CO_2 release from faeces and litter were similar. CO_2 release was higher in maple litter than Douglas-fir litter, and this difference persisted in the faeces from litter that millipedes fed on. Differences in bacterial abundance between litter types were also retained during millipede gut passage. Grinding of litter increased CO_2 release, as did grinding of faeces, indicating that structure of litter and millipedes' faecal pellets may restrict microbial access and thus decrease the decomposition rates. Microbial activity and abundance did not differ between leaf litter and faeces incubated alone vs together.

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List of Abbreviations

ANOVA	analysis of variance
BMF	millipedes' faeces derived from bigleaf maple litter
BML	bigleaf maple litter
BMm	mixture of litter and faeces derived from bigleaf maple
BMmF	faeces from a microcosm with BMm
BMmL	litter from a microcosm with BMm
BMp	pure litter or faeces derived from bigleaf maple
C	carbon
^{14}C	carbon-14
˚C	degrees Celsius
cm	centimetre
C/N ratio	carbon to nitrogen ratio
conc	concentration
CO_2	carbon dioxide
DFF	millipedes' faeces derived from Douglas-fir litter
DFFg	ground faeces derived from Douglas-fir litter
DFL	Douglas-fir litter
DFLg	ground Douglas-fir litter
DFm	mixture of litter and faeces derived from Douglas-fir
DFmF	faeces from a microcosm with DFm
DFmL	litter from a microcosm with DFm

DFp	pure litter or faeces derived from Douglas-fir
DNA	deoxyribonucleic acid
g	gram
Gt	gigatonne
ml	millilitre
mm	millimeter
N_2	nitrogen
PLFA	phospholipid fatty acid
SAS	Statistical Analysis Systems
µm	micrometre

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To my father who taught me the joy of science

Chapter 1: Introduction

1.1 Background

Understanding the fluxes of carbon within ecosystems has become an important topic due to the increasing demand for knowledge about how to mediate climate change. Since carbon dioxide is an important greenhouse gas, it is important to determine how we could reduce the release of carbon to the atmosphere. The terrestrial biosphere contains 2500 Gt of carbon, which is the third largest carbon reservoir after the lithosphere (75,000,000 Gt) and ocean (38,400 Gt) (Falkowski *et al.* 2000). In the terrestrial biosphere, more than half of the total carbon is in organic forms (1550 Gt; inorganic carbon is 950 Gt) (Lal 2004). Increasing accumulation of carbon in soils is one strategy, but as Janzen (2006) pointed out, organic carbon enhances microbial activities which increased the release of respiratory CO_2, so we also need to consider strategies to reduce the release of CO_2 back to the atmosphere through the process of litter decomposition (Prescott 2010).

Litter decomposition has been measured mainly with litter bags (Huhta 2007). In this method, a known mass of litter is confined within mesh bags, and remaining mass is periodically measured in order to estimate the decomposition rate. One criticism of this technique is that the mesh of the litter bag may restrict faunal access to the litter. If the mesh is too fine for soil animals to go through, fragmentation of litter would slow down. Coarse – meshed bags have been used to estimate the degree to which decomposition is stimulated by soil fauna, but this may overestimate decomposition by allowing litter fragments to fall out of the bag (Kampichler and Bruckner 2009, Prescott 2005). The importance of faunal activities in litter decomposition processes is well documented. For instance, in a microscopic study of

pine needle litter decomposition, Ponge (1991) concluded that fauna such as nematodes digests needles after two stages of fungal attacks. A variety of soil animal species was observed also in a field experiment using litter bags implying that the animals feed on the plant residues and thus influence litter decomposition (Anderson 1975). Several microcosm experiments also demonstrate the significance of fauna in decomposition and mineralization. For example, the presence of meso-fauna increased plant growth in microcosms relative to defaunated microcosms, indicating that nematodes, enchytraeids and microarthropods enhance nutrient mineralization (Setälä and Huhta 1991). Collembolans enhanced microbial cellulase production although no significant change was observed in other factors such as fungal biomass and microbial respiration (Faber *et al.* 1992). Kandeler *et al*. (1999) demonstrated an increase in protease production in the H layer in a field mesocosm experiment in a spruce forest in Australia due to the presence of some soil microarthropods. Microcosms with mixtures of micro-, meso- and macro-fauna enhanced N uptake by poplar compared to those with micro- and meso-fauna or micro-fauna only (Setälä *et al*. 1996). These studies indicate the importance of faunal communities in litter decomposition and nutrient mineralization although the specific functions of individual faunal species are not yet clear.

According to Visser (1985), the effects of faunal activities on microbes can be classified into either reduction of fungal diversity, changes in fungal community structures or transportation of microbes. Soil invertebrates homogenize microhabitats through litter breakdown and soil agitation, resulting in the presence of only a limited number of microbial species that are adapted to the environment. Fungal community structure may be modified through selective feeding of soil fauna on particular species of fungi. Finally, microbes

attached to invertebrates' body and/or living in their gut are carried to different habitats, resulting in the spread of the microbes (Harinikumar and Bagyaraj 1994). The significance of these functions varies among faunal species because each species has different capabilities. Fragmentation is strongly related to the body size of animals; most meso- and micro-fauna feed on plant residues that are already fragmented by macro-fauna. Only macro-fauna modify soil structure; earthworms create soil aggregates by burrowing (Frouz 2011) and supply habitat for meso- and micro-fauna that dwell in soil pores (Szlavecz 1985). Therefore, macro-fauna may affect litter decomposition by influencing the activities of smaller animals and microbes (Frouz 2006, Gómez-Brandón *et al*. 2010).

Among the many species of macro-fauna in soils, earthworms are widely recognized as a key species in litter decomposition (Huhta 2007, Scheu 2003). They alter soil profiles by ingesting both organic and inorganic materials, burrowing, and excreting faecal pellets (Lavelle 1997; Staaf 1987). Millipedes, like earthworms, feed on decayed litter. Smaller invertebrates such as collembolans, mites, enchytraeids and isopods are often found with millipedes' faeces (Nicholson *et al*. 1966) indicating that millipedes' faeces are the important food source for those animals. Few earthworms but many millipedes are found in coastal rainforests in British Columbia (Battigelli *et al*. 1994, Setälä *et al*. 1996), and this may be a situation in which functionally similar species undertakes the role in case of absence of a key species (Huhta 2007). One of the millipede species in coastal rainforests in BC, *Harpaphe haydiniana,* was estimated to consume about 36 % of annual aboveground litter fall (Cárcamo 2000). This means that more than one-third of above-ground litter passes through millipedes' gut, inferring that the effects of millipedes on an ecosystem are significant. Cárcamo (2000) also reported that *H. haydiniana,* assimilate only about 10% of their

consumption, so their overall effect on decomposition and C flow depends largely on the fate of their faecal pellets.

1.2 Biology of Millipedes

The general biology of millipedes has been well summarized by Hopkin and Read (1992). Millipedes are arthropods with 10,000 species within the Class Diplopoda. They have two pairs of legs in one body segment, from which the word "diplopods" is derived. Class Diplopoda consists of two subclasses and 14 orders, which can be classified into 5 groups based on their ecomorphological types: long, cylindrical millipedes with hard cuticles which are suitable for burrowing; shorter millipedes with a flat head which are suitable for penetration; millipedes with compressible segments such that they good at broadening gaps; millipedes that protect themselves by rolling up into a ball; and hairy, flat millipedes that cannot burrow. Millipedes' body size also varies from 2 mm and 30 cm. Most millipedes dwell under leaf litter or rocks on the soil surface and in soil pores, although some species are reported to live under bark or even climb trees. These habitats relate to their food source, which is mostly decayed litter and plant residues. Although not common, some species of millipedes are reported to be geophagous as well as coprophagous. Millipedes generally have a long life - for example, some species in the genus Parafontara, known as "train millipedes" in Japan, take 7 to 8 years to mature (Niijima and Shinohara 1988). *Julus scandivavius* moult 9 to 13 times in 2 years to mature and overwinter three times during their life (Blower 1970).

The amount of food consumption varies among and in each development stadium (Kheirallah 1978); most food is consumed at the beginning of each instar (Blower 1974). Consumption also depends on the degree of decay and/or microbial colonization of the

residue (Hafidi *et al*. 1998). The woodlouse, *Procellio scaber*, prefers more-decayed pine needles over partially decayed ones (Soma and Saito 1983). Likewise, millipedes feed upon decayed litter, not fresh fallen leaves (David and Gillon 2002). Uneven consumption of leaf litter of different tree species due to the differences in their quality has been observed for various faunal species (Cotrufo *et al*. 2005, Hättenschwiler and Gasser 2005, Hendriksen 1990, Tian *et al*. 1995). Palatability seems to be related to C/N ratio and phenol content of the food (Hendriksen 1990, Kadamannaya and Sridhar 2009); earthworms removed more litter of willow species with low tannin content from a litter bag than that with high tannin content (Šlapokas and Granhall 1991). Millipedes also prefer litter species with relatively low tannin content (Cárcamo 2000, Edwards 1974, Kadamannaya and Sridhar 2009). On the other hand, the feeding rate of woodlouse on *F. silvatica* decreased after two weeks of increasing consumption, coinciding with the increase and decrease of abundance of bacteria colonized in the litter (Daniel *et al.* 1997). The depletion of available carbon in the later stage of decomposition was confirmed by Maraun and Scheu (1996) who demonstrated that in spring when fresh carbon was abundant in the litter, microbial activity and biomass were enhanced by litter fragmentation, whereas in fall when fresh carbon was less abundant, microbes did not flourish regardless of the increased surface area. The preference of soil fauna for partially decayed "conditioned" litter may also reflect soil animals' preference for litter with abundant fungal tissue. Mites were reported to selectively feed on colonized fungi on decayed litter (Hubert *et al*. 2000). Hyphal material was much more abundant than pine needles in the gut of isopods *Porcellio scaber* (Soma and Saito 1983).

Millipedes influence litter decomposition processes both directly and indirectly. During gut passage, litter is fragmented into smaller particles, chemical substances in litter

are degraded, some nutrients are assimilated into the millipede's body, microbes colonized in litter are digested while gut microbes flourish, and faecal pellets are formed. Each event results in accelerated or decelerated microbial activities, and the subsequent rate of decomposition is influenced by these events.

1.3 Fragmentation of Leaf Litter

Faunal grazing significantly enhances litter breakdown (Tian *et al*. 1995). Litter fragmentation may be related to size and structure of mandibles; species with large mandibles can graze on large particles of litter while others can only feed on fine fragments. This is supported by the correlation between feeding habits and cheliceral size among oribatid mites, indicating that animals with large chelicerae feed on large pieces of plant residues while those with small chelicerae feed on fragments (Kaneko 1988). Anatomical observation of millipedes' mouthparts revealed that millipedes are able to destroy cells of consumed litter as well as microbes (Köhler and Alberti 1990). The most significant effect of litter fragmentation on decomposition processes is an increase in surface area, which is broadened between one and 20 million times through millipedes' grazing (Kheirallah 1990). This increase in surface area of litter enables microbes to access to their food sources and thus, accelerates decomposition. In addition to the physical change, litter breakdown increases available glucose, resulting in high initial respiration of microbes (Maraun and Scheu 1995). Because millipedes consume a large amount of litter, the effect of fragmentation must be significant.

1.4 Biochemical Degradation

Biochemical changes of consumed litter through gut passage result from two antagonistic phenomena: degradation of complex compounds and assimilation of simple compounds. Enzymes in the gut fluid can degrade chemical compounds to some degree, resulting in an increase of easily decomposable substances, while millipedes selectively absorb simple compounds, resulting in stabilization of the remaining substances. The digestive system of millipedes consists of glands such as salivary and maxillary glands and a long pipe linking their mouth to their intestine. The intestine can be divided into three parts: foregut, midgut and hindgut. Digestion of consumed litter takes place mainly in the foregut where salivary glands excrete enzymes (Hopkin and Read 1992). In studies of enzymes in guts of collembolans and mites, amylase, xylanase, laminarinase, lichenase, cellulase complex and trehalase were detected (Urbášek and Rusek 1994; Urbášek and Starý 1994, Hubert *et al*. 1999). As for millipedes, catalase, cellulase and peroxidase were found in the gut of three species of Class Diplopoda, while aldehyde oxidase, which was found in guts of Isopoda and Mollusca, was not detected (Hartenstein 1982). The presence of cellulase is consistent with a microscopic observation, in which microbial cell walls dipped in millipedes' midgut fluid were destroyed, indicating that the fluid has the ability to degrade chemical compounds by hydrolytic activities (Byzov *et al.* 1998a). The origin of these enzymes could be either the animals or the microbes that are capable of surviving in their gut. Studies using transmission electron microscopy and epifluorescent microscopy found bacteria and actinomycetes in earthworm intestine (Krištůfek *et al*.1994, 1995). In guts of enchytraeids, some ingested bacteria survived and produced enzymes that degrade chitin (Krištůfek *et al*. 1999). Microbes have also been found in millipedes' guts (Guzev and Byzov

2006). Knapp *et al.* (2009a) analyzed the DNA samples from millipedes' gut contents and showed that the microbial community in the gut is not affected by millipedes' food, indicating the possibility of microbial symbioses. Therefore millipedes may take advantage of microbial extracellular enzymes.

1.5 Assimilation of Nutrients

Despite enzymatic activities in millipedes' gut to break down chemical compounds in ingested litter, stabilization of soil organic matter is reported. Cárcamo (2000) demonstrated that millipedes assimilate only about 10 % of the litter consumed, which is similar to David and Gillon's (2002) estimate of 6 %, indicating that most of the litter consumed by millipedes stays intact throughout gut passage. More detailed comparison of chemical composition of leaf litter and millipedes' faeces indicated that millipedes utilize only water-soluble and easily degradable compounds such as lipids, carbohydrates and short-chain amino acids (Rawlins *et al.* 2006); therefore, the remaining substances consist of only recalcitrant materials. Enhanced stabilization of material resulting from gut passage was demonstrated in an experiment with oak leaf litter and millipedes' faeces (Rawlins *et al*. 2007). Addition of oak leaf litter to a dry sieved soil significantly increased the microbial respiration rate in the early stage of the decomposition while addition of pill millipedes' faeces did not differ from the control, indicating a low microbial activity on faeces. Generally, microbes specific to degrading recalcitrant substances ("K-strategists") grow slowly whereas those limited to decomposition of easily degradable substances ("r-strategists") grow rapidly (Ekschmitt *et al.* 2005). This may explain the low respiration rate in microcosms with millipedes' faeces because most microbes in those microcosms would be K-strategists. Gram-negative bacteria

such as pseudomonads are r-strategists and Gram-positive bacteria such as corynebacteria are K-strategists (Margesin *et al.* 2003). Microbial activity on faecal material may also be hindered by the physical properties of faecal pellets which may have low surface area : volume ratio and coatings that are resistant to microbial or enzyme attack (Martin and Marinissen 1993).

1.6 Effects of Microbes

As mentioned earlier, millipedes influence microbial activities and communities by increasing surface area of leaf litter and access to carbon and nutrients, decomposing complex chemical compounds, and taking up simple compounds. Millipedes can also directly influence microbes – by digesting microbes and by providing microbial habitats. In a study of collembolans and microbial activities, the presence of collembolans differentially influenced fungal and bacterial biomass; fungi decreased while bacteria increased with collembolan grazing (Hanlon and Anderson 1979). Maraun and Scheu (1996) pointed out that ergosterol (an indicator of the presence of fungi), decreased by 71 % during millipede gut passage while loss of total microbial biomass was 42 %, indicating more intense digestion of fungi relative to to bacteria. Similarly, Byzov *et al.* (1998a) observed rapid reduction of certain fungal species in millipedes' midgut fluid. In contrast, comparisons of bacteria from leaves and faeces showed that gut passage increased the bacterial population (Anderson and Bignell 1980). This supports the finding of Byzov *et al*. (1996) that although most bacteria are killed in the midgut fluid of millipedes, some survived cells grow in the hindgut. A more detailed study using ^{14}C-labelling showed that some microbes, especially fungi, were killed in millipedes' midgut, while the remaining organisms recovered in the

hindgut, resulting in an increase in bacterial population and decrease in fungal population during millipedes' digestion (Byzov *et al.* 1998b).

1.7 Questions and Hypotheses

It is evident from this review of the literature that soil fauna such as millipedes have complex effects on the process of litter decomposition and on the microbial communities involved in decay. Millipedes may enhance decomposition by fragmenting litter, but gut passage appears to stabilize the organic matter and faecal pellets may restrict microbial access, hence reduce its rate of decay. Millipede feeding reduces populations of some micro-organisms (especially fungi) but increases others (especially bacteria), and alters the composition of the microbial communities (Visser 1985). This makes it difficult to generalize and so to predict the net effects of millipede activities on fluxes of carbon and nutrients.

In this study, I assess the influence of feeding by the millipede on decomposition of leaf litter by comparing rates of CO_2 release from leaf litter which has been ingested by millipedes and transformed into faecal pellets with that from litter which has not been ingested by millipedes during laboratory incubation. Changes in litter microbial communities as a consequence of millipede ingestion are assessed by comparing the PLFA profiles of faeces and uningested litter during incubation. Based on studies to date, I hypothesized that CO_2 release from millipede faeces would be less than that from uningested litter. I also hypothesized that millipede faeces would have smaller fungal but larger bacterial PLFA abundance relative to uningested litter.

Since millipedes egest 90% of the litter mass that they consume, the nature of their faecal pellets is likely to differ depending on the nature of litter that they consume. To

determine if the effects of millipede ingestion depends on the type of litter consumed, I compared effects of millipede ingestion of two contrasting leaf litters common in coastal forest – Douglas-fir (*Pseudotsuga menziesii*)needles and bigleaf maple (*Acer macrophyllum*) leaves. I hypothesized that the litter type difference in microbial activity and abundance would persist through millipede ingestion.

I attempt to tease apart the effects of millipede activity in fragmenting litter and thereby increasing surface area (hence accessibility to microbes) from that of ingestion by comparing CO_2 release and PLFA profiles in intact and ground litter. I also address the possibility that the physical nature of faecal pellets inhibits microbial abundance and decay by comparing CO_2 release and PLFA profiles in intact and ground millipede faeces. I hypothesize that grinding of litter and faecal pellets will increase microbial activity and decay as a consequence of increased accessibility to microbes.

In forests, millipedes feed on leaf litter and excrete faeces on the litter, such that the litter and the faeces decompose together. It is therefore possible that the presence of litter affects the decomposition of faeces and vice versa. I address the possibility that mixing litter and faeces alters microbial communities and activity on either litter or faeces by comparing CO_2 release and PLFA profiles of pure and mixed litter and faeces.

Chapter 2: Materials and Methods

2.1 Sampling

Leaf litter and millipedes were collected from Pacific Regional Park, Vancouver, Canada in forest containing Douglas-fir and bigleaf maple. Millipedes were captured from May to October in 2009 and in July and August in 2010. Juvenile individuals, whose bodies were grey and sometimes had fewer segments and premature reproductive organs, were excluded. Millipedes were kept in plastic containers with silica sands at the bottom and fed either Douglas-fir needles or bigleaf maple leaves. Their faeces were collected with forceps within 48 hours of defecation and stored in grass jars at -20 ˚C. Douglas-fir needles and maple leaves for feeding millipedes were collected at the same time that millipedes were captured. The needles and leaves were stored in a refrigerator and rinsed with tap water and distilled water before feeding. Leaf litter samples for microcosm experiments were collected in July and August 2010 and stored at 4 ˚C. Maple leaves were cut to approximately 5 cm x 3 cm using a knife and the thick veins were removed as millipedes did not consume veins. Some of the Douglas-fir litter and faeces derived from Douglas-fir were ground using a mill (Wiley mill, mesh size 20) and a mortar and a pestle, respectively.

2.2 Microcosms

Silica sand (Target Products Inc., Canada) was put in 500-ml glass jars to leave airspace of 400 ml. The jars with the sand were then autoclaved for 45 minutes. Nylon mesh with 0.5 µm pores (Plastok, UK) was placed on top of the silica sand in the jars to prevent small particles of samples from falling through the gaps between sand particles.

Water content of the litter and faeces samples was calculated by measuring fresh and oven-dry weights. A sample weighing 1.0-1.2 g (dry weight equivalent) was placed on top of the nylon mesh in microcosms. Each microcosm had one of the following 9 treatments: 1) DFL, Douglas-fir litter; 2) DFF, faeces derived from Douglas-fir; 3) DFm, litter and faeces of Douglas-fir; 4) DFLg, Douglas-fir litter, ground; 5) DFFg, faeces derived from Douglas-fir, ground; 6) BML, bigleaf maple litter; 7) BMF, faeces derived from bigleaf maple; 8) BMm, litter and faeces of bigleaf maple; 9) none. Litter and faeces from DFm and BMm were sorted for PLFA analyses (DFmL, DFmF, BMmL, BMmF, respectively).There were 4 replicate jars of each treatment.

Microcosms were covered with a thin polyethylene bag to allow gas exchange and prevent moisture loss (Eno 1960) and watered every 2 to 3 days to maintain moisture. They were kept in the laboratory at room temperature.

Time (days) / Treatment			0		1	2	3		4	7		14		28		56	
DF	L		C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
	F		C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
	m	L	C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
		F		P				P			P		P		P		P
	g	L	C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
	g	F	C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
BM	L		C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
	F		C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
	m	L	C	P	C	C	C	P	C	C	P	C	P	C	P	C	P
		F		P				P			P		P		P		P
Blank			C		C	C	C		C	C		C		C		C	

Figure 2.1. Schematics of experiments. DF, Douglas-fir; BM, bigleaf maple; L, litter; F, faeces; m, mixture of litter and faeces; g, ground; C, CO_2 release measured; P, PLFA analyzed.

2.3 CO_2 Release

Prior to gas sampling, the relative humidity of each microcosm was measured so that the influence of partial vapour pressure could be calculated later (Rochette & Bertrand 2008). Microcosms were sealed with lids equipped with rubber septa and gas samples were taken every 10 minutes after sealing up to 5 samples per jar. The 20-ml syringe was flushed with compressed air 3 to 4 times before each sampling to clean the inside of the syringe, and then 12 ml of compressed air was injected to the microcosm and the air inside the microcosm was mixed by pumping 4 times. Then 12 ml of gas was extracted and injected into a vacuum Exetainer of 5.9 ml (719W, Labco Limited, UK) with teflon/silicon septa (C401560, Fisher Scientific, USA) and rubber septa (VW101, Labco Limited, UK). The Exetainers were evacuated prior to sampling. The CO_2 concentration in each sample was measured using a gas chromatograph (HP 5890 Series II, GMI Inc., USA). A linear regression of CO_2 concentrations at 4 or 5 sampling times during the hour was used to calculate CO_2 release in μg CO_2 per g dry sample per hour.

2.4 Phospholipid Fatty Acid (PLFA) Analyses

Phospholipid Fatty Acid analysis (PLFA) was used to estimate the abundance of total bacteria, Gram-negative bacteria, Gram-positive bacteria and actinomycetes as described by Frostegård *et al*. (1993). Following extraction of the gas samples from the microcosms, the remaining litter and faeces were placed in plastic bags and freeze-dried. Mixed samples (DFm and BMm) were sorted into litter and faeces. The freeze-dried samples were ground using a mortar and a pestle.

Fatty acids were extracted from samples (0.15 g of litter, 0.30 g of faeces) following the procedure of Dewi (2009). Fatty acids are designated by the ratio of the total number of carbon atoms:number of double bonds, followed by the position (ω) of the double bond from the methyl end of the molecule. Cis and trans configurations are indicated by *c* and *t*, respectively. The prefixes *a* and *i* indicate anteiso- and iso-branched fatty acids, respectively. Cyclopropane fatty acids have the prefix 'cy' (Bååth *et al*. 1995, Steer & Harris 2000). Fatty acids identifying Gram-positive bacteria include *i*15, *a*15:0, *i*16:0, *i*17:0 and *a*17:0; those identifying Gram-negative bacteria include *i*16:1ω7*c*, 16:1ω9*c*, 16:1ω7*c*, i17:1ω8*c*, cy17:0, 18:1ω7*c*, 18:1ω5*c*, cy19:0. Total Bacteria is calculated as the sum of Gram-positive and Gram-negative fatty acids, plus 15:0, 17:0 18:0. Actinomycetes are identified by a methyl group on the tenth carbon atom from the carboxyl end of the molecule and include 10Me16:0, 10Me17:0, 10Me18:0, 10Me19:0. Biomarkers were identified using a gas chromatograph (Agilent 6890N, Agilent Inc., USA) equipped with a mass selective detector (Agilent 5973N, Agilent Inc., USA).

2.5 Data Analyses

The data were analyzed using an ANOVA model for split-plot designs since the dates are repeated-measures (Kuehl, 1994) (see Tables in the Appendix) using SAS (SAS Institute Inc., Version 9.2, Cary, NC, USA). A 95% of confidence interval was used to determine significant differences. All data were corrected for dry weight and averaged among the replicates.

Chapter 3: Results

3.1 Litter and Faeces

The ANOVA comparing CO_2 release from litter and faeces indicated significant time x treatment interactions, therefore sampling dates are compared individually (Table A.1). Likewise, differences between litter and faeces were distinct for the two litter types (Table A.2 and Figure 3.1). In Douglas-fir there were no significant differences in CO_2 release between litter and faeces throughout the incubation. In bigleaf maple, CO_2 release from litter was greater than that from faeces on Days 0, 1 and 2 but declined to similar levels after two days. On Days 4 and 28, significantly more CO_2 was released from faeces than from litter of maple (Figure 3.1).

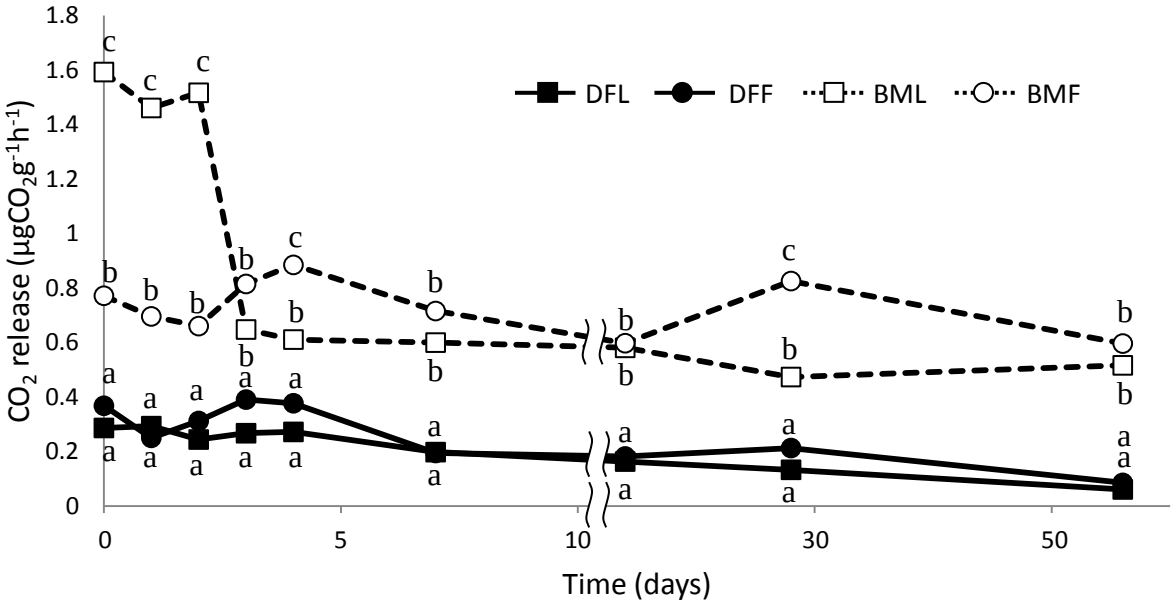

Figure 3.1. CO_2 release from litter and faeces of Douglas-fir and bigleaf maple during an eight-week laboratory incubation in µg CO_2 per g dry mass sample per hour. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

The ANOVA comparing PLFA biomarkers for total bacteria, Gram-positive bacteria, Gram-negative bacteria and actinomycetes indicated significantly higher abundance of these groups of microbes in litter than in faeces (Tables A.3, A.5, A.7 and A.9). Biomarkers for total bacteria and Gram-negative bacteria were significantly more abundant in maple litter than in faeces derived from maple litter at all sampling times (Figures 3.2 and 3.3, Tables A.4 and A.6). There were significantly higher abundance of total bacteria in Douglas-fir litter than in faeces derived from Douglas-fir (Figure 3.2 and Table A.4), but there were no significant differences between Douglas-fir litter and faeces in abundances of Gram-negative bacteria (Figure 3.3 and Table A.6).

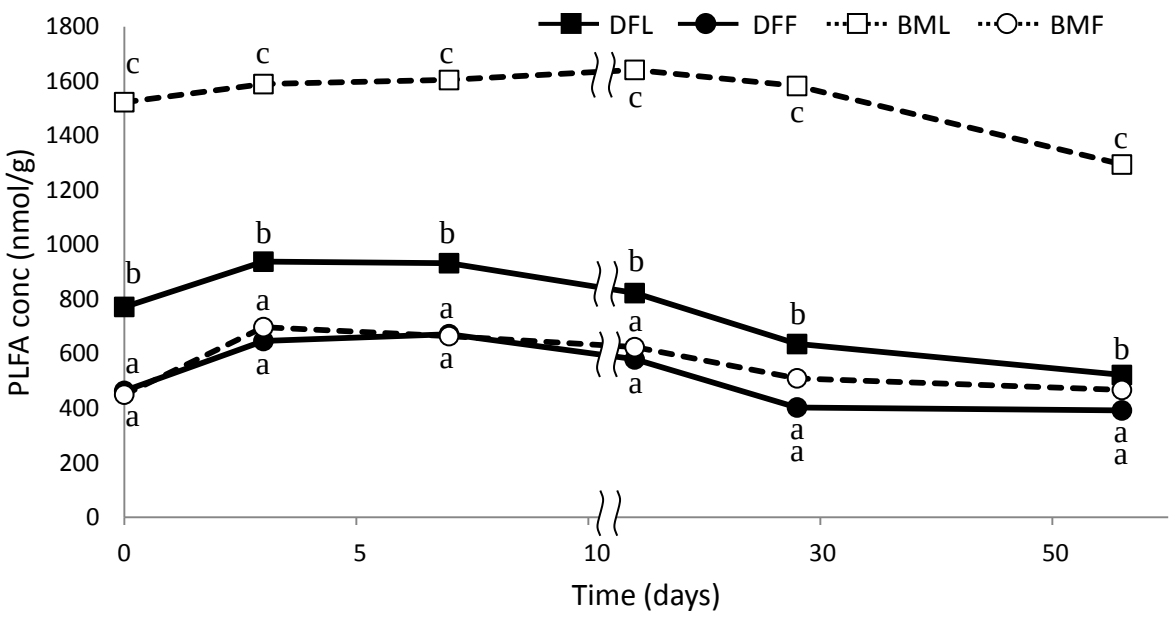

Figure 3.2. Abundance of total bacteria in litter and faeces of Douglas-fir and bigleaf maple during the eight-week laboratory incubation in nmol PLFA per gram dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

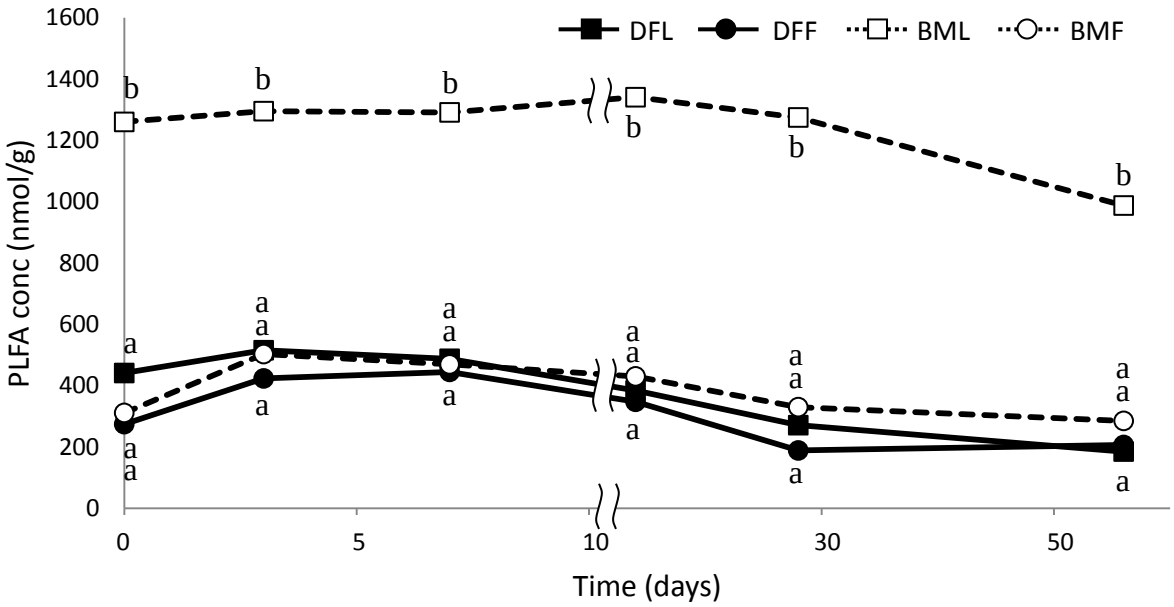

Figure 3.3. Abundance of Gram-negative bacteria in litter and faeces of Douglas-fir and bigleaf maple during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

Gram-positive bacteria were more abundant in Douglas-fir litter than faeces on all sampling dates (Table A.8 and Figure 3.4). In maple, Gram-positive bacteria were significantly more abundant in litter than in faeces on Days 0, 28 and 56 (Table A.8 and Figure 3.4). Actinomycetes were more abundant in litter than in faeces in maple throughout the incubation, as well as in Douglas-fir on Days 0 and 3 (Table A.10 and Figure 3.5).

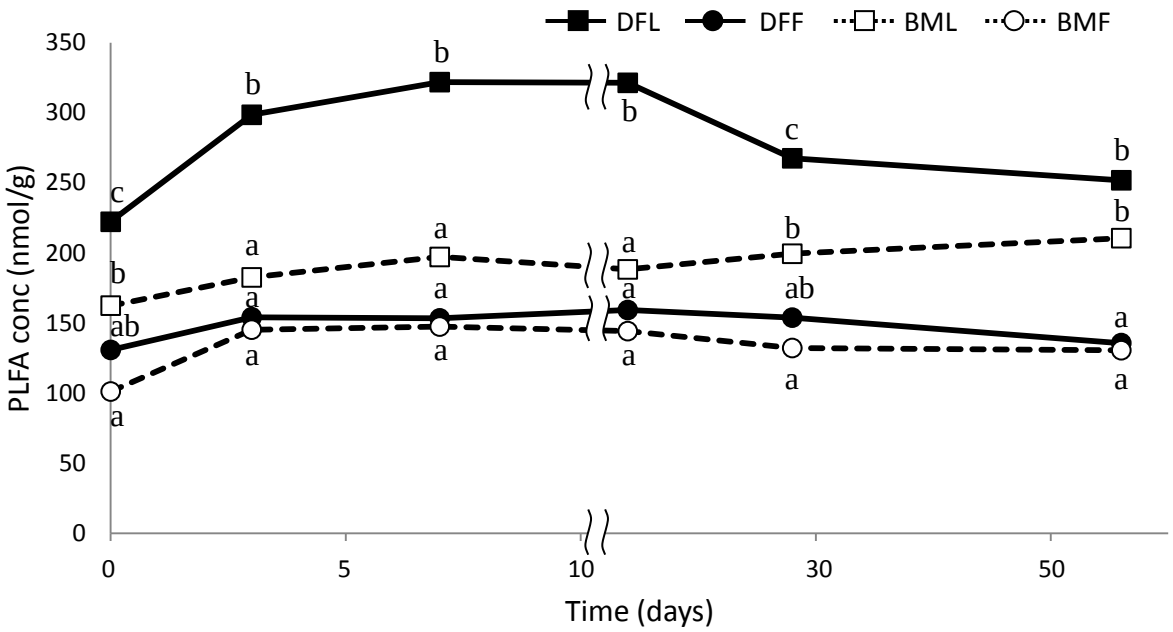

Figure 3.4. Abundance of Gram-positive bacteria in litter and faeces of Douglas-fir and bigleaf maple during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

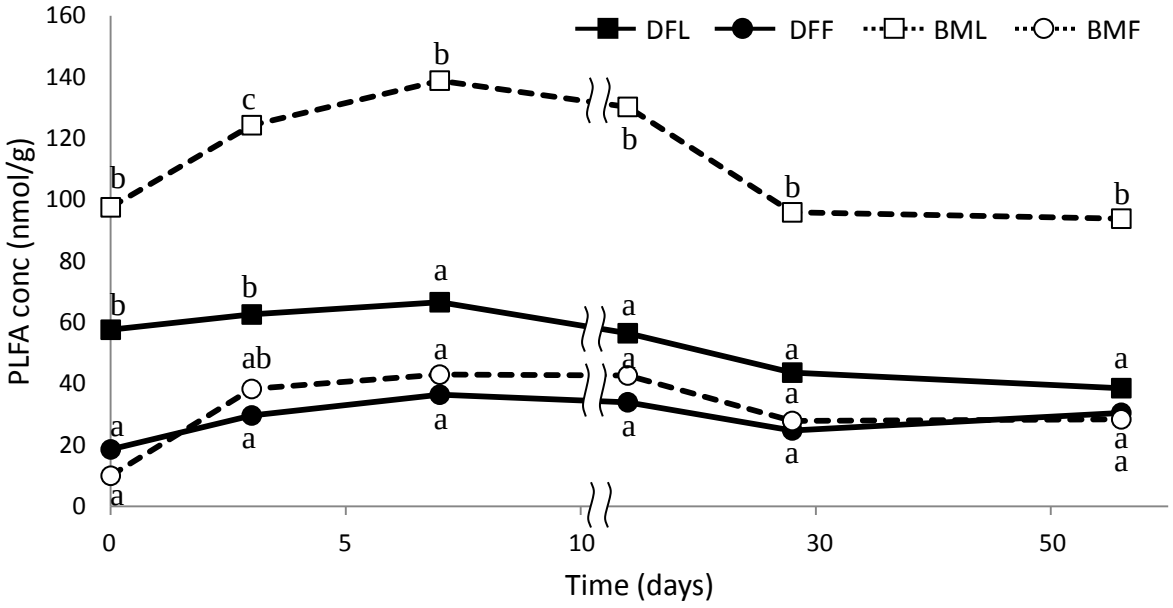

Figure 3.5. Abundance of actinomycetes in litter and faeces of Douglas-fir and bigleaf maple during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

3.2 Douglas-fir and Bigleaf Maple

The ANOVA comparing CO_2 release from the two litter types indicated significant differences but significant treatment x time interaction, therefore sampling dates are compared individually (Table A.1). CO_2 release from maple, both litter and faeces, was significantly higher than that from Douglas-fir throughout the incubation (Figure 3.1).

There was no significant difference in abundance of total bacteria, Gram-positive bacteria and actinomycetes between Douglas-fir litter and faeces and maple litter and faeces (Tables A.12, A.14 and A.15). Gram-negative bacteria were significantly more abundant in bigleaf maple than in Douglas-fir (Table A.13). This difference resulted from the high abundance of Gram-negative bacteria in maple litter relative to Douglas-fir litter on all sampling occasions (Figure 3.3). In a comparison of litter, Gram-positive bacteria were significantly more abundant in Douglas-fir than in maple during the first four weeks of incubation (Figure 3.4), and actinomycetes were significantly more abundant in maple than Douglas-fir on all occasions except Day 0 (Figure 3.5). In contrast, there were no significant differences in bacterial group abundances between faeces derived from Douglas-fir and maple (Tables A.14 and A.15).

The proportions of Gram-negative bacteria and Gram-positive bacteria in the maple and Douglas-fir litter and faeces are provided in Figure 3.6. Gram-negative were the dominant bacteria in maple litter while Gram-positive bacteria composed a greater proportion of the bacterial community in Douglas-fir than in maple throughout the incubation. Bacterial community structures of faeces were more uniform between the two litter types.

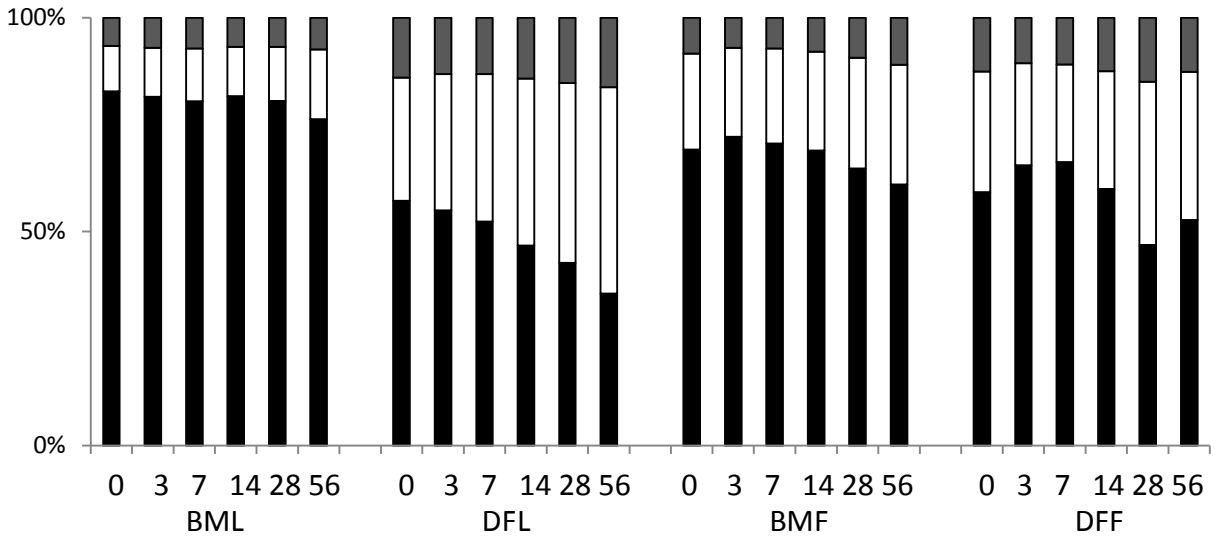

Figure 3.6. Proportion of abundance of each group of bacteria on each sampling date in DFL, DFF, BML and BMF. Gram-negative bacteria, Gram-positive bacteria, unspecified bacteria.

3.3 Grinding

During two months of incubation, I observed fungal mycelium growing on the surface of ground litter. It became visible on Day 3 in all four replicate microcosms and disappeared before the incubation ended.

CO_2 release from ground samples was significantly higher than that from unground samples in both litter and faeces from Douglas-fir overall (Table A.16) and on each sampling date (Table A.17 and Figure 3.7).

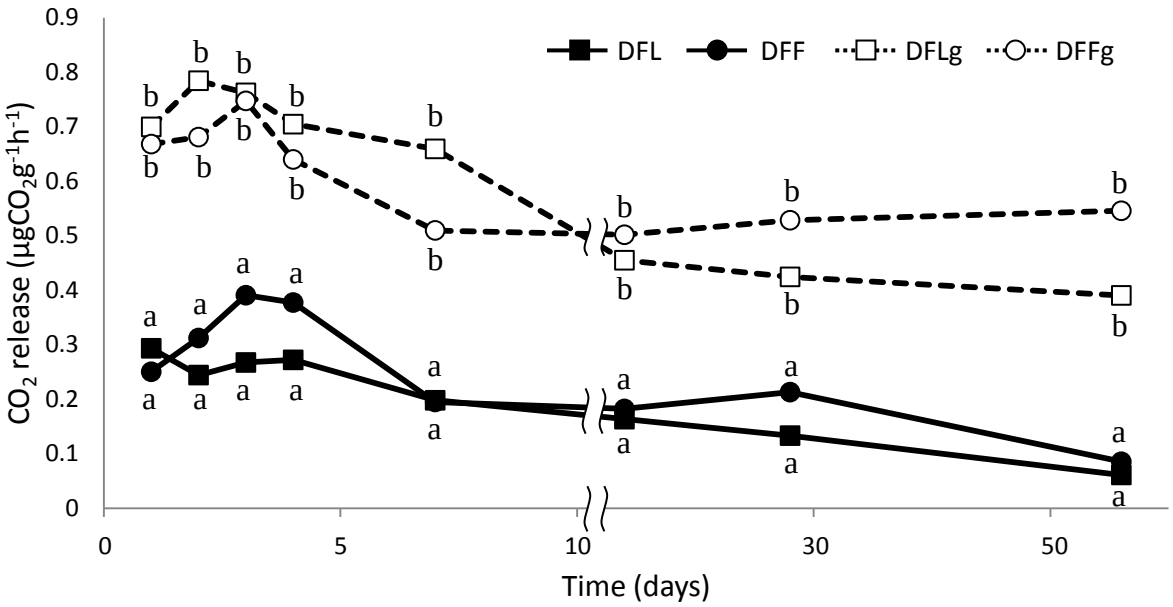

Figure 3.7. CO_2 release from ground and unground Douglas-fir litter and faeces during the eight-week laboratory incubation in µg CO_2 per µg dry mass sample per hour. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

There was no significant difference in abundance of total bacteria, Gram-negative or Gram-positive bacteria between averages of ground litter and faeces and unground litter and faeces (Tables A.18, A.20 and A.22). Abundance of total bacteria and Gram-negative bacteria in both litter and faeces were not significantly different between ground and unground treatments (Tables A.19 and A.21 and Figures 3.8 and 3.9). Unground litter had significantly higher abundance of Gram-positive bacteria than ground litter, but abundances in ground and unground faeces were not significantly different (Table A.23 and Figure 3.10). Actinomycetes were significantly more abundant in unground litter than in ground litter and on Days 3 and 7, but similar in both ground and unground faeces (Tables A.24 and A.25 and Figure 3.11).

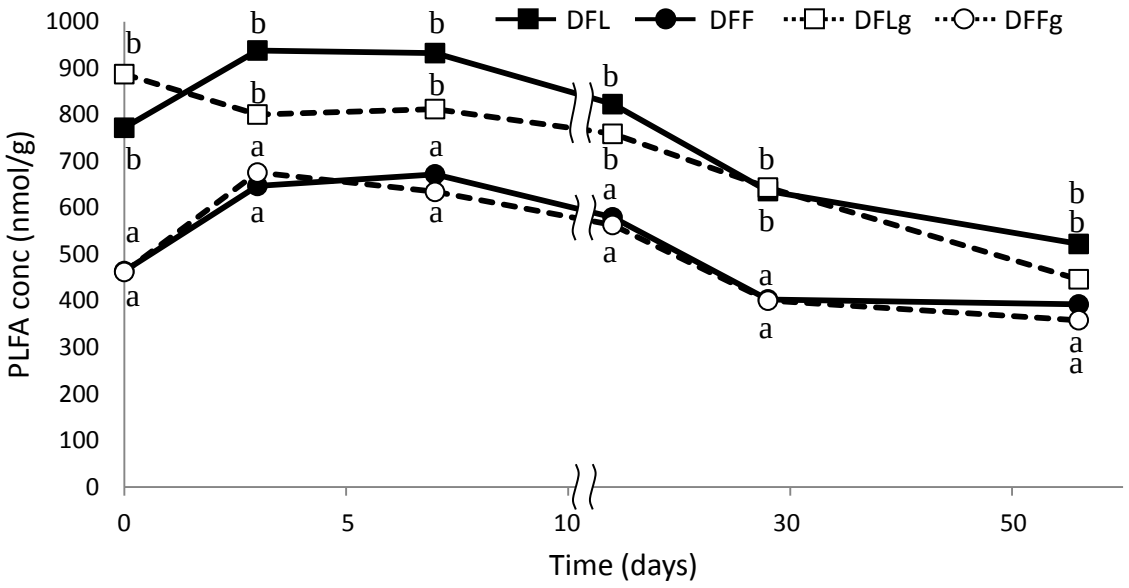

Figure 3.8. Abundance of total bacteria in ground and unground Douglas-fir litter and faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

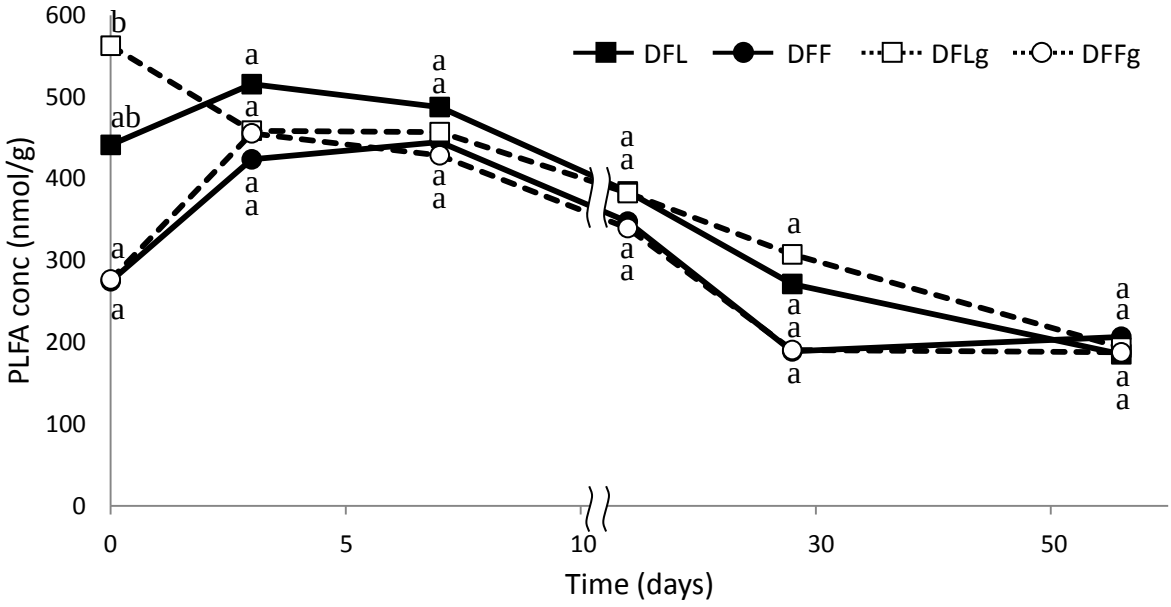

Figure 3.9. Abundance of Gram-negative bacteria in ground and unground Douglas-fir litter and faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

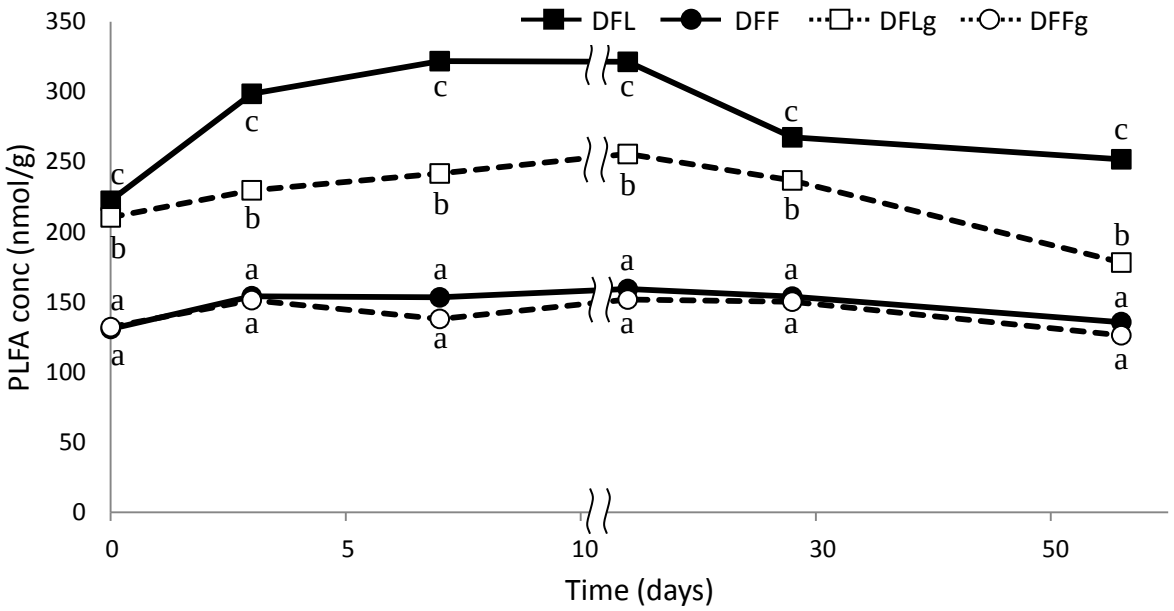

Figure 3.10. Abundance of Gram-positive bacteria in ground and unground Douglas-fir litter and faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

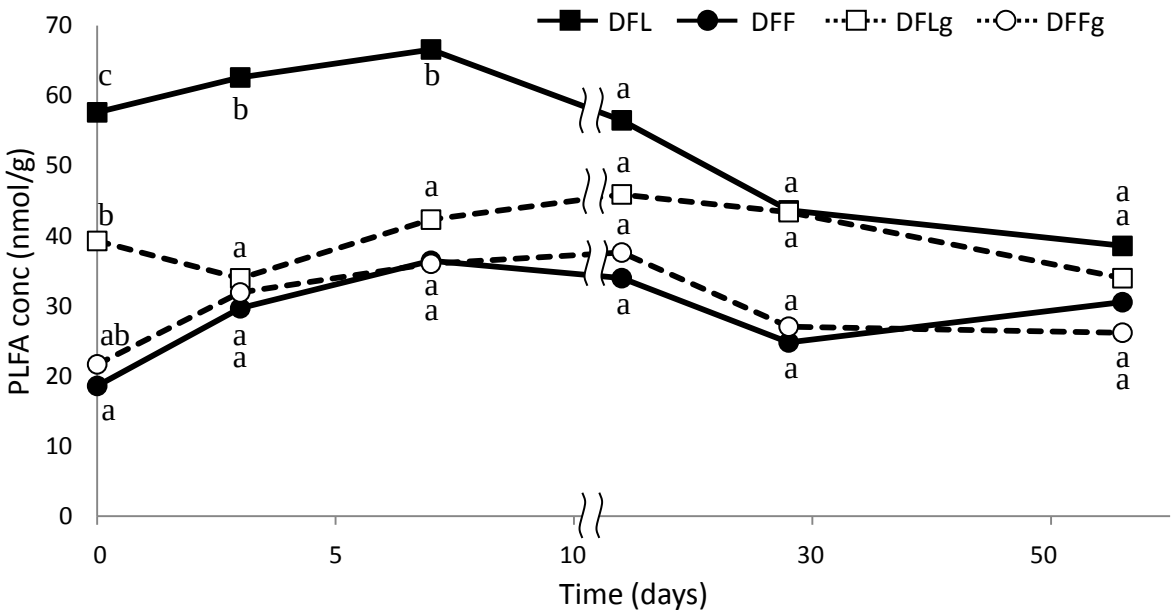

Figure 3.11. Abundance of actinomycetes in ground and unground Douglas-fir litter and faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date (α<0.05).

3.4 Mixing

CO_2 release was compared between microcosms containing both litter and faeces (mixed; DFm and BMm) and the sum of those containing either litter or faeces (pure; DFp and BMp). Average CO_2 release of mixed treatments was higher than pure treatments in the latter half of the two-month incubation (Table A.26). Mixing affected the two litter types differently, although the normality assumption was not met for this analysis. CO_2 release from Douglas-fir was higher in mixed microcosms except on Days 0 and 2. In bigleaf maple, CO_2 release was lower in the mixed microcosms than in pure microcosms on Day 1 but did not differ on the other days (Table A.27 and Figure 3.12). Significant difference could not be detected because residuals were not normally distributed.

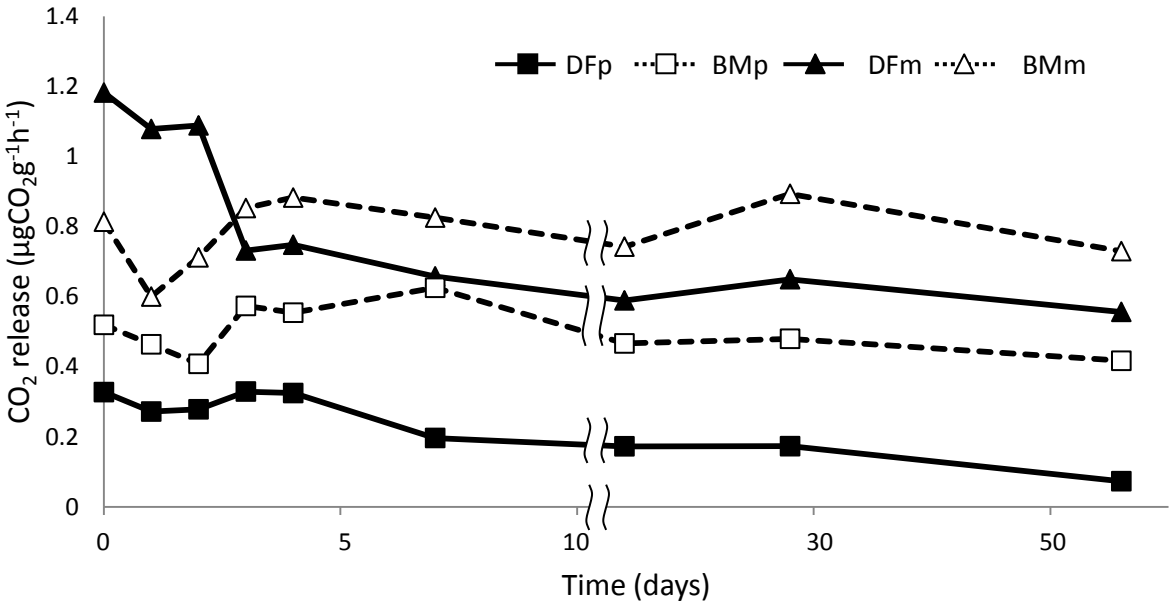

Figure 3.12. CO_2 release from microcosms containing both litter and faeces and those containing either litter or faeces during the eight-week laboratory incubation in µg CO_2 per g dry mass sample per hour. DFp, average of DFL and DFF; BMp, average of BML and BMF.

Mixing litter and faeces did not significantly affect the abundance of total bacteria, Gram-negative bacteria, Gram-positive bacteria or actinomycetes (Tables A.28, A.29, A.30, A.31, A.32, A.33, A.34 and A.35 and Figures 3.13, 3.14, 3.15 and 3.16).

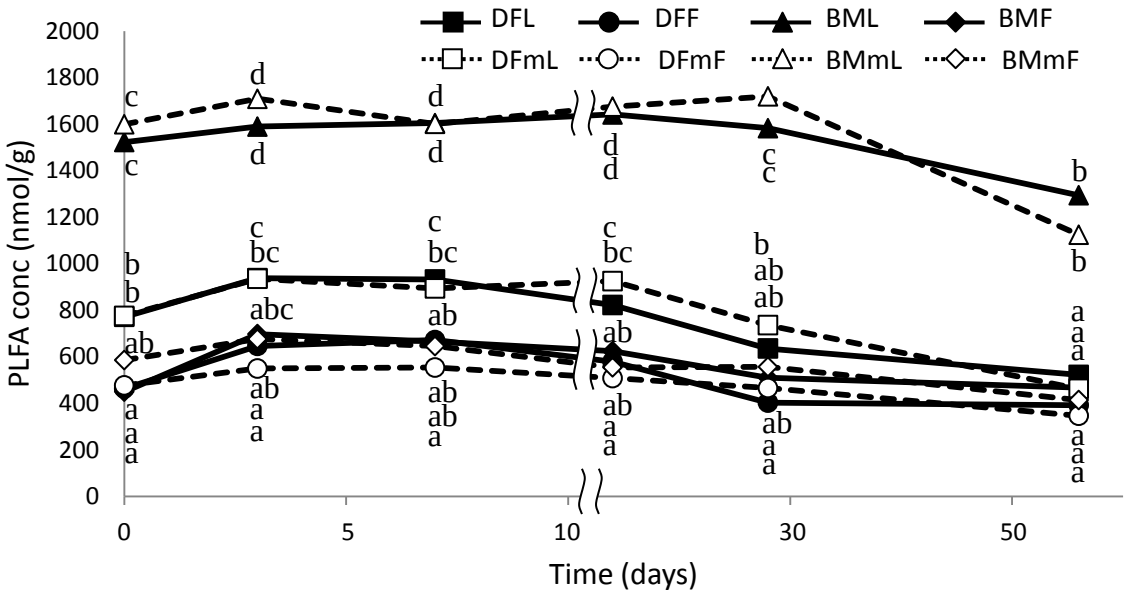

Figure 3.13. Abundance of total bacteria in microcosms containing both litter and faeces and those containing either litter or faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date ($\alpha<0.05$).

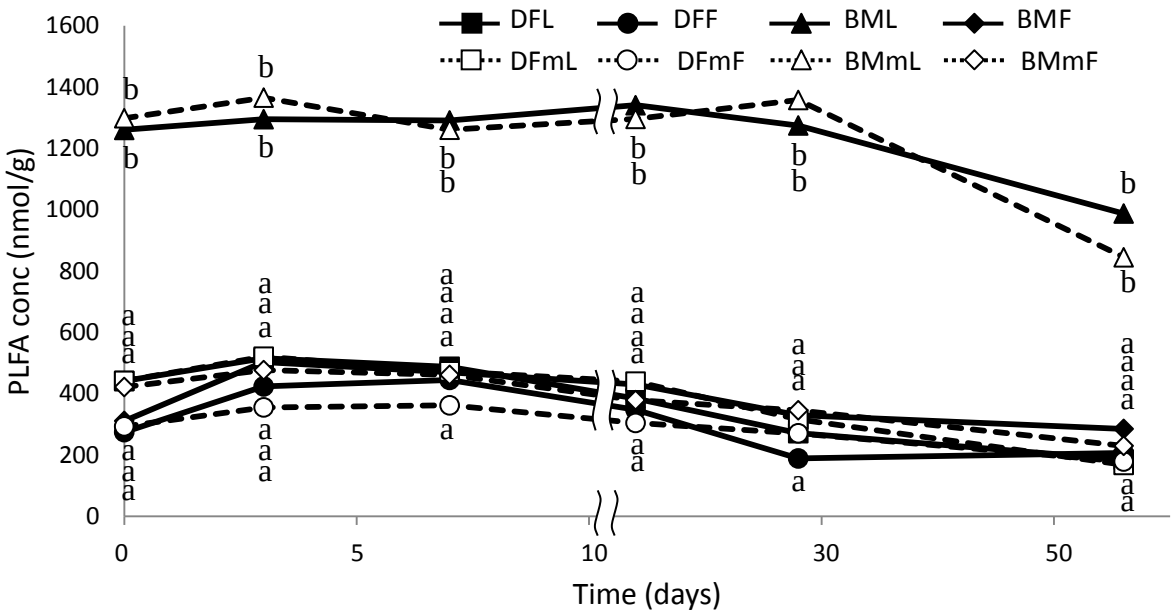

Figure 3.14. Abundance of Gram-negative bacteria in microcosms containing both litter and faeces and those containing either litter or faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date ($\alpha<0.05$).

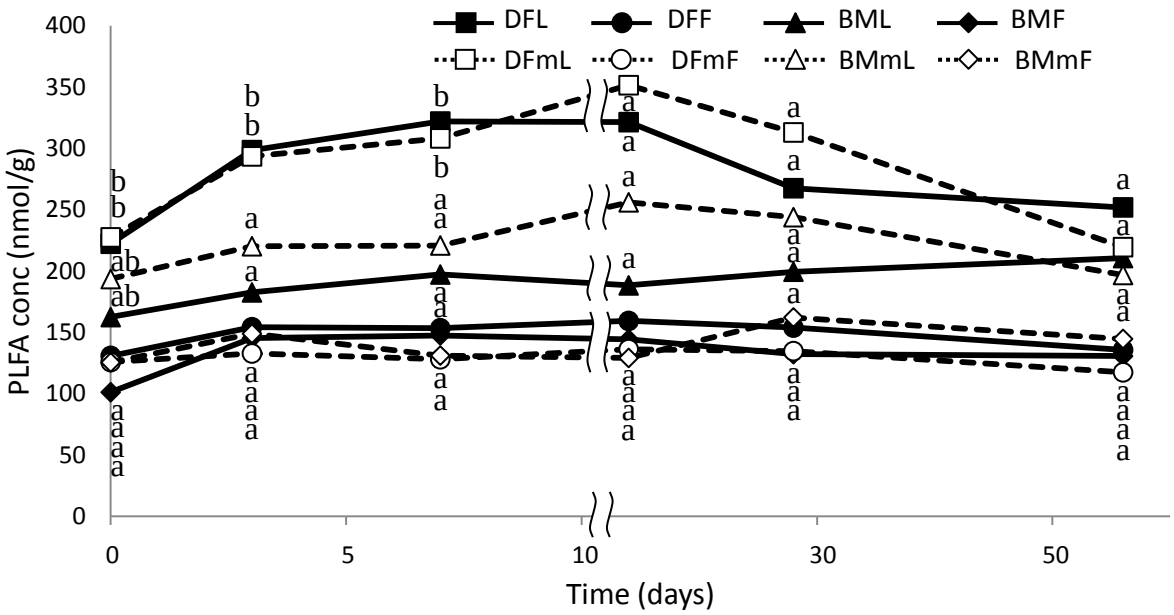

Figure 3.15. Abundance of Gram-positive bacteria in microcosms containing both litter and faeces and those containing either litter or faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date ($\alpha<0.05$).

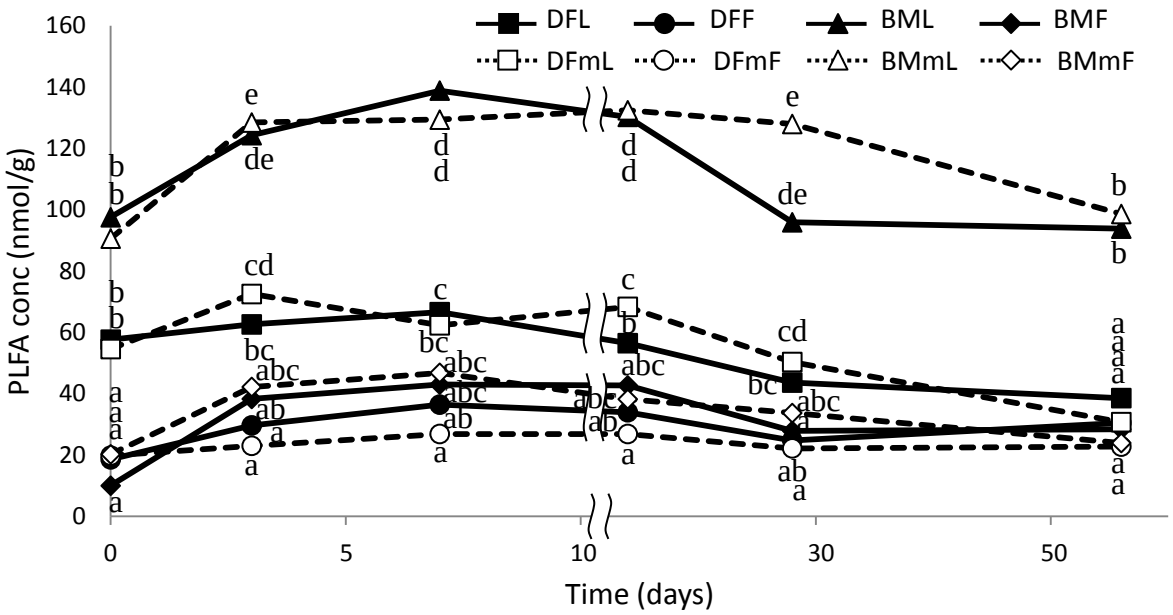

Figure 3.16. Abundance of actinomycetes in microcosms containing both litter and faeces and those containing either litter or faeces during the eight-week laboratory incubation in nmol PLFA per g dry mass sample. Different letters indicate significant differences among treatments within each sampling date ($\alpha<0.05$).

Chapter 4: Discussion

4.1 Litter and Faeces in Two Litter Types

The hypothesized greater CO_2 release from litter compared with faeces occurred only in the maple samples and only during the first 2 days of the incubation. At other times and at all times in the Douglas-fir comparison, CO_2 release was similar in litter and faeces. Rawlins *et al.* (2007) also measured higher rates of CO_2 release from soil amended with oak leaf litter than that amended with millipede faeces only during the first few days of the incubation. The short-lived increase in CO_2 release from litter may have been caused by the leaf litter being cut into pieces prior to placing in the microcosms in both of these studies, which would increase access of microorganisms and enzymes to labile material. Fyles and McGill (1987) showed that pine needles were decayed faster when they were cut in to 1-cm pieces and that litter structures were as important as their chemical composition. I also moistened the leaf litter just before putting it into microcosms, which could have led to a flush of microbial activity, although the faeces were similarly remoistened. Maple litter was drier than other samples before moistened (approximate water content of Douglas-fir litter 76%, faeces derived from Douglas-fir 70%, maple litter 10% and faeces derived from maple 83%), so it is possible that moistening more strongly affected the maple litter than faeces and Douglas-fir needles. The lack of early stimulation of microbial activity in faeces indicates that the initial flush of activity from microorganisms which colonize litter during gut passage is not significant in millipede faeces. It may also indicate that the most easily decomposable organic materials had been removed during gut passage.

Despite incubation under identical conditions, the two litter types used in this study differed in microbial activity during incubation, and these differences persisted in the faeces

of the millipedes. Leaf litter of maple and faeces of millipedes that consumed maple litter had higher rates of CO_2 release than litter of Douglas-fir and faeces of millipedes that consumed Douglas-fir litter. Thus, differences in decay rates of different litter types persisted despite consumption and gut passage by millipedes. This is consistent with many studies that have indicated that litter-type differences influence litter decomposition rates and that broadleaf litter such as maple initially decays more quickly than needle litter (Laganière *et al*. 2010, Prescott *et al.* 2004). Digestion of litter by millipedes changes the chemical composition, as the millipedes assimilate nutrients and labile compounds rendering their faeces more recalcitrant (Gillon and David 2001, Rawlins *et al*. 2006). However, the low assimilation rate of millipedes (<10%; Carcamo 2000), would allow many biochemical components to remain intact throughout gut passage, such that differences in initial chemistry of litter between species are maintained in the faeces.

Contrary to my hypothesis that millipede faeces would have larger bacterial PLFA abundance relative to uningested litter, the greatest abundances of all PLFA biomarkers for bacteria were in the leaf litter. The hypothesis was based on the studies reporting that fungi in litter were selectively consumed during gut passage and surviving bacteria flourished in the hind gut of millipedes. Therefore, decrease of bacterial biomarkers may be explained by slow growth of bacteria in the hind gut, but I did not examine this in this study. Another possible explanation is that symbiotic microbes which could have been excreted with faeces could not adjust to the environment outside the gut and died soon after defaecation; the faeces were not collected for 48 hours at longest. However, it is not yet clear if PLFAs are decomposed immediately after cell death, and the PLFA analyses cannot distinguish PLFAs in dead cells from those in living cells (Frostegård *et al.* 2011). Other techniques such as ergosterol

analysis for fungal abundance (Högberg 2006) and DNA analyses (Oravecz *et al*. 2002) are required for better understanding of microbial communities.

The proportion of Gram-negative bacteria and Gram-positive bacteria appeared to be different between two litter types. The bacterial community in Douglas-fir litter consisted of about 50 % of Gram-negative bacteria and 30% of Gram-positive bacteria while maple litter had about 80 % of Gram-negatives and 10 % of Gram-positives. Dominance of Gram-negative bacteria, r-strategists, in maple litter compared to Douglas-fir litter implied that maple litter contained more easily utilizable substances. Contrary to this study, Gram-positives decreased through digestion of earthworms (Knapp *et al*. 2009b). Since it is not yet clear where these microbes originate from, further studies including labeling microbes in the litter prior to faunal digestion are needed to understand the changes in microbial community.

It is interesting that whilst there were significant differences in CO_2 release and total bacteria between maple litter and faeces, relatively small differences were observed between Douglas-fir litter and faeces (Figure 3.2). Different rates of decay have been reported for different litter types; high nitrogen content of litter usually results in rapid decomposition in short-term experiments. This is consistent with greater CO_2 release from maple than Douglas-fir in the current study. On the other hand, in long-term experiments, decay rates would be extremely slow after some time and organic materials stay in soils for a long time (Prescott 2010). The amount of the stabilized organic matter ("maximum decomposition limits"; Berg *et al*. 1996) has been reported to be positively correlated with nitrogen content in the original litter (Berg 2000); litter with high nitrogen content such as broadleaf litter decompose fast at the beginning but reach to the decomposition limit quickly while litter with low nitrogen content such as coniferous litter decompose slowly but keeps decaying at

similar rates (Prescott *et al*. 2004). The significant change of microbial activity in maple litter during a single gut passage may be related to the quick retardance of decomposition in long-term decomposition processes.

In this study, microbial community was assessed only using PLFA biomarkers. The biomarker for fungi is found also in many eukaryotes (Frostegård *et al.* 2011) and, as my samples contained many plant cells, abundance of fungi could not be estimated.

4.2 Grinding

Grinding of Douglas-fir litter to increase the surface area : volume ratio of the particles significantly increased rates of CO_2 release. Stimulation of decomposition or microbial activity in ground litter has been reported by Maraun and Scheu (1995) and Fyles and McGill (1987). However, PLFA biomarkers indicated that abundances of total bacteria and Gram-negative bacteria were not significantly different between unground and ground samples except for Day 0 and Gram-positive bacteria and actinomycetes were more abundant in unground litter than in ground litter. Therefore, the increase of CO_2 release might be explained by respiration of fungi. This is supported by the observation of fungal mycelium on the surface of ground litter.

The stimulation of CO_2 release caused by grinding faeces indicates that microbial activity and decomposition of faeces is also inhibited by the tendency for faecal material to remain enclosed and protected within pellets. Webb (1977) pointed out that surface area : volume ratio increased as particle size decreased, but when the particles were sufficiently small, the interparticle cohesive forces would be strong enough to generate compact pellets and the total microbial accessible area decreased. Some other studies reported that faecal

pellets retain their structure well in soils (reviewed in Coleman *et al*. 2004). In addition, tough coatings difficult to penetrate fungal hyphae growing on faecal pellets may further prevented physical breakdown of the pellet structure (Martin and Marinissen 1993). Therefore, part of the recalcitrance of faecal material may be related to inaccessibility of contents to microbial attack.

4.3 Mixing

In forests, millipedes *H. haydeniana* were usually found in the litter layer, and when they were present, their faeces often clung to the bigleaf maple leaf litter on the forest floor. It was therefore of interest to know if there were interactions between the decay of the two materials. It is possible that the micro-environment such as moisture and pH in which litter and faeces are in contact might be different from the surroundings, and thus change the microbial decomposition processes. There was little evidence in my experiment that mixing litter and faeces altered microbial communities and activity of either litter or faeces.

4.4 Future Studies

One aspect of faunal activity that was not examined in these short-term incubations is subsequent ingestion of faeces by coprophagous organisms and effects of secondary digestion on subsequent decomposition of faecal material. Coprophagy is common in woodlice (Kautz *et al*. 2002), enchytraeids, mites and earthworms (Martin and Marinissen 1993) and has been observed in a few species of millipedes (Hopkin and Read 1992) and may be related to growth of fungus/microbes on faeces. Nicholson *et al.* (1966) observed substantial increases in microbial biomass on faecal pellets 20 days into the laboratory incubation. Considering that millipedes selectively digest fungi, growth of fungi on faecal

pellets might induce consumption of the faeces by millipedes as well as other soil animals. Because assimilation rate of millipedes are very low, faunal digestion and fungal growth can be repeated several times and may influence microbial litter decomposition processes. However, due to the few studies of biology of *H. haydeniana*, their feeding habit such as coprophagy and geophagy is not yet clear.

Another aspect I did not test in this study is the effects of mixed species of litter. Since litter type differences were retained during the gut passage of millipedes, consumption of multiple food sources may influence the decomposition of the faeces. Including the effects of mixed species of litter would help us fully understand the effects of millipedes on litter decomposition.

Chapter 5: Conclusion

This study indicated that millipedes significantly influenced microbial litter decomposition processes only when they feed on some types of litter. CO_2 release from bigleaf maple litter was significantly greater than that from faeces derived from maple litter while a similar amount of CO_2 was released from Douglas-fir litter and faeces. The litter type difference in microbial community structure persisted in the faeces of the millipedes although the abundances of bacteria were not consistent with the amount of CO_2 release throughout incubation. Greater release of CO_2 from ground litter and faeces indicated that their physical structure may prevent litter and millipedes' faeces from decomposition. The presence of litter did not significantly influence the microbial litter decomposition processes of faeces, and vice versa. Therefore, including faunal effects on various types of litter is important to understand terrestrial litter decomposition processes.

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Appendix

Table A.1. ANOVA for comparing CO_2 release between litter and faeces. The data was transformed to the power of 0.9.

Source	DF	MS	F	P
T	1	0.06550727	2.60	0.1100
R(T)	14	0.79815452	31.63	<.0001
D	8	0.033127661	13.13	<.0001
T*D	8	0.22073159	8.75	<.0001

Table A.2. ANOVA for comparing CO_2 release among Douglas-fir litter, Douglas-fir faeces, bigleaf maple litter and bigleaf maple faeces. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	3	3.35761715	936.96	<.0001
R(T)	12	0.00385286	1.08	0.3896
D	8	0.28911109	80.68	<.0001
T*D	24	0.13809081	38.53	<.0001

Table A.3. ANOVA for comparing abundance of total bacteria between litter and faeces.

Source	DF	MS	F	P
T	1	8843946.212	731.92	<.0001
R(T)	14	517975.703	42.87	<.0001
D	5	230083.833	19.04	<.0001
T*D	5	21106.589	1.75	0.1353

Table A.4. ANOVA for comparing abundance of total bacteria among Douglas-fir litter, Douglas-fir faeces, bigleaf maple litter and bigleaf maple faeces. The data was transformed to the power of 0.9.

Source	DF	MS	F	P
T	3	1081405.195	453.28	<.0001
R(T)	12	2433.558	1.02	0.4427
D	5	49714.087	20.84	<.0001
T*D	15	3423.972	1.44	0.1609

Table A.5. ANOVA for comparing abundance of Gram-negative bacteria between litter and faeces. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	1	233220.5307	664.68	<.0001
R(T)	14	29337.9420	83.61	<.0001
D	5	9363.0469	26.68	<.0001
T*D	5	1118.8380	3.19	0.0119

Table A.6. ANOVA for comparing abundance of Gram-negative bacteria among Douglas-fir litter, Douglas-fir faeces, bigleaf maple litter and bigleaf maple faeces. The data was transformed to the power of 0.65.

Source	DF	MS	F	P
T	3	19656.64576	680.48	<.0001
R(T)	12	26.63184	0.92	0.5313
D	5	1006.03376	34.83	<.0001
T*D	15	83.22350	2.88	0.0019

Table A.7. ANOVA for comparing abundance of Gram-positive bacteria between litter and faeces. The data was transformed to the power of 0.9.

Source	DF	MS	F	P
T	1	60511.25789	341.58	<.0001
R(T)	14	2159.50643	12.19	<.0001
D	5	1578.58578	8.91	<.0001
T*D	5	124.85972	0.70	0.6217

Table A.8. ANOVA for comparing abundance of Gram-positive bacteria among Douglas-fir litter, Douglas-fir faeces, bigleaf maple litter and bigleaf maple faeces. The data was transformed to the power of 0.7.

Source	DF	MS	F	P
T	3	2101.701516	218.92	<.0001
R(T)	12	16.733176	1.74	0.0799
D	5	116.619617	12.15	<.0001
T*D	15	20.369321	2.12	0.0207

Table A.9. ANOVA for comparing abundance of actinomycetes between litter and faeces. The data was transformed to the power of 0.2.

Source	DF	MS	F	P
T	1	4.41224645	362.75	<.0001
R(T)	14	0.11044269	9.08	<.0001
D	5	0.18155191	14.93	<.0001
T*D	5	0.07470901	6.14	<.0001

Table A.10. ANOVA for comparing abundance of actinomycetes among Douglas-fir litter, Douglas-fir faeces, bigleaf maple litter and bigleaf maple faeces. The data was transformed to the power of 0.1.

Source	DF	MS	F	P
T	3	0.21782352	168.85	<.0001
R(T)	12	0.00128223	0.99	0.4655
D	5	0.02246335	17.41	<.0001
T*D	15	0.00489025	3.79	0.0001

Table A.11. ANOVA for comparing CO_2 release between Douglas-fir and bigleaf maple. The data was transformed to the power of 0.01.

Source	DF	MS	F	P
T	1	0.47974647	695.91	<.0001
R(T)	14	0.00073057	1.06	0.4016
D	8	0.01499064	21.75	<.0001
T*D	8	0.00349110	5.06	<.0001

Table A.12. ANOVA for comparing abundance of total bacteria between Douglas-fir and bigleaf maple.

Source	DF	MS	F	P
T	1	39494.9080	325.26	<.0001
R(T)	14	867579.66	71.45	<.0001
D	5	230083.83	18.95	<.0001
T*D	5	20273.51	1.67	0.2218

Table A.13. ANOVA for comparing abundance of Gram-negative bacteria between Douglas-fir and bigleaf maple. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	1	245036.1040	677.26	<.0001
R(T)	14	28493.9725	78.76	<.0001
D	5	9363.0469	25.88	<.0001
T*D	5	965.8869	2.67	0.0288

Table A.14. ANOVA for comparing abundance of Gram-positive bacteria between Douglas-fir and bigleaf maple.

Source	DF	MS	F	P
T	1	65948.6876	110.96	<.0001
R(T)	14	18592.1988	31.28	<.0001
D	5	5602.1390	9.43	<.0001
T*D	5	1362.9619	2.29	0.0546

Table A.15. ANOVA for comparing abundance of actinomycetes between Douglas-fir and bigleaf maple. The data was transformed to the power of 0.45.

Source	DF	MS	F	P
T	1	35.4820222	68.16	<.0001
R(T)	14	13.9152035	26.73	<.0001
D	5	5.6478788	10.85	<.0001
T*D	5	0.8816274	1.69	0.1476

Table A.16. ANOVA for comparing CO_2 release between ground and unground treatments. The data was transformed to the power of 0.4.

Source	DF	MS	F	P
T	1	2.39176904	1023.11	<.0001
R(T)	14	0.00340785	1.46	0.1421
D	7	0.09999527	42.77	<.0001
T*D	7	0.01016537	4.35	0.0003

Table A.17. ANOVA for comparing CO_2 release among ground litter, ground faeces, unground litter and unground faeces. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	3	1.42845193	476.94	<.0001
R(T)	12	0.00322654	1.08	0.3897
D	7	0.15882899	53.03	<.0001
T*D	21	0.01096157	3.66	<.0001

Table A.18. ANOVA for comparing abundance of total bacteria between ground and unground treatments. The data was transformed to the power of 0.4.

Source	DF	MS	F	P
T	1	1.3343941	1.77	0.1880
R(T)	14	6.6813609	8.85	<.0001
D	5	21.7047595	28.75	<.0001
T*D	5	0.6067912	0.80	0.5508

Table A.19. ANOVA for comparing abundance of total bacteria among ground litter, ground faeces, unground litter and unground faeces.

Source	DF	MS	F	P
T	3	419664.196	40.22	<.0001
R(T)	12	8614.785	0.83	0.6238
D	5	289976.982	27.79	<.0001
T*D	15	15321.460	1.47	0.1467

Table A.20. ANOVA for comparing abundance of Gram-negative bacteria between ground and unground treatments. The data was transformed to the power of 0.3.

Source	DF	MS	F	P
T	1	0.01094278	0.08	0.7811
R(T)	14	0.29583376	2.10	0.0218
D	5	5.64915724	40.17	<.0001
T*D	5	0.06903267	0.49	0.7820

Table A.21. ANOVA for comparing abundance of Gram-negative bacteria among ground litter, ground faeces, unground litter and unground faeces.

Source	DF	MS	F	P
T	3	43850.0379	9.91	<.0001
R(T)	12	3358.0104	0.76	0.6885
D	5	199069.5890	45.01	<.0001
T*D	15	11831.6308	2.68	0.0036

Table A.22. ANOVA for comparing abundance of Gram-positive bacteria between ground and unground treatments.

Source	DF	MS	F	P
T	1	22586.5886	25.10	<.0001
R(T)	14	21809.8909	24.24	<.0001
D	5	6881.5023	7.65	<.0001
T*D	5	1037.7379	1.15	0.3409

Table A.23. ANOVA for comparing abundance of Gram-positive bacteria among ground litter, ground faeces, unground litter and unground faeces.

Source	DF	MS	F	P
T	3	105952.8850	127.18	<.0001
R(T)	12	838.8672	1.01	0.4541
D	5	6881.5023	8.26	<.0001
T*D	15	1212.8532	1.46	0.1520

Table A.24. ANOVA for comparing abundance of actinomycetes between ground and unground treatments.

Source	DF	MS	F	P
T	1	1072.718713	15.54	0.0002
R(T)	14	685.535604	9.93	<.0001
D	5	457.144060	6.62	<.0001
T*D	5	119.757972	1.73	0.1381

Table A.25. ANOVA for comparing abundance of actinomycetes among ground litter, ground faeces, unground litter and unground faeces.

Source	DF	MS	F	P
T	3	3283.823713	60.95	<.0001
R(T)	12	68.228836	1.27	0.2621
D	5	457.144060	8.48	<.0001
T*D	15	146.650224	2.72	0.0031

Table A.26. ANOVA for comparing CO_2 release between mixed and pure treatments. Residuals were not normally distributed (Kolmogorov-Smirnov test; p=0.313). Three outliers were eliminated for the analysis and the data was transformed to the power of 0.1.

Source	DF	MS	F	P
T	1	0.06294623	119.45	<.0001
R(T)	22	0.02205247	41.85	<.0001
D	8	0.00582004	11.04	<.0001
T*D	8	0.00478367	9.08	<.0001

Table A.27. ANOVA for comparing CO_2 release among mixed Douglas-fir, mixed bigleaf maple, pure Douglas-fir and pure bigleaf maple. Residuals were not normally distributed (Kolmogorov-Smirnov test; p=0.0189). Three outliers were eliminated for this analysis and the data was transformed to the power of 0.1.

Source	DF	MS	F	P
T	3	0.18228289	455.06	<.0001
R(T)	20	0.00057376	1.43	0.1147
D	8	0.00639990	15.98	<.0001
T*D	24	0.00298120	7.44	<.0001

Table A.28. ANOVA for comparing abundance of total bacteria between mixed and pure treatments. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	1	1.116	0.00	<.0001
R(T)	30	45613.480	94.51	<.0001
D	5	22385.330	46.38	<.0001
T*D	5	1417.364	2.94	0.0147

Table A.29. ANOVA for comparing abundance of total bacteria among mixed Douglas-fir litter, mixed Douglas-fir faeces, mixed bigleaf maple litter, mixed bigleaf maple faeces, pure Douglas-fir litter, pure Douglas-fir faeces, pure bigleaf maple litter and pure bigleaf maple faeces. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	7	193884.939	498.50	<.0001
R(T)	24	467.122	1.20	0.2550
D	5	22385.330	57.55	<.0001
T*D	35	937.307	2.41	0.0002

Table A.30. ANOVA for comparing abundance of Gram-negative bacteria between mixed and pure treatments. The data was transformed to the power of 0.8.

Source	DF	MS	F	P
T	1	34.366	0.10	0.7508
R(T)	30	43001.003	126.68	<.0001
D	5	19857.805	58.50	<.0001
T*D	5	891.688	2.63	0.0262

Table A.31. ANOVA for comparing abundance of Gram-negative bacteria among mixed Douglas-fir litter, mixed Douglas-fir faeces, mixed bigleaf maple litter, mixed bigleaf maple faeces, pure Douglas-fir litter, pure Douglas-fir faeces, pure bigleaf maple litter and pure bigleaf maple faeces. The data was transformed to the power of 0.45.

Source	DF	MS	F	P
T	7	593.094946	666.97	<.0001
R(T)	24	1.014985	1.14	0.3110
D	5	91.779005	103.21	<.0001
T*D	35	3.174634	3.57	<.0001

Table A.32. ANOVA for comparing abundance of Gram-positive bacteria between mixed and pure treatments.

Source	DF	MS	F	P
T	1	1832.8780	2.24	0.1366
R(T)	30	24144.1432	29.50	<.0001
D	5	11168.8650	13.65	<.0001
T*D	5	1686.9351	2.06	0.0734

Table A.33. ANOVA for comparing abundance of Gram-positive bacteria among mixed Douglas-fir litter, mixed Douglas-fir faeces, mixed bigleaf maple litter, mixed bigleaf maple faeces, pure Douglas-fir litter, pure Douglas-fir faeces, pure bigleaf maple litter and pure bigleaf maple faeces. The data was transformed to the power of 0.7.

Source	DF	MS	F	P
T	7	2032.49480	175.18	<.0001
R(T)	24	14.97621	1.29	0.1849
D	5	220.15388	18.98	<.0001
T*D	35	30.07394	2.59	<.0001

Table A.34. ANOVA for comparing abundance of actinomycetes between mixed and pure treatments. Residuals were not normally distributed (Kolmogorov-Smirnov test; p=0.0268). Two outliers were eliminated for the analysis and the data were transformed to the power of 0.1.

Source	DF	MS	F	P
T	1	0.00002446	0.02	0.8939
R(T)	30	0.04784730	34.90	<.0001
D	5	0.02984528	21.77	<.0001
T*D	5	0.00271622	1.98	0.0847

Table A.35. ANOVA for comparing abundance of actinomycetes among mixed Dougla-fir litter, mixed Douglas-fir faeces, mixed biglaf maple litter, mixed bigleaf maple faeces, pure Douglas-fir litter, pure Douglas-fir faeces, pure bigleaf maple litter and pure bigleaf maple faeces. One outlier was eliminated for this analysis and the data was transformed to the power of 0.1.

Source	DF	MS	F	P
T	7	0.20458317	208.75	<.0001
R(T)	24	0.00099836	1.02	0.4488
D	5	0.03342609	34.11	<.0001
T*D	35	0.00359582	3.67	<.0001