The effects of forest degradation on soil carbon dynamics in the tropics

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Declaration

This thesis has not been submitted in support of an application for another degree at this or any other university. It is the result of my own work and includes nothing that is the outcome of work done in collaboration except where specifically indicated. Many of the ideas in this thesis were the product of discussion with my supervisors Emma Sayer, Julia Drewer and Eleanor Slade.

This thesis is 34743 words and therefore does not exceed the permitted maximum

Abstract

Plant-soil interactions and soil carbon dynamics are an essential part of soil function. Land-use change can affect the soil's ability to accumulate and store carbon. Deforestation and conversion to croplands has decreased tree species cover and diversity in the tropics resulting in degraded and secondary forests becoming the dominant forested habitat. Understanding the effects of forest degradation on soil carbon dynamics is vital if we are to remediate these ecosystems under climate change.

The overarching aim of this thesis was understanding how changes in tree and plant species composition at different levels of degradation affect soil carbon dynamics and litter decomposition in the old and neo-tropics using litter transplant experiments in the field. Malaysia is one of the two biggest producers of palm oil in the world with Indonesia. Borneo is a biodiversity hotspot, but this ecosystem is decreasing at an alarming rate. Sabah, in norther Malaysian Borneo is converting its tropical forest to oil palm plantations resulting in vast expanses of oil palm monocultures containing secondary forest fragments at various degrees of degradation. In Central America, the tree cover is also dominated by secondary forests and timber plantations; in Panama, only 21 % of the tree cover classified as intact forest and there are remediation projects in place to encourage reforestation of degraded landscapes into plantations using native timber species.

The level of degradation in the habitats changed the microclimate which affected soil properties, microbial activity and litter decomposition. Litter properties also had an effect on the rate of litter decomposition and microbial activity. The loss of tree cover in the deforested habitats and monocultures resulted in lower microbial activity and decomposition rates whereas the secondary forests has similar microbial activity and decomposition rates as the old growth forests. Overall, my results suggest that mixed litter inputs are crucial for maintaining soil function and that tropical forest soils might be more resilient to change than expected.

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CHAPTER 1 FOREST MODIFICATION AFFECTS SOIL CARBON DYNAMICS VIA ALTERED LITTER INPUTS

1.1 Soil is an essential component of the carbon cycle

Carbon dioxide (CO_2) from anthropogenic activity is the greenhouse gas released in the highest concentrations contributing 75 % of the anthropogenic greenhouse gas emissions (IPCC, 2014). In 2010, anthropogenic activities released 35 Gt CO_2 to the atmosphere, of which 11 % originated from deforestation and other land use changes (IPCC, 2014). Deforestation for conversion to agricultural lands has resulted in an estimated loss of 41 % of the soil organic carbon (SOC) in the tropical regions (Wei et al., 2014). If we want to mitigate our impact on climate change, it is essential that we reduce the amount of CO_2 released to the atmosphere and carbon (C) storage by natural systems is an essential part of this endeavour (IPCC, 2018).

As part of the terrestrial biogeochemical C cycle, soils play a key role in sequestering C as a C sink. Soil is the largest terrestrial biomass C pool containing an estimated 1460 Pg C in the first metre depth and is has been theorised that it might hold double this amount at a depth of three metres, especially concentrated in Boreal and tropical areas (Scharlemann et al., 2014). Primary forests have been replaced by secondary forests or agricultural lands throughout the world (Global Forest Watch, 2018); maintaining the soil C pool is essential to limiting the amount of CO_2 released to the atmosphere but human activities, in particular land-use change, have multiple direct and indirect effects on soil C storage and release.

1.2 The importance of tropical forests in the carbon cycle

It has been estimated that 30 % of the soil terrestrial biomass C is in tropical regions (Sayer et al., 2011) and approximately 16 to 20 % of the soil organic carbon (SOC) is

specifically in tropical evergreen forests (Stockmann et al., 2013). Consequently, although tropical forests only cover about 6% of the world's land surface area, they are the second largest forest C sink in the world behind boreal forests with an estimated C sink of 1 to 3 Pg C y^{-1}, which makes them an essential part of the global biogeochemical cycle and a key player in the mitigation of anthropogenic CO_2 emissions (Pan et al., 2011; Sayer et al., 2011). Tropical forests contain two thirds of the worlds' plant and animal biodiversity, which is currently under great threat from human activities (Chazdon, 2014). Understanding the functional interactions between the above- and below-ground soil food web that control soil C storage is essential to determine the links between changes in biodiversity and ecosystem function during forest degradation. To date, the functional properties of leaf litter, especially in tropical forests are understudied (Fanin et al., 2014; Hättenschwiler and Jørgensen, 2010; Liang et al., 2016), which is largely due to the difficulties involved in characterising ecosystem processes in highly diverse tropical forests and the lack of ecological information on the vast majority of species.

1.2.1 Deforestation

Anthropogenic disturbances, especially deforestation for conversion to croplands in tropical regions, has resulted in losses of 20 to 30 % of soil organic C (Don et al., 2011). More worryingly, C losses due to forest conversion has converted tropical soils from a sink to a source of CO_2 (Sodhi et al., 2004; Wilcove et al., 2013). Deforestation causes wholesale changes in aboveground plant and tree species composition as well as invertebrates and soil microbial communities (Ashford et al., 2013; Berenguer et al., 2014; Hättenschwiler and Gasser, 2005; Sayer, 2006). Furthermore, forest disturbance drastically modifies abiotic conditions; the removal of trees releases C from the soil in the form of CO_2 and increases the amount of direct sunlight by opening the canopy, which in turn increases the variation in air and soil temperature as well as soil moisture (Dechert et al., 2004; Houghton, 2012). Logging causes significant losses in soil C and reduces the capacity of the forest to act as a C sink by changing soil organic C content and the loss of trees reduces the soils' ability to store C (Cramer et al., 2004; Don et al., 2011; Gibbs et al., 2007). As a result of widespread deforestation, secondary or degraded forest have now become the dominant forest type in the tropics (FAO, 2016)

This is concerning because, besides the losses of C incurred during forest conversion, secondary forests store an estimated 9 % less C than primary forests (Don et al., 2011).

1.2.2 Reforestation and Forest Regeneration

In order to reduce the extent of deforestation in the tropics, a number of international initiatives have been set-up to reforest and restore previously deforested land. For example, the Bonn challenge and REDD++ are particularly centred around reforesting degraded landscapes in and with rural communities (IUCN, 2018). Regenerating secondary forests undergo successional changes, starting with the emergence of grasses and shrubs, which are adapted to growing in low-nutrient and low-shade environments. This is followed by the establishment of pioneer tree species, which are generally characterised as fast-growing and light-demanding with high foliar nutrient concentrations, and that are better adapted to the drier microclimate (Chazdon, 2014; Swaine and Whitmore, 1988). By contrast, undisturbed old-growth tropical forests are generally characterised by slow-growing plant species that are more shade-tolerant and have greater below-ground biomass and structural stability. Although, secondary forests in Panama have been found to recover aboveground biomass species richness within 40 years of regeneration (Dent et al., 2013) other studies across the tropics show that the recovery of forest biomass and diversity can take 80-100 years (Martin et al., 2011). Given that both forest disturbance and regeneration involve concerted changes in tree diversity and the quality and quantity of plant inputs to the soil, it is essential to understand how belowground ecosystem functions recover during forest regeneration in order to take adequate actions for forest and soil C restoration.

1.2.3 Effect of tropical forest modification on soil carbon

Forest modification in the tropics, as in other forest environments, causes changes in microclimate and soil ecosystem function. Research at the landscape level comparing forests at different levels of degradation have found that the more degraded forests contained less C and had lower microbial activity (Dinesh et al., 2003; Don et al., 2011; Gomez-Acata et al., 2014). A study of SOC losses during conversion of forest to tree cash crops in three countries (Indonesia, Cameroon and Peru) found losses of up to 50

% of SOC, even in mineral soils (van Straaten et al., 2015). Studies comparing soil respiration in forest and oil palm plantations have variously found that CO_2 fluxes were lower in oil palm plantations than in forest which was related to soil organic matter quality and quantity (Hassler et al., 2015). or that respiration rates were similar, but soil microbial communities differed, with greater microbial diversity in the forest compared to the oil palm (Adachi et al. 2004). One study found that soil respiration in primary forest was strongly related to fine root biomass, whereas in secondary forests it was related to the soil C:N ratio and variation was largely controlled by soil water content; in oil palm plantations soil respiration was related to the soil C:N ratio and fine root biomass but the main driver of respiration was the quantity of soil C (Adachi et al. 2004). This demonstrates that forest conversion and regeneration not only influence the amount of C stored belowground, but also affect the processes governing soil C storage and release. Thus, although it is clear that deforestation and forest conversion reduce SOC content and soil microbial activity and significantly increase atmospheric concentrations of CO_2 (Houghton and Hackler, 1999; Kirschbaum and Paul, 2002; Murty et al., 2002), the influence of altered tree diversity and tree species composition also need to be fully understood and quantified in order to assess future consequences of such land use change.

1.3 Organic matter, microbial activity and the soil carbon pool

1.3.1 Litter

The accumulation and retention of C in the soil is a key component of the biogeochemical cycle and is related to the quantity and quality of organic matter inputs (Fanin et al., 2011; Hättenschwiler and Jørgensen, 2010; S. Xu et al., 2013). One of the key sources of organic matter in forest ecosystems is litterfall from trees (Bréchet et al., 2017, 2018; Leff et al., 2012; Sayer et al., 2011, 2007; Sayer & Tanner, 2010). Plant litter is the main source of substrate and habitat for a wide range of decomposer organisms from invertebrate decomposers to microbial communities (Sayer, 2006), which in turn play a key role in mineralising C and other nutrients in the litter, ultimately leading to the formation of SOC (Paudel et al., 2015). The accumulation of SOC in turn sustains decomposer communities, and higher SOC

content can result in higher microbial activity (Lagomarsino et al. 2012). Anthropogenic activities that alter the amount and type of plant litter inputs to the soil will therefore also affect soil C dynamics and storage. Different plant species have leaves with distinct characteristics such as C, nitrogen (N), and phosphorus (P) content and secondary plant compounds such as lignin, tannins and phenolics (Cornwell et al., 2008; Pérez-Harguindeguy et al., 2000). These characteristics affect the decomposition rate of plant litter via the quality of C available to decomposers, and secondary plant compounds such as phenols and tannins can also strongly inhibit microbial activity (Cornwell et al., 2008; Fanin et al., 2011; Freschet et al., 2013; Huang et al., 2007; Powers et al., 2009). Hence, litter of varying decomposability will interact with the rhizosphere to influence the soil microenvironment, which can in turn affect microbial activity and therefore soil C cycling (Fornara et al., 2009; Hättenschwiler et al., 2005; Hobbie, 1992). Consequently, changes in tree species composition as a result of disturbance, selective logging, or secondary succession could have a substantial influence on decomposition processes and soil C dynamics.

A review of leaf traits and leaf decomposition showed that litter properties have the greatest effect on leaf litter decomposition rates, due to the differing ability of microbes at decomposing various qualities of litter: leaf functional traits can also create plant-soil feedbacks, where nutrient-rich rapidly decomposing litter tends to maintain high soil fertility, microbial biomass, and soil C while slow decomposing, nutrient-poor litter will form infertile soils (Hättenschwiler et al., 2010). This is likely to be particularly important during secondary forest succession or forest restoration, because fast-growing pioneer tree species often have rapidly decomposing litter, whereas old-growth forest species generally have tougher nutrient-poor leaves (Aiba and Nakashizuka, 2009; Laird-Hopkins et al., 2017; Lavorel et al., 2011).

Reduced species diversity in disturbed tropical forests or tropical plantations can also affect soil C cycling via decomposition processes. Natural tropical forests contain a high diversity of plant species and, the forest floor will usually contain the litter of many different species, as well as more distinct patches of litter of varying quality, which may affect the overall decomposition rates of the forest litter mixtures (Gessner et al., 2010; Hättenschwiler et al., 2010; Sayer, 2006) Due to the high variability of litter

properties, especially the C:N ratio, lignin content and the possible presence of tannins, mixing different litter species can sometimes have so-called non-additive effects, which can be either facilitative or antagonistic, so that a given litter combination may accelerate or hinder decomposition and microbial activity compared to expected rates derived from experiments using single-species litter (Laird-Hopkins et al., 2017; Makkonen et al., 2013; Meier and Bowman, 2010). Decomposition experiments are often carried out with a limited number of species, using species ratios that are not found in the field, so it is unsure whether the results can be extrapolated to explain ecosystem-level processes (Handa et al., 2014; Laird-Hopkins et al., 2017). For example, a study in temperate systems found that if the litter mixtures placed in litterbags are at the same weight ratio as the natural leaf litter from the forest, they decomposed differently than if they were in a simulated 50:50 ratio (Gartner and Cardon, 2012). Importantly, most of the research on non-additive effects has been carried out in temperate forests with a different climate and much lower plant diversity than in tropical forests. Tropical forests are by definition warmer and more humid than temperate forests, which means that they have a faster organic matter turnover and may react differently to environmental changes (Bréchet et al., 2018). Hence, there is a need to investigate how forest modification affects soil C cycling and other important ecosystem functions via changes in tree diversity and species composition.

1.3.2 Links between plant and microbial communities

Just as changes in plant species composition has repercussions for soil functions, changes in microbial communities can also alter decomposition processes and nutrient cycling, which has repercussions for plant growth (Sayer et al., 2017). The reciprocal exchange of resources between plant and microbial communities can also develop into plant-soil feedbacks. There are multiple lines of evidence from temperate forests that specific microbial communities will develop in the vicinity of specific tree species (Sayer et al., 2017; Vivanco and Austin, 2008). Therefore, litter decomposition, microbial biomass, and microbial community composition are expected to differ below different tree species. As a consequence, litter will decompose faster close to its parent tree and slower if it is in a foreign environment; this has been named the

"home-field advantage" (Ayres et al., 2009; Freschet et al., 2013; Veen et al., 2015). Another hypothesis that also involves the interaction of microbial communities with plant species states that plants will release chemicals in their surroundings designed to hinder other plants and microbial communities from growing in their environment; thus, preventing other species from establishing themselves in their environment (Cummings et al., 2013). However, the applicability of these theories to highly diverse tropical forests is uncertain. For example, a reciprocal litter transplant experiment in a subtropical pine and broadleaf plantations in Southeast China found that although microbial community composition differed between plantations, the decomposition rates were similar (He et al., 2016). Hence, although the microbial communities were distinct, they were functionally similar and were stimulated by the addition of new substrate, regardless of origin. Furthermore, although the overall decay rates were the same, early-stage decomposition of the litter was faster in one habitat whereas late-stage decomposition was more rapid in the other habitat. Hence, distinct microbial communities may result in the same overall decay rate of litter, but the pattern of decomposition can differ according to the predominant microbial functional groups (He et al., 2016).

Changes in plant diversity could also affect ecosystem function via the quantity of litter inputs. A meta-analysis of litter manipulation studies found a positive correlation between soil respiration rates and the amount of litter added and a significant decrease in total C from mineral soil when litter was removed. In general, microbial biomass C declined significantly with the removal of litter in tropical forests indicating that microbes are reliant on C supply from fresh litter. This may be particularly important in tropical forests, where the mean residence time of surface litter is 0.25 to 1 year. Hence, tropical forests are highly responsive to changes in litter production, and soil C pools are affected even over short periods of time (Xu et al., 2013).

Therefore, both litter quantity and litter diversity play a crucial role in soil C dynamics and sequestration. However, we still know very little about how litter properties interact with the soil microbial communities and affect soil C dynamics in tropical forests (Hättenschwiler et al., 2010; Laird-Hopkins et al., 2017). It is especially important to understand the linkages between plant diversity, productivity and soil

processes in tropical forests to assess the full impact of anthropogenic activities on forest C dynamics and ecosystem function.

1.4 Two case studies: oil palm agriculture in Sabah and timber plantations in panama

Deforestation and conversion to plantations account for an estimated 75 % of total CO_2 emissions from tropical Asia since the 1980s (Laurance 1999; Houghton & Hackler 1999; Miettinen et al. 2011). In Southeast Asia deforestation is so widespread that three quarters of its forest might be lost by the end of the 21st century (Sodhi et al., 2004). In 2002, Borneo had already lost 57 % of its original forest cover (Saner et al., 2012) and in Sabah, only 53 % of the original forest cover remains, of which only 15 % is undisturbed primary forest (Global Forest Watch, 2018). Oil palm agriculture is one of the main drivers of deforestation in Southeast Asia. Worldwide, the area covered by oil palm plantations increased by 2.6 times between 1990 and 2010 and oil palm plantations currently occupy 15.9 Mha of land (FAO. 2015), and Malaysia and Indonesia in Southeast Asia produce 85 % of the world's palm oil (RSPO, 2013). In Sabah alone, around 714,000 ha of forest were logged and converted to oil palm monocultures between 1990 and 2010 (Figure 1.1).

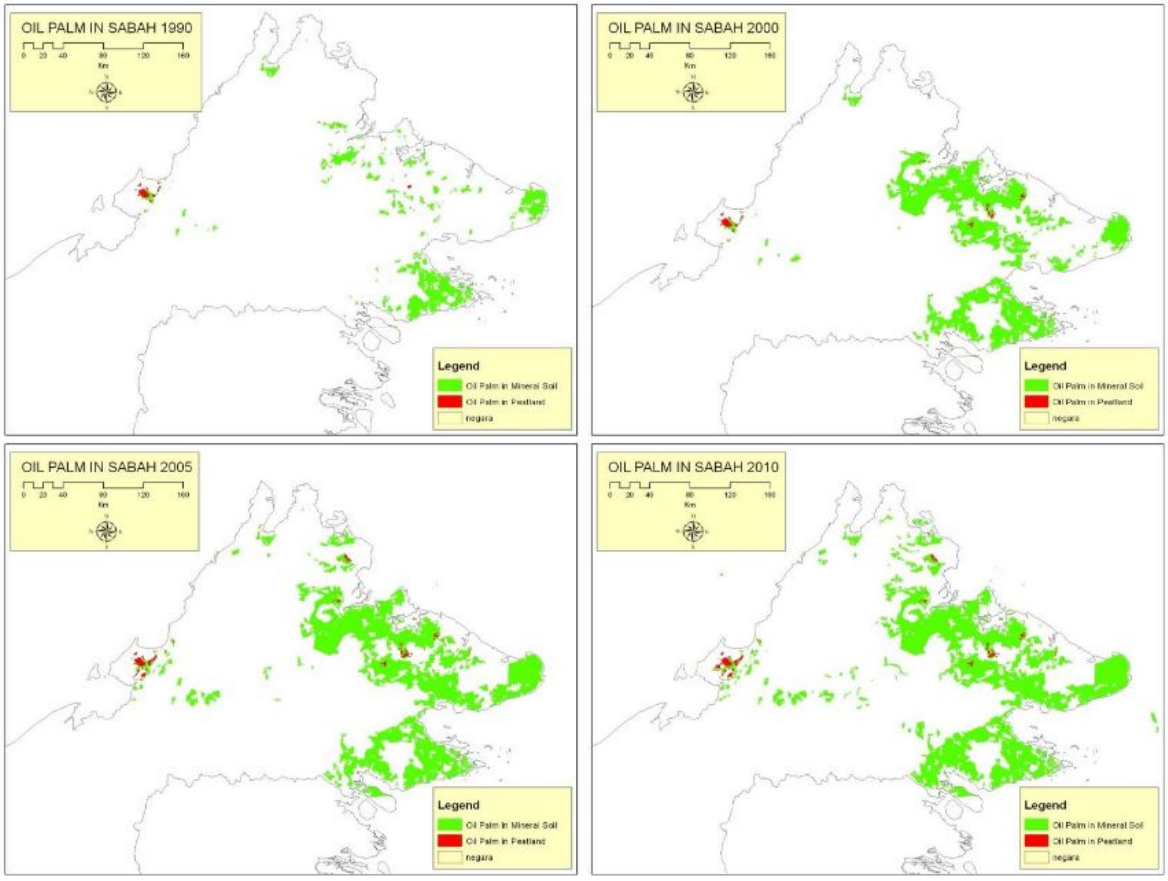


Figure 1.1 The expansion of oil palm plantations on mineral and peat soil in Sabah between 1990 and 2010 (RSPO, 2013).

In Sabah, deforestation and conversion to oil palms has resulted in the creation of small isolated fragments of forest (Reynolds et al., 2011). It is in this context that the Stability of Forest Ecosystems was set up to study the effects of forest habitat fragmentation on biodiversity (Ewers et al., 2011). The project was established within an area of degraded forest that had been selectively logged in the 1980s (Cannon et al., 1999; Stibig et al., 2014). Selective logging involves the removal of trees above a specific diameter, which not only increases the frequency of forest gaps but also causes extensive damage through the construction of temporary roads for access to the trees (Achard et al., 2002; Berry et al., 2010; Sodhi et al., 2004). This has resulted in the removal of key species in the dipterocarp family, which have a highly specialised ectomycorrhizal symbiotic fungal relationship (Both et al., 2017; Kenzo et al., 2006). Such changes in forest cover and disturbance is expected to have significant impacts on the biogeochemical cycle, including the soil C cycle. Logging, complete deforestation and terracing changes the above and below ground diversity of the

fauna and flora in the ecosystem. As such, the SAFE project allows for the study of soils over a gradient of forest degradation from pristine old-growth forest to oil palm monocultures.

In contrast to Malaysian Borneo, over 60 % of the land in Panama, Central America, comprises tree cover (4.49 Mha, Global Forest Watch 2018), although only 937 kha is considered to be old-growth forest. Between 2001 and 2017, an estimated 352 kha (6 % of the total tree cover) were deforested resulting in emissions of 33.8 Mt of CO_2 into the atmosphere. Many reforestation projects such as the Bonn challenge or REDD+ are encouraging small landowners to grow timber plantations on old croplands in an attempt to restore soil quality for fertility and C storage (Hall et al., 2011b, 2011a; Mayoral et al., 2017). As a result, 40 % of the tree cover in Panama now comprises timber plantations and secondary forest (Global Forest Watch, 2018).

Although sustainably managed timber plantations can help maintain soil C stocks and ecosystem function, one of the most common timber species in plantations in Panama is Teak (*Tectona grandis,* L;f) an introduced species from South and Southeast Asia. Teak plantations represented 76 % of the timber plantation area in Panama planted between 1992 and 2002 (Pandey and Brown, 2000; Wolfe et al., 2015). However, teak does not grow well on the acidic clay soils which are common around the Panama Canal Area (Mayoral et al., 2017), which creates an ecological and economical issue. Failing plantations represent a risk to employment and income in local communities and also affect the microclimate and ecosystem functions of planted areas. More recently, a number of projects have instead started growth trials with native timber species (Hall et al., 2011a, 2011b; Healey and Gara, 2003; Mayoral et al., 2017; Piotto et al., 2010) but there is still a lack of information on the possible positive effects of native as opposed to non-native timber plantations on soil C dynamics, which is essential for planning sustainable timber production and reforestation projects. The Agua Salud Project in Panama is part of the Smithsonian Tropical Research Institute and aims to study how degraded landscapes can be restored through forest regeneration, timber plantations, or livestock ranches (STRI, 2018). As part of this aim, in 2011, they set up five native timber plantations and a teak plantation as comparison to find a sustainable alternative to the underperforming teak plantations (Mayoral et

al., 2017). These plantations provided the ideal system to study the effects of teak compared to native tree species on soil microbial activity.

1.5 Thesis objectives

Previous studies have demonstrated the negative impacts of deforestation and forest modification on C storage in aboveground biomass and soil, but whereas the recovery trajectory of aboveground biomass during forest regeneration appears largely consistent, there is no clear pattern in soil C storage during forest regeneration (Telles et al., 2003; Wiesmeier et al., 2013). We are currently unable to predict how forest modification will influence soil C storage over the longer term because our understanding of how anthropogenic disturbance influences soil C dynamics is still lacking. In addition, we do not know why results differ depending on the geographical location of the studied area. To gain a more complete understanding of soil C dynamics and storage, it is important to understand how plant diversity and litter inputs influence microbial processes in the soil and how these are affected by forest modification. Such knowledge will help us assess which areas will be more affected by forest degradation and determine suitable regeneration or management strategies. The body of work presented in this thesis aims to address some of these challenges by characterising plant-soil interactions and quantifying changes in soil C dynamics in three different types of human-modified tropical forests: degraded forest with oil palm agriculture in Sabah, Malaysian Borneo; naturally regenerating secondary forest in Panama, Central America, and in timber plantations with native vs. non-native species in Panama. I used litter transplant experiments to assess the effects of forest changes on soil activity. I investigated how differences in microclimate, soil and litter properties influence decomposition processes and soil respiration at all sites, and I compare and contrast the results to identify general patterns.

1) In chapter 3, I compared the influence of microclimate and litter inputs on soil C dynamics along a deforestation gradient, including oil palm plantations in Sabah, Malaysia. Using a mesocosm experiment with reciprocal litter transplants I show that litter properties and microclimate both affect microbial activity.

2) In chapter 4, I compared the influence of microclimate and litter inputs on soil C dynamics along a forest modification gradient, including a deforested habitat, a regenerating secondary forest and an Old-Growth forest. Using a mesocosm experiment with reciprocal litter transplants I show that litter properties and microclimate both affect microbial activity and that regenerating secondary forests regain some soil C cycling function.
3) In chapter 5, I compared how changes in litter diversity affect soil C dynamics in one non-native and two native monoculture plantations in Panama. Using a mesocosm litter transplant experiment using single and mixed species. I show that litter properties and microclimate but also tree species characteristics such as herbivory rates and the release of allelopathic chemicals affect litter decomposition and microbial activity.

CHAPTER 2 METHODS

2.1 Overview of Experiments

In this chapter I will describe the field sites as well as the set-up of field mesocosms, litter transplants, and general methods used in the quantification of soil characteristics, gas fluxes, decomposition rates throughout this thesis.

I conducted manipulative field and lab experiments in which specific mixtures of leaf litter (thereafter referred to as "litter") were left to decompose on different soils in field mesocosms or lab microcosms. I measured soil carbon dioxide (CO_2) efflux to determine microbial activity and analysed soil characteristics linked to carbon (C) turnover to assess the effects of the different litter mixtures on C dynamics.

Mesocosms are enclosed experimental areas that can be manipulated while retaining field environmental conditions (Figure 2.1). Due to their being in the field and therefore undergoing natural climatic conditions such as seasonal temperature and rainfall, the mesocosms are considered to be an accepted method to study changes in ecosystems in a more realistic setting than under laboratory conditions (Stewart, 2013).

My first field experiment was a pantropical study, in which I compared the effects of forest disturbance between the old-world tropics (Borneo) and the neo-tropics (Panama). My second field experiment aimed to establish specifically how litter functional traits (lignin, foliar C and nutrients) from different tree species affect soil C dynamics during decomposition. My lab experiments aimed to narrow down functional effects of changes in litter by controlling environmental factors such as temperature and moisture.

Figure 2.1 Example of experimental mesocosms made out of grey PVC pipes and placed 4 cm into the soil. The mesocosms were covered with large mesh to prevent litter from falling in while allowing rain to penetrate.

2.2 Study Locations

2.2.1 Malaysia

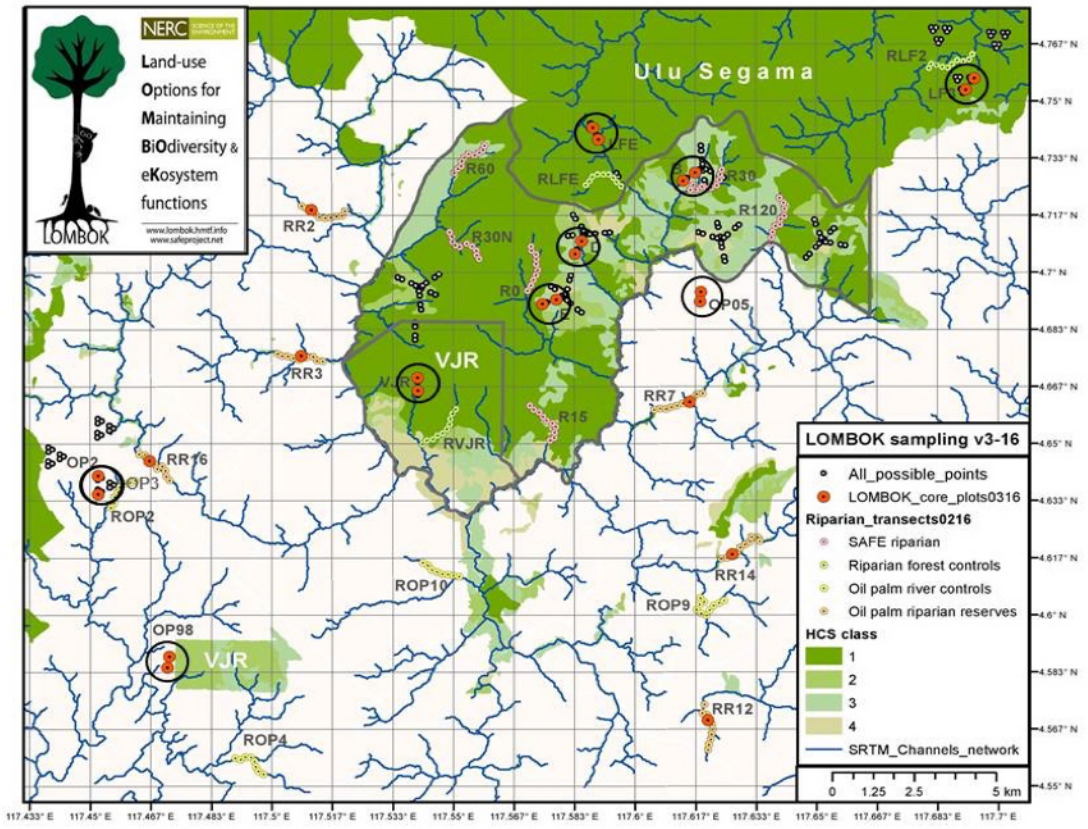


Figure 2.2 Map of the Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysia showing the areas selected for the present experiment circles in black; the red dots within the circles represent the replicate blocks which are at least 300 m apart.

The Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysian Borneo was designed as a long-term landscape ecological experiment. The project covers 32 km^2 of land including secondary forest, watersheds and oil palm plantations up to 30 years old (Figure 2.2). Specifically, it contains forests at different levels of disturbance from lightly logged to forest fragments to oil palm monocultures. The aim of the SAFE project is to study how biodiversity and ecosystem function change as a result of anthropogenic activity, specifically how forest fragmentation and conversion to oil palm monocultures affects ecosystem services (SAFE, 2018). The forest fragmentation experiment uses a fractal design creating circular forest fragments of different sizes ranging from 1 ha to 10 ha and 100 ha around which the rest of the forest is completely logged followed by conversion to oil palm plantations; the control for this experiment

are larger areas of forest that have been selectively logged in the 1980s (Ewers et al., 2011). Within each experimental fragment, oil palm site and control forest site, blocks of 12 m^2, 150 m apart have been set up as sampling points (for a total of 18 sampling points).

My experiment was set up in collaboration with the "Land-use options for maintaining biodiversity and ecosystem functions" (LOMBOK) consortium, an interdisciplinary programme of research from plant and animal biodiversity to biogeochemical cycles of tropical forest ecosystems (LOMBOK, 2018). I used the sites selected by the LOMBOK consortium so that data collected can be added to the long-term project. Due to the nature of the LOMBOK consortium experiments, a subsample of sites within the SAFE project setup were selected. The LOMBOK consortium sites that were chosen are described below (Table 1.). The soils are orthic Acrisols or Ultisols (Riutta 2018), nutrient content is described in chapter 3.

I focused on three levels of forest disturbance: Control logged forest (CLF), Fragmented forest (FF), Oil palm plantations (PLT). The control logged forest and forest fragments were selectively logged for hardwood first during the 1970s then again between one and three times between 1990 and 2008 (Ruitta 2018, Fisher 2011, Struebig 2013), the sites chosen for the forest fragments are in the SAFE 10 ha plots. Logging started out around the forest fragments since 2012, however at the time of setting up the experiments logging hadn't been completed and most fragments had not been isolated. The oil palms (*Elaeis guineensis* (Jacq.), an introduced palm species from western Africa) were planted in 1998, 2001 and 2005.

Table 2.1 Description of the sites used in Borneo

SAFE Terminology		Project terminology		Coordinates		
SAFE Fragment Name	**SAFE Site Name**	**Habitat**	**Block**	**Latitude**	**Longitude**	**Site Description**
FFB	605	SF	A	04.729191	117.616669	Reasonably flat
FFB	607	SF	B	04.726849	117.613297	Steep slope
FFD	637	SF	C	04.709145	117.585473	Very steep, close to river
FFD	639	SF	D	04.705456	117.583625	In a small valley, no stream
FFE	653	SF	E	04.692142	117.578478	Very sleep, adjacent to gravel logging road
FFE	655	SF	F	04.690841	117.574562	Slight slope, near stream
LF3	697	CLF	A	04.756744	117.693649	Slight slope
LF3	699	CLF	B	04.753379	117.69129	On flattened out surrounded by steep slope
LFE	704	CLF	C	04.725539	117.596573	Very near stream, waterlogging
LFE	706	CLF	D	04.729039	117.595033	Reasonably Flat
VJR	770	CLF	E	04.668068	117.540289	On a flattened area along a steep hill
VJR	772	CLF	F	04.664244	117.540304	On flattened area along middle of a hill
OP3	760	PLT	A	04.635258	117.4522	Flattened by terracing, many stones
OP3	761	PLT	B	04.640706	117.452279	Flattened by terracing, many stones
OP98	OP98A	PLT	C	04.58468	117.47129	Flattened by terracing, many stones
OP98	OP98B	PLT	D	04.58785	117.4718	Flattened by terracing, many stones
OP05	OP05A	PLT	E	04.69430	117.61827	Flattened by terracing, many stones

OP05	OP05B	PLT	F	04.69157	117.61803	Flattened by terracing, many stones

The average monthly temperature for 2015 in Tawau, the closest town to the sites, is 32.5 ± 0.67 °C with a minimum of 28.3 ± 0.78 °C and a maximum of 35.08 ± 0.67°C in 2015. The year is approximately divided into a wet (November till March) and a dry season (April till October); mean monthly rainfall was 164.1 ± 24.34 mm, ranging from *ca.* 44.9 mm during the driest month (March 2015) and *ca.* 342.8 mm during the wettest month (May 2015; R. Walsh, unpublished data).

2.2.2 Panama

Barro Colorado Nature Monument (BCNM)

The Barro Colorado Nature Monument (BCNM, Figure 2.3) in Panama is administered by the Smithsonian Tropical Research Institute (STRI, 2017) and scientific experiments have been conducted there since the 1920s (STRI, 2018). My experiment focussed on three forest types with different levels of disturbance similar to those described for the SAFE project (Figure 2.2) old growth forest, over 200 year old (Wright, 2011), lowland tropical forest on the Gigante Peninsula (09.1067 N 079.8515 W), 2) 30-year old secondary forest in nearby Rio Gigantito in areas that were used for some form of agriculture prior to abandonment less than 100 years prior (Dent, 2013; 09.1085 N 079.8232 W) and 3) disturbed forest at the field station on Gigante (09.1283 N 079.855 W). The Gigante field station was deforested to create the station in the 1960s and has been regularly disturbed since by the construction and maintenance of buildings (E. Leigh, personal communication 2016). I was able to pick plots at each habitat such that the relief and aspect were consistent across plots so that they were all on reasonably flat surfaces and away from waterways.

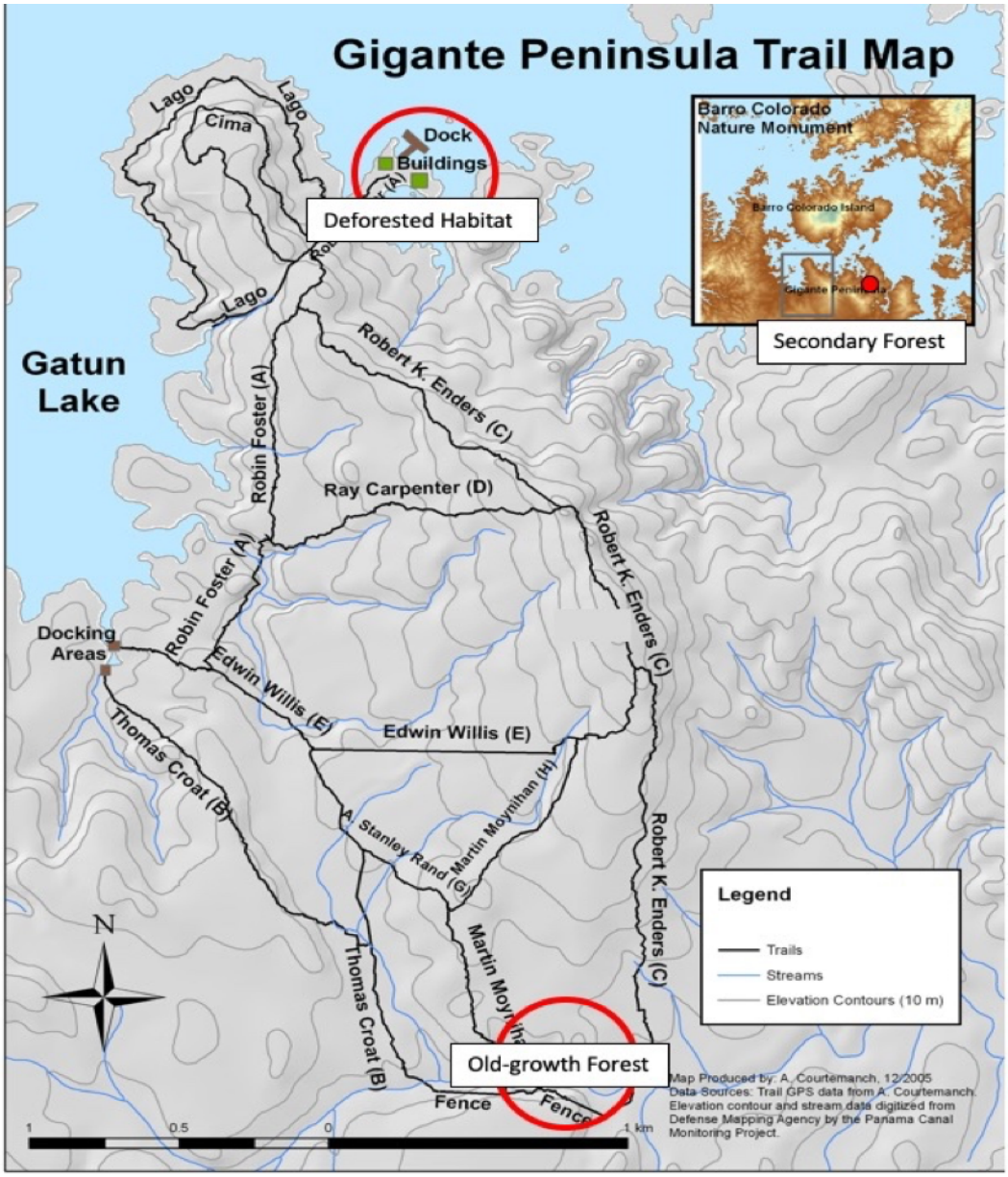


Figure 2.3 Map of the field experiment on Gigante Peninsula, Panama showing the location of the three habitats.

The weather conditions are similar to the island of Barro Colorado, *ca.* 5 km from the field sites. Between October 2016 and June 2017 (the duration of the experiment), the mean temperature was 26.9 ± 0.74 °C with a minimum of 22.8 °C in November 2016 and a maximum of 31 °C in October 2016 and received a mean rainfall of 231 ± 89.74 mm with a minimum of 8.6 mm in February 2017 and a maximum of 835.9 mm in November 2016 (Data sets provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute, 2016 and 2017). The experiment started during the dry season and the wet season started in May 2017. The soil at the study

sites is classed as an oxisol with a pH of 4.5–5.0 with low phosphorus availability (Sayer 2006, Wright & Turner 2014).

Agua Salud

The Agua Salud (AS) Project (Figure 2.4) is a large-scale experiment studying ecosystem functions and services in different forest land-use types from undisturbed forest to agricultural and forestry plantations. The project has planted five native timber species (monocultures and mixtures) as well as teak, *Tectona grandis* (L.f) (TECTGR), an introduced timber species from Southeast Asia. I established a transplant experiment in monoculture plantations of three different species: *T. grandis*, *Dalbergia retusa* (Hemsl.) (DALBRE) and *Terminalia amazonia* (J.F.Gmel). Exel) (TERMAM), which were planted in 2008 and maintained regularly by removing the native vegetation four times a year (Mayoral et al 2017). The soil type is an infertile Oxisol; previously the land had been used either as cattle pasture or secondary forest less than five years old (Mayoral et al, 2017). All blocks were chosen so that they were on reasonably flat terrain.

Figure 2.4 Map of the Agua Salud project, the experiment is set up in the Teak area and the adjacent native species area.

Between January and July 2017 (when the experiment took place), Chilibre, the city closest to Agua Salud had an average temperature of 30.43 ± 0.84 °C with a minimum of 25 ± 0.48 °C (January and February 2017) and a maximum of 33 ± 0.39 °C (March, April and July 2017).The experiment started during the dry season and the wet season started in May 2017 The average rainfall was 190.79 ± 68.97 mm with a minimum of 12.9 mm in February and a maximum of 464.8 mm in July.

2.3 Experimental Design

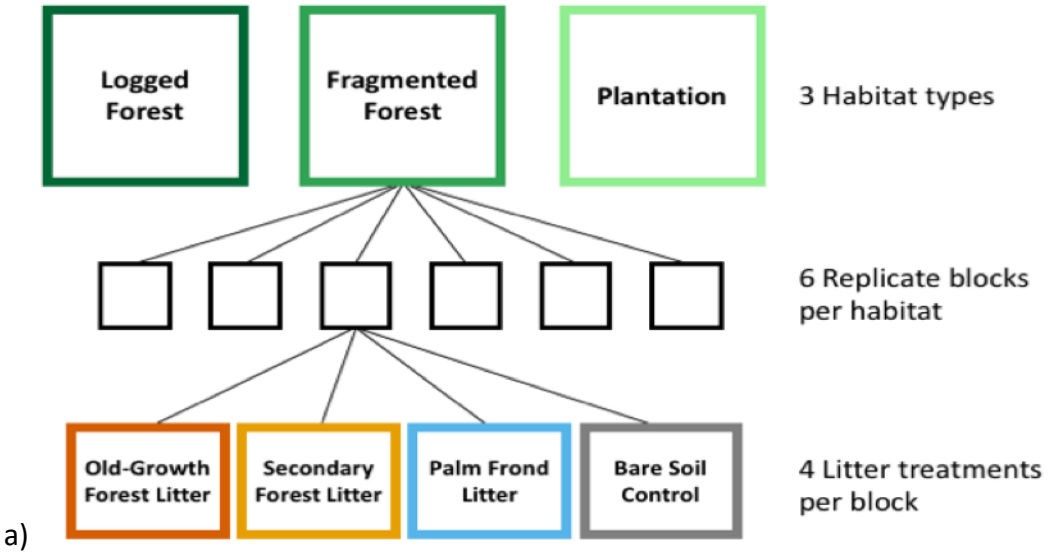
2.3.1 Pan-Tropical Comparison of Borneo and Panama forests

To understand how different levels of forest degradation affect soil C dynamics I carried out a litter transplant mesocosm field experiment in the old and the new tropics. Both Malaysia and Panama lie in the tropics, Malaysia is situated in the Indomalayan realm and Panama in the neotropical realm. The Malaysian forest is classified as a dipterocarp rainforest and the Panama as a tropical evergreen forest. They have similar average temperatures of 27°C and 28°C.

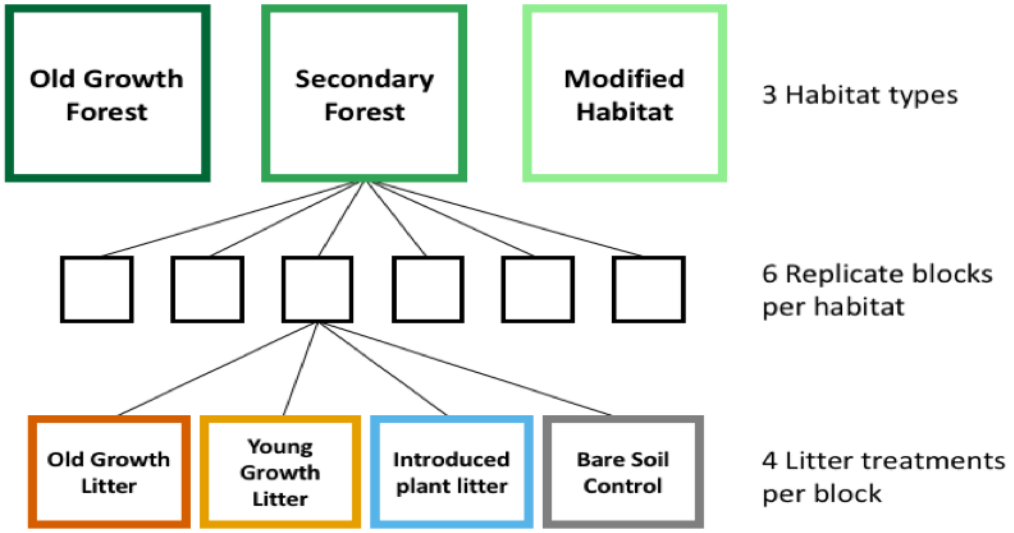
Table 2.2 Comparison of temperatures, rainfall and soil characteristics between the Malaysian and Panamanian experimental sites.

	Malaysia	Panama
Coordinates	4°66'N 117°56'E	9°11'N 79°82'W
Mean air temperature over the duration of the experiment (°C)	27.8 ± 0.09	26.9 ± 0.74
Minimum air temperature over the 9 months duration of the experiment (°C)	23.1 (July 2015)	22.8 (November 2016)
Maximum air temperature over the 9 months duration of the experiment (°C)	31.4 (August 2015)	31 (October 2016)
Mean monthly rainfall over the 9 months duration of the experiment (mm)	164.1 ± 24.34	231 ± 89.74
Minimum monthly rainfall over the 9 months duration of the experiment (mm)	45 (March 2015)	8.6 (February 2017)
Maximum monthly rainfall over the duration of the experiment (mm)	343 (May 2015)	835.9 (November 2016)
Soil type	Acrisols/Ultisols	Oxisol
Soil pH	4 to 6.5	4.5 to 5

To determine the relative influence of litter and soil properties on soil C dynamics along a forest disturbance gradient, I established a reciprocal litter transplant experiment in mesocosms within forest sites with different levels of disturbance. I selected three different forest types to represent a comparable disturbance gradient at SAFE and BCNM (Figure 2.5).



a)



b)

Figure 2.5 Design for the reciprocal litter transplant in SAFE a) and BCNM b). The green squares are the three different forest types (sites); the brown, orange, yellow and grey squares are the litter treatments.

I established six blocks of mesocosms per forest type. The mesocosm were made of 20cm diameter PVC pipes cut at a height of 13 to 14 cm. Each block consisted of two mesocosms per litter treatment to allow for destructive sampling of mesocosms after 6 and 9 months. I also had two control mesocosms per block in which all the litter was removed for a total of 144 mesocosms in each experiment. Each mesocosm received the equivalent of the mean litter standing crop in the forest sites in Borneo (16 g, unpublished data). The litter was collected by hand from the ground of the plots then rinsed using stream water and air dried at 40°C in a drying cupboard fitted with 40 W electric bulbs.

I also prepared 108 decomposition bags of each litter mixture, 16 g litter in 1.4 mm mosquito mesh of 18 cm^2 (which is equivalent to the surface inside the mesocosms), which were placed in each block (Figure 2.1). One bag of each litter mixture was harvested from each block after 6 and 9 months (in both BCNM and SAFE) to calculate litter decomposition rates (see section 2.4).

I measured CO_2 efflux over each mesocosm with a soil chamber attached to an EGM-4 infrared gas analyser (PP systems, Amesbury, USA) in SAFE and with a Li-8100 (LI-COR Bioscience, Lincoln, USA) in BCNM once a month over 9 months to measure soil microbial activity. In the SAFE and BCNM experiments, CO_2 efflux was measured over the leaves to avoid disturbance to the soil caused by removal of the leaves.

I sampled soil from the mesocosms using a 5 cm diameter punch corer to a depth of 10 cm after 6 and 9 months from the start of the experiment for SAFE and BCNM. One core was used on site for extractable N and two cores were sent off for analysis. As there were limited facilities on-site, two cores were kept and refrigerated at approx. 5°C for up to 7 days prior to microbial extraction and pH measurement as described in section 2.4. The SAFE soil samples were extracted at the Forest Research Centre in Sandakan (Sabah, Malaysia) and the BCNM soil samples were shipped to Lancaster University (U.K) for extraction (see section 2.4).

At SAFE, the sites were: OG - old growth forest that was selectively logged and left to regrow since the 1990s; FF - fragments of degraded forest that are surrounded by a deforested area; and OP - oil palm plantations, which were terraced before being planted in 1998, 2001 and 2005 (Figure 2.5). To represent the oil palm site, I used fronds of *E. guineensis,* an introduced palm species*,* cut from the trees in the OP98 plantation (Figure 2.2).

At BCNM, the sites were OF - old-growth forest, YF - young secondary forest, and DF - a deforested area (Figure 2.3). I used different litter mixtures to represent the sites; for the old-growth, secondary forest and disturbed forest sites, I used native litter collected at each site. To represent the disturbed forest at BCNM I used leaves from *Saccharum spontaneum* (L.)*,* commonly known as wild sugarcane, an introduced and fast-growing C4 grass used for stabilising the soil along the canal.

2.3.2 Effect of leaf functional traits on soil carbon dynamics

To further understand how tress with different functional traits interact to affect soil C dynamics I carried out a mesocosm field experiment comparing three known species of tree litter.

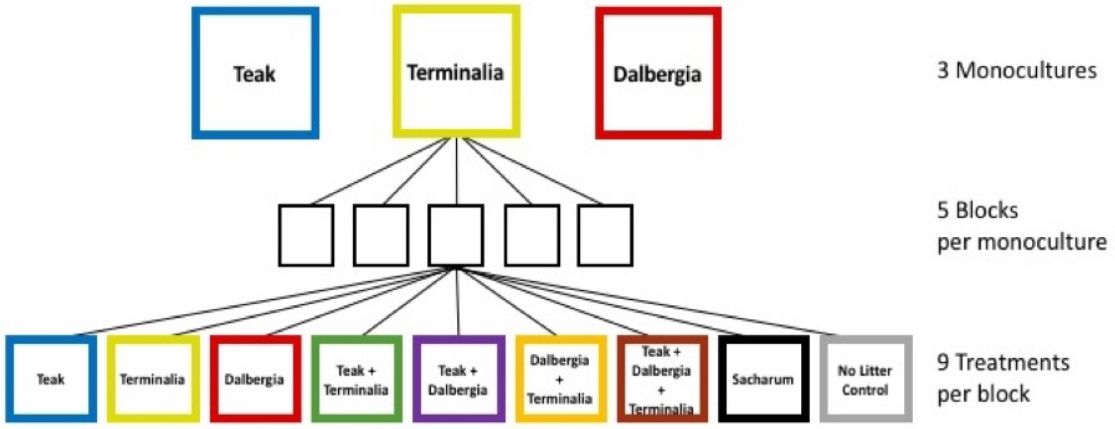


Figure 2.6. Experimental design for Agua Salud. The top three squares are the three monoculture sites and the lower nine squares are the litter treatments in the mesocosms.

I established a mesocosm experiment with reciprocal litter treatments comparing 7 different mixtures of litter from timber species (Figure 2.6) in Agua Salud. The litter mixes were:

- *Tectona grandis* (TECTGR) Only (6 g)

- *Dalbergia retusa* (DALBRE) Only (6 g)

- *Terminalia amazonia* (TERMAM) Only (6 g)

- TECTGR + DALBRE (3 g of each species)

- TECTGR + TERMAM (3 g of each species)

- DALBRE + TERMAM (3 g of each species)

- TECTGR + DALBRE + TERMAM (2 g of each species)

- Bare soil

In order to compare these sites with the BCNM sites, I established additional mesocosms with *Saccharum spontaneum* litter as a standard (6 g).

I established 5 blocks of mesocosms per forest type. The mesocosms were made out of PVC pipes 20 cm in diameter and 15 cm tall. Each block consisted of two sets of mesocosms to allow destructive sampling after three and six months. To determine the amount of litter to add to each mesocosm, I estimated the litter standing crop by placing three mesocosms on the ground in each plantation and collecting the litter inside them to determine air-dry weight. Accordingly, each mesocosm received 6 g air-dried litter, which was equivalent to half the mean litter standing crop. I also added 1 g of litter each month to simulate the monthly litterfall (determined from data from the Agua Salud project).

To characterise soil microbial biomass and soil chemistry (total C and N, inorganic N), I collected soil samples from each replicate block at the start of the experiment and then from within the harvested mesocosms after three and six months. Three soil cores were taken in each block or mesocosm to a depth of 10 cm using a 5 cm diameter punch-corer; one core was used to determine extractable nitrogen (N) and two cores were used for all other chemical analyses (see section 2.4.). Each month, I measured soil CO_2 efflux using a Li-8100 (LI-COR Bioscience, Lincoln, Nebraska, USA) from the mesocosms by removing the litter and then taking the measurement to estimate soil microbial activity. In Agua Salud, small, removable, mesh baskets were made to hold the leaves, in order to minimise the disturbance caused by litter removal

In addition to the mesocosm experiments, I used litterbags to assess changes in litter properties and measure nutrient release during decomposition. 210 litterbags, made of PVC mosquito mesh with a width of 1.4 mm, of 8 cm^2 containing 3 g of air-dried material (half the surface of the mesocosms and half the amount of litter) from each species and mixture were placed on the forest floor in each replicate block and 1 bag per mixture per block were collected after three and six months (at the same time as the mesocosms). To track the dynamics of C and nutrients from the litter during decomposition, I characterised specific litter properties (lignin and cellulose content, foliar C and nutrients) at the start of the experiments (see section 2.4).

2.4 Soil CO_2 efflux, soil sampling, and chemical analyses

2.4.1 Analyses of soil and litter for site characteristics

To determine the initial characteristics of the soil at each site (SAFE, BCNM and AS), I collected three soil cores from each block at 10 cm depth using a 5 cm diameter punch corer. All samples were air-dried at room temperature for analysis of total C, (N) and soil pH (see below).

Soil sampling

For site characterisation, I took four 10 cm cores from each block using a 5 cm diameter punch-corer and air dried them at room temperature. For the soil sampling protocol of the mesocosm see sections 2.3.1 and 2.3.2.

Soil pH

I measured soil pH on 3 g fresh soil in 9 ml of deionised water to give a soil to water ratio of 1:3. The slurry was shaken and left to rest for 30 min before measurement with a pH meter (Mettler Toledo® Seven Compact®, Leicester, UK or Corning® Model 430, Edison, USA). I chose to measure pH in water for comparison to previous research at the sites and because the method is easily conducted in remote sites without lab access.

Total soil C and N

For analysis of total soil C and N, I ground subsamples of air-dried soil and litter from each sample using ball mill (Mixer Mill 400, Retsch®, Haan, Germany). Total C and N was analysed by high temperature combustion gas chromatography on a Vario El III C/N analyser (Elementar, Stockport, UK) at Lancaster University using 30 mg soil and 15 mg litter.

2.4.2 Experiment analyses

Soil water content

I measured soil water content (SWC) using subsamples of 10 g fresh soil weight for the field experiments and 2 g for the lab incubations. All subsamples were oven-dried at 105°C for 24 h and then weighed (Eq 2.1) to determine percentage SWC using the equation:

$$\frac{F-D}{D} \times 100 \qquad \textbf{Eq 2.1}$$

Where: F is the soil fresh weight in g, D is the soil dry weight in g.

Soil and litter respiration

Pumpanen et al (2004) compared the EGM-4 with the SRC-1 chamber with a LI-6400-09 infrared gas analyser and found that both infrared gas analysers give similar readings that underestimate CO_2 flues by about 4% of the reference sample which is considered acceptable for field sampling. The LI-6400 infrared gas analyser is an older version of the Li-8100 LI-COR gas analyser, a study by Madsen et al (2005) showed that both the old and the new infrared gas analyser gave similar flux rates. The fluxes from EGM-4 and the LI-8100 are therefore comparable.

Soil microbial biomass by fumigation-extraction

I determined microbial biomass C and N on paired 8 g subsamples of fresh soil by the fumigation-extraction method described in Vance et al. (1987) and modified by Jones and Willett (2006). One 8 g subsample per pair was fumigated for 24 h with ethanol-free chloroform stabilised with Amylene and all samples were extracted in 40 ml 0.5 M K_2SO_4, then centrifuged and filtered through Whatman 42® (GE Healthcare, Chicago, USA) filter paper, or equivalent, previously rinsed with 0.5 M K_2SO_4 solution. Total organic C (TOC) and total N in the extracts were analysed on a TOC-L combustion analyser (Read, 1921) coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Microbial biomass was calculated (Eq 2.2) by subtracting the fumigated from the unfumigated samples (correcting with blanks but not correcting for extraction efficiency). The equation used to calculate microbial biomass is:

$$\frac{(M-b)\times V}{D} = C \qquad \textbf{Eq 2.2}$$

Where: M is the carbon or nitrogen reading, b is the average of the blanks, V is the volume of K_2SO_4 in ml, D is the soil dry weight and C is the microbial biomass carbon or nitrogen in µg per g of dry weight soil

Soil extractable ammonium and nitrate

I determined extractable ammonium and nitrate (extractable N) by adding 6 g of fresh soil to 20 ml of 1M KCl solution in the field straight after soil core extraction as described in Turner & Romero (2009). The samples were shaken by hand every 15 min for a length of 1 min over 1 hour then filtered through Whatman 42® (GE Healthcare, Chicago, USA) filter paper, or equivalent, previously rinsed with 1 M KCl solution. Each sample received 2 drops of 70% HCl and was frozen until analysis on an Astoria Pacific (Astoria Pacific©, Clackamas, USA) auto-analyser in Sabah and a Seal auto-analyser AA3 (Seal-Analytics©, Southampton, UK) in Lancaster. The equation used to convert the results from ppm to mg/ g dry weight soil is (Eq 2.3):

$$\frac{[(\mathrm{NH3+NO3.NO2})\text{-b}]\times \mathrm{V}}{\mathrm{D}} = \mathrm{N} \qquad \textbf{Eq 2.3}$$

Where NH3 and NO3.NO2 are the ammonium and nitrate readings, b is the average of the blanks, V is the volume of KCl in ml and N is the KCl extractable N in mg per g of dry weight soil.

Anion and cation exchange using resin probes

I placed anion and cation exchange resins (PRS Probes®, WesternAG, Saskatchewan, Canada) flat on the soil beneath the litter inside of the mesocosms at the start of the experiment (exposure time 30 days as per manufacturer instructions) to measure differences in nutrient availability at the litter-soil interface during the early stages of decomposition in BCNM and in Agua Salud. The probes were only installed for one month and would therefore have had minimal impact on decomposition over the duration of the experiment. On collection from the mesocosms, the probes were cleaned with distilled water then sent to WesternAG® (*www.westernag.ca)* for

analysis. The nutrients analysed were: Total N, NO_3N, NH_4N, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, Cd.

Phospho-lipid fatty acids (PLFA)

Phospho-lipid fatty acids (PLFAs) were extracted from c. 1g freeze-dried soil following the high-throughput method of Buyer and Sasser (2012). Extracts were analysed by gas chromatography and peaks were identified using the Sherlock 6.2™ Microbial Identification System (MIDI, Newark, DE, USA) at a commercial laboratory (Microbial ID Inc., Newark, USA). The individual peaks are interpreted as biomarkers for different microbial groups (Frostegård, Tunlid & Bååth, 2011), with the relative intensity of peaks representing the relative abundances of each group in the soil microbial community.

Litter decomposition rate

To clean the litter bags and the litter from the mesocosms after removal from the field I selected the dirtiest litter bag and timed how long it took to remove as much soil as possible without damaging the leaves. I then cleaned all other litter samples for the same amount of time and air dried them at 40°C for at least 48 hours then weighed separately. The equation used for calculating the decay rate (Eq 2.4) *k* according to Olson (1963) is:

$$\ln\left(\frac{X}{X_0}\right) = kt \qquad \textbf{Eq 2.4}$$

Where: t is time in months since the bags were placed in the field, X is litter dry mass at collection, X_0 is the initial litter dry mass.

Acid detergent fibre (ADF) and acid detergent lignin (ADL) from litter

Plant fibre and lignin analysis was determined using the method described in Van Soest et al (1991). The method has two steps described below: Acid detergent fibre (ADF) extraction is the first step which extracts all fibre and lignin, the second step is acid detergent lignin (ADL) which is the extraction of the lignin.

Samples of dried litter mixtures used in all the experiments were ground using ball mill (Mixer Mill 400, Retsch®, Germany). Then 1 g of sample was placed in a crucible with 1 g of acetanilide and placed in a FOSS fibertec™ 8000 fibre analysis system (FOSS, Hilleroed Denmark). The samples were boiled with 100 ml an acid detergent solution (ADS) and 4 drops of n-Octanol for 1 hour (FOSS, AN3429) then the samples were rinsed with distilled water until acid free and soaked in reagent grade acetone. The samples were then dried at 105°C overnight before weighing.

Once dried and weighed, 25 ml of 72% H_2SO_4 was added to each crucible which was stirred for every hour over a 3 hour period. The crucibles were rinsed with hot distilled water and dried at 105°C overnight before being placed in a furnace at 525°C for 3 hours. The samples were finally left to cool to room temperature in a desiccator before weighing. The equations used to calculate %ADF (Eq 2.5) and ADL (Eq 2.6) are as follows:

$$\text{ADF} = \frac{(\boldsymbol{W_3}-\boldsymbol{W_1})-(\boldsymbol{b_3}-\boldsymbol{b_1})}{\boldsymbol{W_2}} \qquad \textbf{Eq 2.5}$$

Where ADF is the fibre content in %, W_1 = Initial crucible weight (g) W_2 = Sample weight (g) W_3 = Crucible + residue weight (g) after ADF extraction b_1 = Initial blank weight (g) b_3 = Blank weight (g) after ADF extraction

$$\text{ADL} = \frac{(W_4-W_5)-(b_4-b_5)}{W_2} \qquad \textbf{Eq 2.6}$$

Where: ADL is the lignin content in %, W_2 = Sample weight (g) W_4 = Crucible + residue weight (g) after ADL extraction W_5 = Crucible + residue weight (g) after ashing step b_4 = Blank weight (g) after ADL extraction b_5 = Blank weight (g) after ashing step

2.5 Data analysis

2.5.1 Response ratio

Response ratio of the soil respiration was calculated for each litter treatment in each replicate block at each month using the following equation (Eq. 2.7):

$$R = \ln\left(\frac{L}{S}\right) \qquad \textbf{Eq 2.7}$$

Where: R is the response ratio, L is the respiration from the mesocosm containing litter and S is the respiration from the bare soil mesocosm from the same plot

2.5.2 Homefield Advantage

I calculated homefield advantage (HFA, Eq 2.8) of the litter decay rates and overall mean soil respiration of the single species litters after three and six months using the same equation used to calculate homefield advantage in sports. The equation described by Ayres et al. (2009) where HFA > 0 indicates that the litter decomposes faster in its home plantation, HFA = 0 indicates no HFA and HFA < 0 indicates that the litter decomposes slower in its home plantation:

$$HFA = (HDD - ADD - H)/(n-2)$$

$$HDD = (D_{aA} - D_{bA}) + (D_{aA} - D_{cA})$$

$$ADD = (D_{aB} - D_{bB}) + (D_{aC} - D_{cC}) \qquad \textbf{Eq 2.8}$$

$$H = \frac{(HDDa + HDDb + HDDc)}{(n-1)}$$

Where HFA is the additional decomposition at home, HDD is the home decomposition difference and ADD is the away from home decomposition difference. D is the measure of decomposition (decay or respiration rate), a, b, and c are the single-species litters, A, B, and C are the plantations for each respective litter species, n is the number of litter species, and H is the sum of all HDD divided by n - 1.

2.5.3 Non-additive effects of species mixtures

To calculate if there were any additive or antagonistic effects from the three different litters used in the experiment, I calculated the mean respiration and mass loss of two or three single species for the expected respiration and mass loss and compared it to its respective species mixture that had been placed in the field (observed mass loss and respiration).

2.5.4 Statistical Analysis

All statistical analyses were conducted in R version 3.4.2 (R Core Team, 2017) using the lme4 package for linear mixed effects models (Bates et al. 2017).

I used linear models to assess differences in individual soil properties among habitats (*lm* function). The effects of habitat and litter treatment on soil temperature, moisture, respiration and response ratios as well as litter mass loss were assessed using nested linear mixed effects models with month and replicate blocks as random effects (*lmer* function). Full models included the fixed effects: habitat and litter treatment and their interaction. Their significance was determined by sequentially dropping terms until a minimum adequate model was reached, using AIC and p-values to check for model improvement. The final models were compared to appropriate null models using likelihood ratio tests and the model fit was assessed using diagnostic plots (Crawley, 2007).

I used principal component analysis (PCA; *rda* function) to visualise the separation between habitats based on soil properties (total C, total N, C:N ratio, P, K and pH). I also used PCA to visualise the separation between habitats or litter treatments on the nutrients released from the PRS resin probes.

I investigated the relationship between decomposition and mean soil respiration at three, six and nine months with Pearson's correlations, using the decay rates calculated from the litterbags.

I used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities to represent shifts in soil microbial communities (metaMDS function) to analyse the PLFA results and the vegan package (Oksanen et al., 2015). Stable solutions with stress scores <0.2 and r2 > .95 were used for subsequent analyses, resulting in a two-dimensional solution. I then used the envfit function for vector fitting to the NMDS ordinations to determine the effects of soil properties; significance values were generated with 9,999 random permutations stratified within experimental blocks. Finally, assessed the effects of site or litter treatment on individual microbial groups

(fungi, Gram+ bacteria, Gram- bacteria and Actinomycetes) using separate linear models (*lm* function).

CHAPTER 3 THE EFFECTS OF FOREST DEGRADATION ON SOIL CARBON DYNAMICS ALONG A DISTURBANCE GRADIENT IN SABAH, BORNEO

3.1 Abstract

Land-use change in general, and deforestation in particular, can alter soil organic carbon content and can also affect the soils' ability to accumulate and store carbon. Sabah, in Malaysian Borneo, is rapidly converting its tropical forest to oil palm plantations resulting in isolated secondary forest fragments of various sizes and degrees of degradation. The Stability of Altered Forest Ecosystems (SAFE) project in Sabah aims to understand the ecological implications of forest degradation and fragmentation and conversion to oil palm monocultures.

I investigated the effects of forest modifications on soil C dynamics at distinct sites within the SAFE project. To determine how changes in forest cover affects soil C through differences in leaf litter composition, I established a reciprocal field experiment. I measured decomposition of three different leaf litter mixtures representing old-growth forest, secondary forest and oil palm plantations, in mesocosms within three different habitats: continuous logged forest, fragmented forest and oil palm plantations. As a measure of microbial activity, I measured soil CO_2 efflux over the mesocosms each month from March until November 2015 and I characterised soil properties at each experimental site.

Litter properties explained litter decomposition rates and microbial activity with the oil palm litter decomposing the fastest regardless of the habitat. Litter from old-growth and secondary forests decomposed at similar rates due to the high species

diversity of the litter. Microclimate and soil properties of the different habitats explained the variation in litter decomposition and microbial activity between habitats. Both microbial activity and litter decomposition were significantly lower in the oil palm plantation, the most degraded habitat, which also showed differences in microbial community composition suggesting a decrease in soil C cycling abilities.

Overall, my results suggest the oil palm habitat has severely reduced microbial activity and ecosystem soil C function and that degraded forests retain some soil function. It is therefore important to apply sustainable management practices in order to improve soil C cycling in oil palm plantations and preserve forest cover.

3.2 Introduction

Tropical forests are one of the largest terrestrial C sinks; they represent up to 30 % of the total forest soil C sink (Sayer *et al.*, 2011), contain an estimated 2344 Gt of organic C (Stockmann *et al.*, 2013), and sequester an estimated 1.1 ± 0.3 Pg C y^{-1} (Malhi, 2010), making them an essential part of the global C cycle. However, disturbance from anthropogenic activity is jeopardising the C sink potential of tropical forests (Lal, 2005; Don, Schumacher and Freibauer, 2011). Tropical deforestation is responsible for the release of 1.7 Pg C per year and logging and forest conversions is responsible for 25 % of anthropogenic CO_2 emissions. Between 2000 and 2005, forest degradation caused tropical forests to become C sources instead of sinks (Malhi, 2010). These degraded or secondary forests now represent 60 % of tropical rainforests (Laurance, Sayer & Cassman, 2014; Hansen *et al.*, 2013).

In 2014, deforestation and forest degradation were responsible for tree cover loss spanning 24 million hectares (Global Forest Watch, 2018). Deforestation is the complete removal of the forest for conversion to anthropogenic land-use such as agriculture, silviculture or urbanisation; it is the main driver of forest loss in the world, especially in the tropics (Wright, 2005). Deforestation in Southeast Asia is so intense that up to three quarter of its forests might be lost by the end of the 21st century (Sodhi *et al.*, 2004), much of which is attributed to conversion of forest to croplands (Wilcove *et al.*, 2013). Many of the remaining forests are considered to be degraded, as partial

logging has removed specific species of trees and increased the incidence of forest gaps (Houghton, 2012). Specifically, it has been estimated that aboveground woody biomass in the secondary forests of Sabah is only one third that of the old-growth forest (Riutta *et al.*, 2018). Furthermore, due to the deforestation, fragments of forest remain isolated from each other, which can have consequences for biodiversity and ecosystem function (Ewers *et al.*, 2011). Selective logging, which is the specific removal of the tree species that have commercial value, is becoming common as it is more sustainable and is often practiced in the tropics. However, the removal of specific, often bigger, trees can also lead to forest degradation due to the loss of key species or an increase in large gaps (Costantini, Edwards and Simons, 2016; Riutta *et al.*, 2018).

Oil palm plantations are one of the main causes of deforestation and forest degradation in southeast Asia (Lee-Cruz *et al.*, 2013; Wilcove *et al.*, 2013). The area occupied by industrial oil palm plantations in Indonesia, Malaysia and Papua New Guinea has increased rapidly in recent decades, from 3.5 Mha in 1990 to 13.1 Mha in 2010; forest prior to conversion 4.1 % of the land was undisturbed forest and 32.4 % was disturbed (RSPO, 2013). In Malaysia in 2000, 88 % (20.8 Mha) of the land was covered by natural forest, by 2010 this had decreased to 69 % (16.6 Mha) and 91 % of the deforestation resulted in complete tree cover loss (Global Forest Watch, 2018). By 2015, the area covered by oil-palm exceeded the area of old-growth tropical forest: old-growth forest occupied 5.02 Mha whereas oil palm plantations occupied 7.22 Mha (FAO, 2015). In Sabah, a region of Malaysia in northern Borneo, total tree cover in 2013 was 7.37 Mha but only 3 % (160 kha) of that was intact forest, while 26 % (1.86 Mha) was oil palm plantations (Global Forest Watch, 2018). Consequently, tropical forests in Southeast Asia now consist mostly of secondary forest or oil palm plantations.

Oil palm agriculture has been shown to be responsible for soil degradation, loss of soil C and overall soil fertility due to the conversion and management methods (Comte et al., 2012b; Guillaume et al., 2016, 2015; Lee-Cruz et al., 2013). To create an oil palm plantation, complete deforestation followed by terracing of the land is required, and this can result in poor drainage due to waterlogging from soil compaction and lack of tree cover, reduced soil fertility from the removal of top soil during the terracing process and increased soil erosion in comparison to forested areas, especially in

environments that have clay-rich soils (Guillaume et al., 2016). Furthermore, management practices such as the use of herbicides and the piling of oil palm fronds can negatively affect soil microbial communities and increase soil erosion by reducing leaf litter and understory plant coverage (Dechert et al., 2004; Hamdan et al., 2000; Luskin and Potts, 2011). As vast areas of forest are being converted into oil palm plantations in response to increasing global demand, we need to improve our understanding of the potential negative effects of oil palm agriculture on tropical soil function.

Logging and conversion to oil palm agriculture changes tree species composition, and therefore the type and amounts of organic matter sources available to soil microbes (Costantini et al., 2016; Tripathi et al., 2016). Litterfall is one of the most important sources of organic matter for nutrient cycling in forest ecosystems (Vitousek, 1984; Attiwill and Adams, 1993; Adachi *et al.*, 2006; Sayer and Tanner, 2010). Litter inputs provide nutrients to the soil microbial decomposers; during decomposition plant-available nutrients such as nitrogen (N) are released and organic C is stored in the soil (Gougoulias et al., 2014). However, it is not clear how changes in the nature of the organic matter provided to the microbes affects them and their C cycling abilities (Fanin et al., 2011; Hättenschwiler et al., 2005; Marichal et al., 2011). If plantations or degraded or secondary forests are to continue acting as a C sink, they must retain similar C cycling conditions as old-growth forests. Therefore, we should seek to understand if and how oil palm plantations differ in their ability to cycle C so that action can be taken to increase their sustainability.

To start addressing questions about the effect of changes in tree and plant composition on soil C dynamics and litter decomposition, I set up a mesocosm experiment under field conditions at the Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysian Borneo (see site description below). My mesocosm experiment used reciprocal litter treatments in three habitats along a forest degradation gradient to establish how different litter mixtures, representing varying degrees of forest degradation, affect soil C dynamics in three habitats: an oil palm plantation, forest fragments and continuous logged forest. Mesocosm experiments are a useful way of manipulating environmental conditions while retaining field

environmental conditions and minimising disturbances (Laird-Hopkins et al., 2017). The mesocosms allowed me to measure CO_2 efflux over the soil and litter ("soil respiration") as it was decomposing to determine if changes in soil respiration could be explained by changes in leaf litter composition (Ayres et al., 2009); the combined measurements of decomposition and soil respiration served as an indicator of soil microbial activity. I aimed to test the following hypotheses:

1) Soil microbial activity will differ among habitats, whereby microbial activity will be greatest in the forest habitats and lowest in oil palm plantations due to changes in microclimate, such as soil temperature and moisture content, soil chemical properties, and litter diversity.

2) Leaf litter decomposition rates will be related to the interactive effects of litter diversity, soil properties, and microclimate.

3) After accounting for microclimate, the differences in litter and soil C cycling among sites and experimental treatments will be attributable to differences in leaf litter functional traits.

3.3 Methods

3.3.1 Site Description

I carried out my study within the Stability of Altered Forest Ecosystems (SAFE) project in Malaysian Borneo (4°49'N, 116°54'E). The SAFE project was started in Sabah in 2011 in a secondary forest area designated by the Malaysian government for conversion to plantations for palm oil production (4°49'N, 116°54'E). It is a long-term landscape-scale experiment designed to study the effects of anthropogenic activity related to deforestation and oil palm agriculture on the ecosystem as a whole (Ewers *et al.*, 2011). A key part of the SAFE project is to study how habitat fragmentation affects the forest ecosystem (Ewers *et al.*, 2011). The SAFE project comprises forest fragments of 1 ha, 10 ha and 100 ha; larger areas of forest, designated as continuous logged forest, that are not part of the conversion plan were selected as a control (Figure 3.1). All forest sites were selectively logged for dipterocarps first in the 1970s then again

between 2000 and 2008, such that the logged forest and forest fragments have a similar land-use history (Ewers *et al.*, 2011). My experiment was set-up in collaboration with the "Land-use options for maintaining biodiversity and ecosystem functions" (LOMBOK) project (Lombok, 2018).

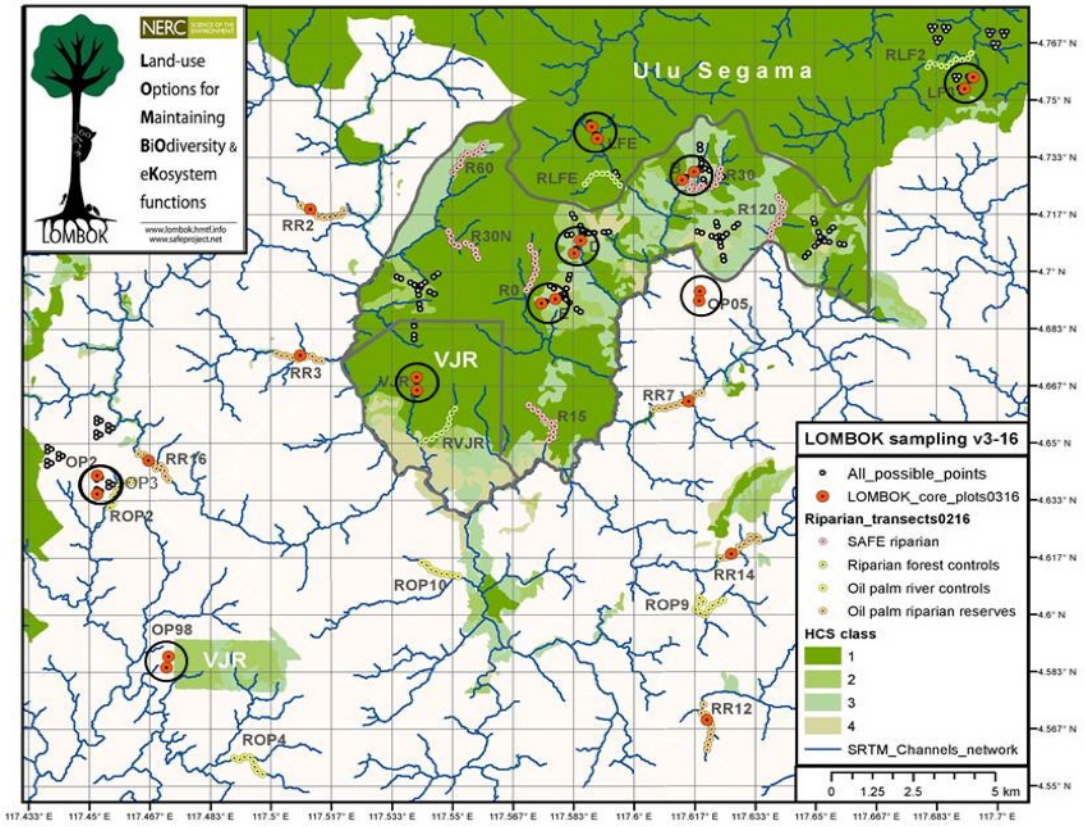


Figure 3.1 Map of the Stability of Altered Forest Ecosystems (SAFE) project in Sabah, Malaysia showing the areas selected for the present experiment circles in black; the red dots within the circles represent the replicate blocks which are at least 300 m apart.

The climate in the study area is wet tropical with a wet season from November to March and a dry season from April to October with average monthly temperatures of 32.5 ± 0.67 and average monthly rainfall 164.1 ± 24.34 mm (climate-data.org, 2018). At SAFE, the mean monthly rainfall during the study period was 164 ± 24 mm, ranging from *c.* 45 mm during the driest month (March 2015) and *c.* 343 mm during the wettest month (May 2015; R. Walsh, unpublished data). The soils at SAFE are classed as orthic Acrisols or Ultisols (Riutta et al., 2018).

3.3.2 Experimental design

I established a mesocosm experiment to test my hypotheses about the influence of habitat type and litter properties on soil C dynamics. I selected three different habitats representing different forest degradation levels: an oil palm plantation, forest fragments and continuous logged forest, with the assumption that their litter diversity decreases from selectively logged continuous forest to fragmented forest to oil palm monoculture (Figure 3.2). To enable comparisons with other relevant studies, I established my experiments in the sites selected by the LOMBOK consortium: three 10 ha sites in each habitat and randomly selected two 12 m x 12 m blocks within each site, each at least 300 m apart, making six replicate blocks per habitat type for a total of 18 blocks (Figure 3.1).

To characterise the soil and leaf litter in each habitat type, I collected five soil cores (0-10 cm depth) from each block using a 5 cm diameter soil corer. I also collected *c*. 50 g of naturally fallen leaf litter from each block in the continuous logged forest and fragmented forest. I was only able to collect palm fronds from one block in the plantation due to sampling restrictions. The samples were air dried at 40°C and stored in ziploc® bags before being shipped to Lancaster University, UK. Subsamples of soils and litter were used for chemical analyses (see section 3.2.3.) and any remaining material was air dried at 40°C and stored in polyethene bags according to DEFRA regulations.

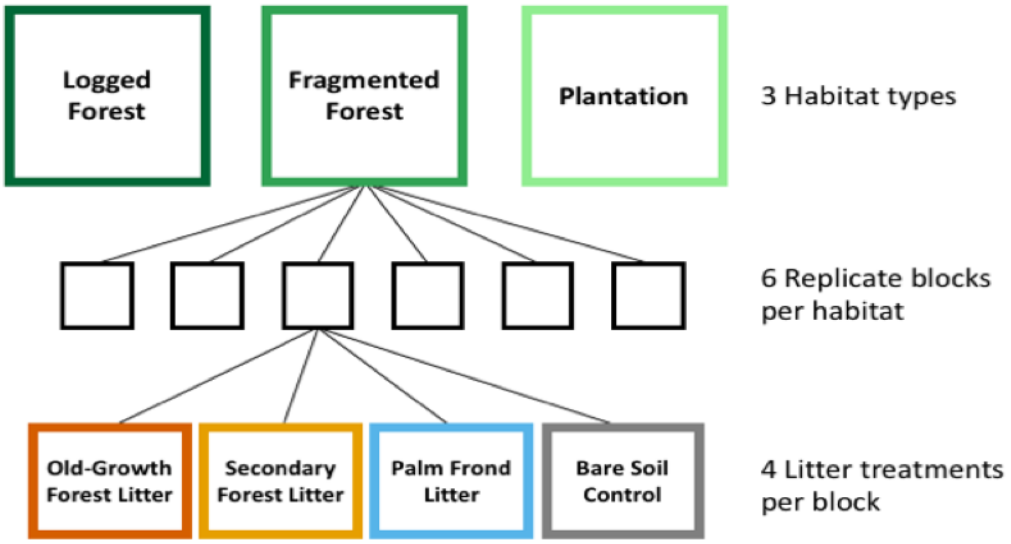


Figure 3.2 Schematic diagram illustrating the set-up of a mesocosm experiment within the landscape-scale Stability of Altered Forest Ecosystems (SAFE) Project in Sabah, Malaysian Borneo; showing the chosen three habitat types: continuous logged forest, forest fragment and palm oil plantation, and four litter treatments: old-growth forest, secondary forest, oil palm and bare soil.

In January and February 2015, I installed 144 mesocosms in a factorial design (Figure 3.2) in six replicate blocks within each of the three habitats. The mesocosms were made of 20 cm diameter plastic PVC pipes cut to a length of *c*. 13 cm. The mesocosms were placed at least 1 m from the nearest tree and at least 0.5 m from the nearest neighbouring mesocosm; they were sunk into the soil so that they all had an above-ground height of 10 cm (Figure 3.2); all mesocosms were left undisturbed for at least two weeks before the start of treatments.

I randomly assigned four different litter treatments to two mesocosms within each block in March 2015: old-growth litter, secondary forest litter and non-native introduced oil palm fronds (palm fronds), plus bare soil (no litter inputs) as a control; two sets of mesocosms per treatment were established in each block to allow for destructive sampling after six and nine months. I therefore had two sets of six replicates of four litter treatments in each of the three habitats, resulting in a total of 144 mesocosms.

To ensure my litter treatments represented a gradient of tree species diversity, I used primary forest litter instead of litter collected in the logged forest sites at SAFE. I collected the old-growth litter for the experiments from the Maliau Basin Forest reserve; a primary forest reserve approximately 80 km in a direct line from SAFE. The

secondary forest litter was collected from four blocks within the SAFE forest fragments. As it was not possible to collect sufficient quantities of freshly fallen litter, both forest litter types were collected by hand from the surface of the forest floor, selecting leaves that were undamaged and showed no visible signs of decomposition. The palm, fronds were collected directly from oil palm trees (*Elaeis guineensis* Jacq.), as they are usually not allowed to fall naturally but are instead cut down by plantation workers when the palm fruits are picked (Luskin and Potts, 2011). All litter was rinsed under a continuous flow of water to remove soil particles, dried at *c.* 40°C in a drying cabinet, then chopped by hand to give pieces < 5 cm diameter. Each mesocosm received 16 g of dried litter, which represented the mean annual litterfall at the SAFE project site (R. Ewers, unpublished data). The litter was placed on the soil surface inside each mesocosm, and the mesocosms were then covered with plastic mesh (mesh size 1 cm^2; Figure 3.3) to exclude natural litterfall and small animals, while allowing rainfall to penetrate.

To measure decomposition rates of the three litter types in all habitats, I made 108 litterbags measuring 18 cm $\times$ 18 cm each, using nylon mosquito mesh (0.4 mm mesh) sealed with a soldering iron. Each bag received 16 g of litter, making them comparable to the mesocosms in surface area (314 cm^2) and litter mass. In each habitat, I placed two bags per litter treatment and block on the soil surface (Figure 3.3). I collected one litterbag per treatment and block after six and nine months; the bags were rinsed gently to remove soil particles, dried at 40°C, and then weighed.

Figure 3.3 Experimental sites in logged forest (left) and oil palm plantation (right), showing the mesocosms and litter bags used in a decomposition experiment at the SAFE project in Sabah, Borneo between March and November 2015.

I measured soil respiration over the decaying litter in the mesocosms once a month for nine months with an SRC-1 soil chamber attached to an EGM-4 infrared gas analyser (PP systems, Amesbury, USA), using a custom-made adaptor (20 cm diameter and 5 cm height) to fit over the mesocosms. At the same time, I measured soil water content at 0-5 cm depth (SM150T soil moisture sensor, Delta-T Devices, Cambridge UK) as well as soil temperature (at 0-10 cm depth) within 10 cm of each mesocosm and air temperature above the mesocosm using a temperature probe (Fisher Scientific AG, Reinach Switzerland).

3.3.3 Chemical analyses of soil and leaf litter

To assess the influence of the litter treatments on soil properties within the mesocosms, I destructively sampled the first set of mesocosms for each litter treatment and block after six months and the second set after nine months. First, I collected the remaining litter from each mesocosm in a ziploc® bag and, once at the field station, rinsed, dried and weighed it. I then collected three soil cores (0-10 cm depth) per mesocosm using a 5 cm diameter punch corer. One soil core was used to determine KCl-extractable soil nitrogen (N) in the field, by placing *c*. 6 g soil (fresh weight) from each core into pre-weighed 50 ml falcon tubes containing 25 ml 1 M KCl solution. The samples were shaken for 1 min every 15 mins for one hour then filtered

through Whatman 42® filter paper (GE Healthcare, Chicago, USA) and frozen after addition of a drop of 75% H_2SO_4 as a preservative. The remaining two cores were refrigerated for up to 72 hours for analysis of soil pH and microbial biomass at the Forest Research Centre in Sandakan (Sabah, Malaysia). All remaining material was dried to constant weight at 40ºC and sieved (2 mm mesh) to remove roots and stones. I analysed the soils and litter for total C and N, soil pH, and litter fibre and lignin at Lancaster University and sent subsamples of soil and litter for nutrient analysis to an external laboratory (SAC Commercial Ltd., Aberdeen, UK).

To determine microbial biomass C and N, I performed chloroform fumigation extraction on paired fresh soil samples (8 g fresh weight) for each mesocosm and microcosm following Vance, Brookes & Jenkinson (1967) with modifications by Jones & Willett (2006). Briefly, one sample per pair was fumigated with ethanol-free chloroform for 24 h and then both samples were extracted in 40 ml K_2SO_4 and centrifuged before analysis for total organic C and total N on a TOC-L combustion analyser coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Microbial biomass C and N was then calculated from the difference in C and N concentrations between paired fumigated and unfumigated samples.

Phospho-lipid fatty acids (PLFAs) were extracted from c. 1 g freeze-dried soil following the high-throughput method of Buyer and Sasser (2012). Extracts were analysed by gas chromatography and peaks were identified using the Sherlock 6.2™ Microbial Identification System (MIDI, Newark, DE, USA). The analyses were performed by a commercial laboratory (Microbial ID Inc., Newark, USA).

I determined KCl-extractable ammonium-N and nitrate-N in the soil extracts on an Astoria Pacific (Astoria Pacific®, Clackamas, USA) auto-analyser.

Soil pH was measured on 3 g soil using a 1:3 ratio of soil distilled H_2O (Mettler Toledo® Seven Compact®, Leicester, UK or Corning® Model 430, Edison, USA) and gravimetric water content was calculated after drying the soil at 105°C for 24h.

Total C and N concentrations in soil and leaf litter collected in March 2015 from all habitats were determined on air-dried, ground samples (30 mg soil and 15 mg litter)

using a Vario El III C/N analyser (Elementar, Stockport, UK) at Lancaster University. I then determined litter fibre and lignin content using the acid detergent fibre and lignin extraction method designed by Van Soest *et al.* (1991) on a hot extraction unit fibre analysis system (bertecTM 1020, FOSS, Hilleroed Denmark). One g of each dried litter sample was placed in a glass crucible with 1 g of celite and total acid detergent fibre (ADF) was obtained by washing the samples in boiling acid detergent solution for 1 h. Then the acid solution was rinsed out and the samples were soaked in acetone for 5 min. The total extracted fibre content (ADF) was calculated by subtracting the weight of the processed sample from the original sample weight (corrected with blanks). The ADF samples were drained and oven-dried at 105°C for a minimum of 5 hours and then weighed. To obtain acid detergent lignin (ADL), the ADF samples were soaked in H_2SO_4 for 3 h, then washed with hot deionised H_2O until acid-free. The samples were dried at 105°C for at least 5 h and ashed in a furnace at 525°C for 3 h. The lignin content (ADL) was calculated by subtracting the weight of the sample at the final stage from the weight of total extracted fibre (ADF) with correction with blanks.

3.3.4 Data analyses

Response ratios for soil respiration were calculated for each litter treatment in each replicate block and each month using the following equation (Eq 3.1):

$$\ln\left(\frac{SR_l}{SR_s}\right) \qquad \textbf{Eq. 3.1}$$

Where SR_l represents the respiration from the mesocosm containing litter and SR_s represents the respiration from the bare soil mesocosm within the same replicate plot

To compare litter decomposition across habitats and treatments, I calculated percentage mass loss by subtracting the remaining litter after three or six months from the weight of the litter at the start of the experiment.

I calculated the litter decay rates for each treatment and block using the equation (Eq. 3.2):

$$\ln\left(\frac{X}{X_0}\right) = kt \qquad \textbf{Eq. 3.2}$$

as defined in Olson (1963) where *t* is time in years, X_0 is the original weight and X is the weight after decomposition.

3.3.5 Statistical analysis

All statistical analyses were conducted in R version 3.4.2 (R Core Team, 2017) using the vegan package for multivariate analyses (Oksanen et al., 2015) and the lme4 package for linear mixed effects models (Bates *et al.* 2017). Principal component analysis (PCA; *rda* function) was used to visualise the separation between habitats based on soil properties (total C, total N, C:N ratio, P, K and pH;). I then used linear models to assess differences in individual soil properties among habitats (*lm* function). I investigated the relationship between decomposition and mean soil respiration at three, six and nine months with Pearson's correlations, using the decay rates calculated from the litterbags collected after nine months.

The effects of habitat and litter treatment on soil temperature, moisture, respiration and response ratios as well as litter mass loss were assessed using nested linear mixed effects models with month and replicate blocks as random effects (*lmer* function). Full models included habitat and litter treatment and their interaction as fixed effects, and significance was determined by sequentially dropping terms until a minimum adequate model was reached, using AIC and p-values to check for model improvement. The final models were compared to appropriate null models using likelihood ratio tests and the model fit was assessed using diagnostic plots (Crawley, 2007).

I used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities to represent shifts in soil microbial communities (metaMDS function); stable solutions with stress scores <0.2 and r2 > .95 were used for subsequent analyses, resulting in a two- dimensional solution. I then used vector fitting to the NMDS ordinations (envfit function) to determine the effects of soil properties; significance values were generated with 9,999 random permutations stratified within experimental blocks. To test the effect of sites or litter treatments on individual

microbial groups, I ran separate linear models for the total PLFA biomarkers of each group.

3.4 Results

3.4.1 Site characteristics

Soil and litter properties varied with habitat type (Table 3.1). Total soil C was lowest (1.88% ± 0.5) in the oil palm plantations and highest (2.23% ± 0.71) in logged forest but none of the soil nutrients examined differed significantly among habitats ($p > 0.1$ for all soil properties). Accordingly, principal components analysis of soil properties showed no separation of the three habitats.

Total C and N in the litter was similar in the forest fragments and the logged forest. Although the litter N content was higher and the litter C:N ratio of the palm fronds was lower than that of the forested habitats, statistical significance could not be established due to a lack of replicates for the oil palm fronds.

Table 3.1 Initial soil properties for three tropical habitats representing different levels of forest degradation at the SAFE Project in Sabah, Malaysia, showing total soil carbon and nitrogen concentrations, extractable phosphorus (P), and potassium (K) and soil pH at 0-10 cm depth; total litter carbon (C) and nitrogen (C) content, and carbon to nitrogen (C:N) ratios. Means and ± standard errors are shown for *n* = 6, except for soil P and K in forest habitats (*n* = 4) and oil palm litter properties (*n* = 1 due to sampling restrictions).

Property / Habitat	Plantation	Fragmented Forest	Logged Forest
Total carbon (%)	1.88 (± 0.50)	2.18 (± 0.65)	2.23 (± 0.71)
Total nitrogen (%)	0.31 (± 0.03)	0.30 (± 0.05)	0.32 (± 0.05)
C:N	5.73 (± 0.97)	6.60 (± 0.76)	6.52 (± 1.00)
Extractable P (mg kg^{-1})	6.58 (± 2.71)	4.20 (± 1.87)	5.80 (± 2.62)
Extractable K (mg kg^{-1})	456 (± 58.9)	532 (± 179.7)	429 (± 270.3)
soil pH	4.77 (± 0.19)	5.53 (± 0.44)	5.39 (± 0.46)

Soil temperature was higher in the oil palm plantation than in the forested habitats (logged forest: 23 ± 0.04°C, forest fragments: 24 ± 0.04 °C, plantation: 25 ± 0.09 °C), and litter treatment had no effect on soil temperature. The model that best explained variation in soil temperature included habitat only ($\chi^2 = 13.25$, $p < 0.05$; Figure 3.6).

Meanwhile, neither habitat nor litter treatment had a significant effect on soil moisture content (Figure 3.4).

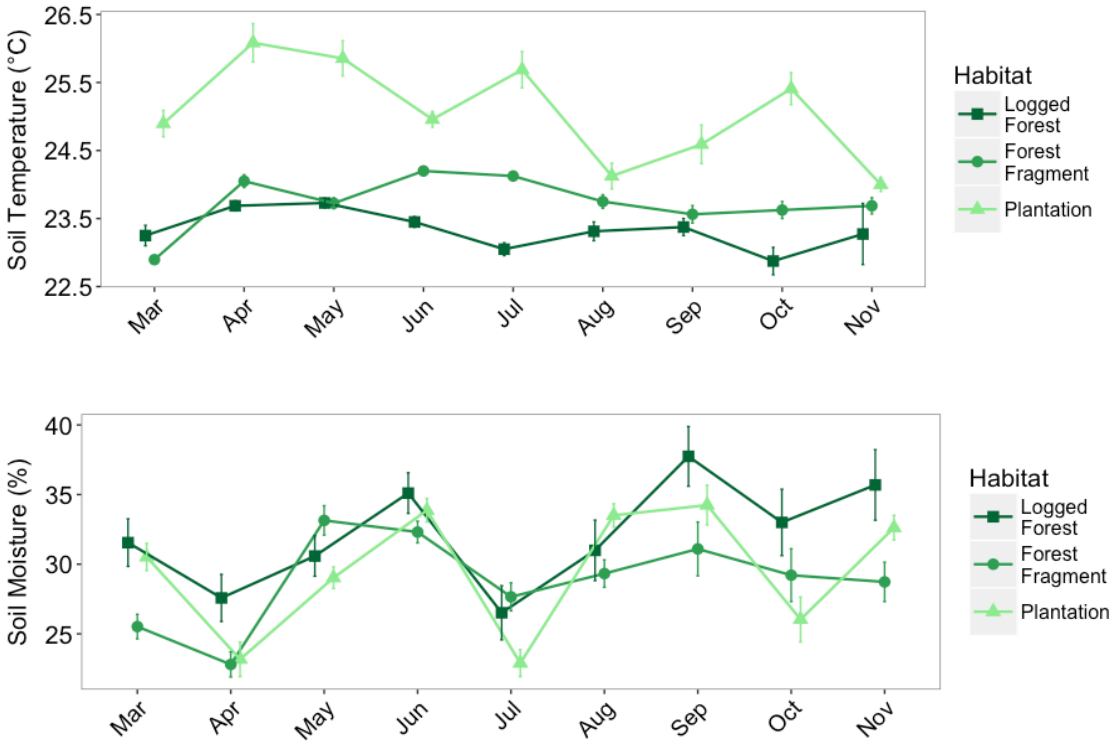


Figure 3.4 Soil temperature (top) and soil moisture (bottom) in three tropical habitats representing different levels of forest degradation, logged forest (dark green squares), fragmented forest (medium green circles) and an oil palm plantation (light green triangles), during a litter decomposition experiment in the SAFE Project in Sabah, Malaysia. The measurements were taken adjacent to experimental mesocosms (*n* = 8 per replicate block from March until July 2015 and n = 4 per block from August until November 2018) every month for 9 months.

3.4.2 Litter decomposition

Oil palm litter had the lowest proportions of fibre (67.53%) and lignin (4.97%) and the lowest lignin:N ratio (L:N; 2.63%), whereas the two forest litters had similar fibre and lignin contents (Table 3.2). Oil palm litter also had a lower C:N ratio and double the concentration of phosphorus compared to the forest litters (Table 3.2).

Table 3.2 Litter nutrient contents for three tropical habitats representing different levels of forest degradation used in a litter decomposition experiment at the SAFE Project in Sabah, Malaysia, showing litter total litter carbon (C) content, total litter nitrogen (N) content, soil and litter carbon to nitrogen (C:N) ratios, extractable phosphorus (P), and potassium (K).

Nutrients\Litter	Introduced	Secondary forest	Old-Growth Forest
Total C (%)	44.65	44.73	46.27
Total N (%)	1.89	1.17	1.38
C:N ratio	23.62	38.24	33.52
P (%)	0.13	0.062	0.058
K (%)	0.86	0.76	0.27
Fibre (%)	19.6	31.4	35.3
Lignin (%)	4.97	17.21	17.5
L:N ratio	2.63	14.71	12.68
Ca (%)	0.62	0.66	1.26
Mg (%)	0.37	0.26	0.29
Zn(mg/kg)	19.3	19	18.7

The introduced oil palm litter and the plantation habitat differed markedly in decomposition rates from the forest litter and habitats: the introduced litter treatment had the highest decay rate regardless of habitat (palm fronds: 1.5 ± 0.24, secondary forest: 0.73 ± 0.12, old-growth forest: 0.74 ± 0.12) but the decay rate was lowest in the plantation habitat regardless of litter treatment. Litter decomposed at similar rates in the two forested habitats, and the old-growth and secondary forest litter also decomposed at similar rates across habitats (Figure 3.5).The model that best explained decay rate therefore included habitat type, litter treatment, and their interaction ($\chi^2 = 84.40$, $p < 0.05$).

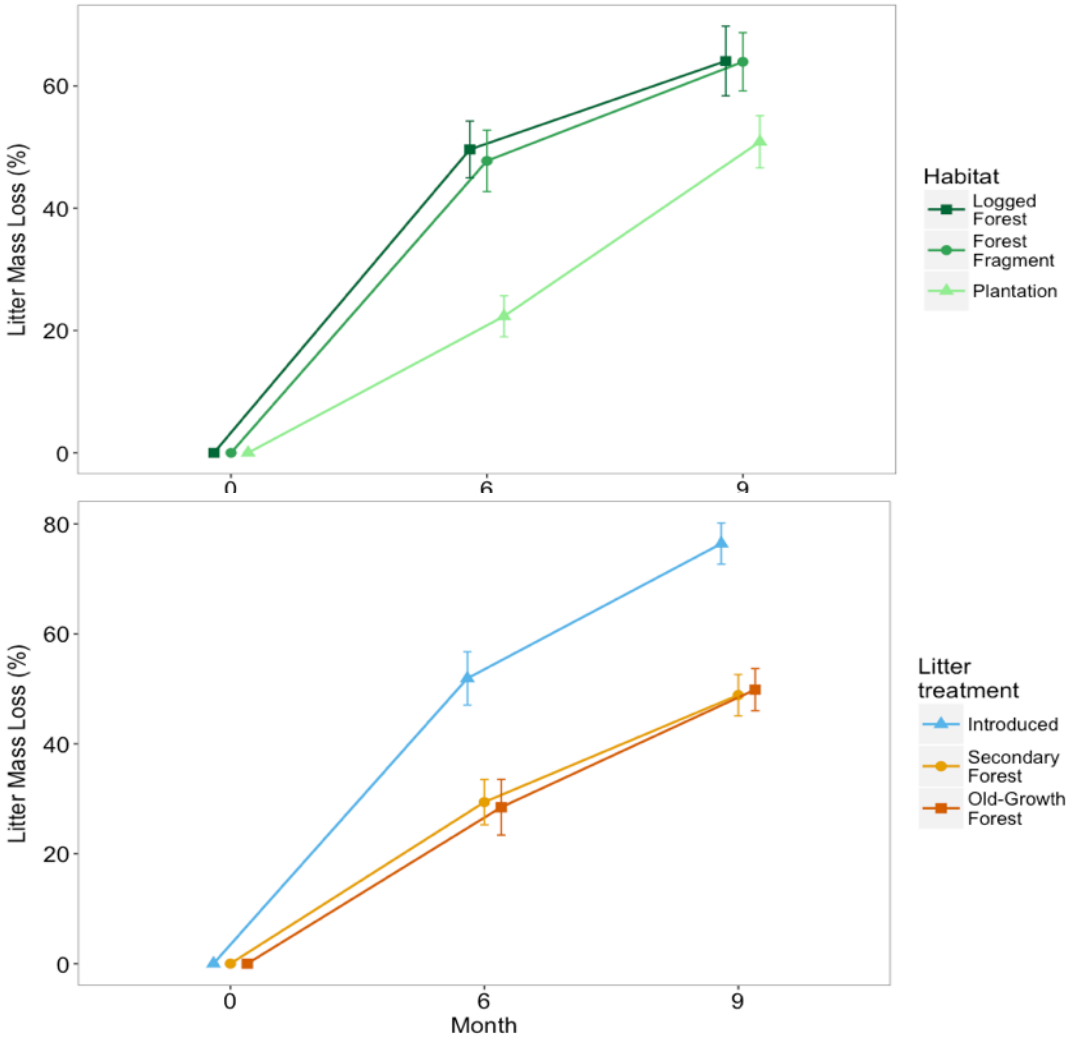


Figure 3.5 Litter mass loss from litterbags placed in three tropical habitats representing different levels of forest degradation and collected after six and nine months. The top panel shows the litter mass loss for each habitat with all treatments combined where old growth forest is in dark green squares, secondary forest is in green circles and oil palm plantation is light green triangles. The bottom panel shows litter mass loss for each litter treatment regardless of habitat, where the oil palm fronds are denoted by blue triangles, secondary forest litter is yellow circles and old growth forest litter is orange squares. The points at each collection time have been separated for clarity.

3.4.3 Microbial activity

Soil respiration was lowest in the plantation habitat (plantation 83 ± 3 mg CO_2-C m^{-2} hr^{-1}, compared to the forest fragments 105 ± 3 mg CO_2-C m^{-2} hr^{-1} and logged forest: 101 ± 3 mg CO_2-C m^{-2} hr^{-1}), regardless of litter treatment. Although soil respiration in mesocosms with litter was higher compared to the bare soil controls in the forest habitats, there was little difference between bare soil and the litter treatments in the oil palm plantation (Figure 3.6). Hence, the model that best explained the variation in soil respiration included habitat type, litter treatment and their interaction (χ^2 =

118.53, $p < 0.05$). There were no clear differences in soil respiration among litter types within the forest fragments or oil palm plantation, but there was a trend towards higher respiration from mesocosms with oil palm litter in the continuous logged forest. Furthermore, after removal of the bare soil control from the analysis litter treatment and habitat, but not their interaction, still had an effect on soil respiration (Figure 3.6) (χ^2 = 12.05, $p < 0.05$), indicating that the influence of decomposition on total soil respiration varied in magnitude depending on litter type and habitat. There was a positive relationship between soil respiration and litter decomposition after three (r = 0.34, $p < 0.05$), six (r = 0.33, $p < 0.05$) and nine (r = 0.32, $p < 0.05$) months but neither habitat nor litter type affected the relationship.

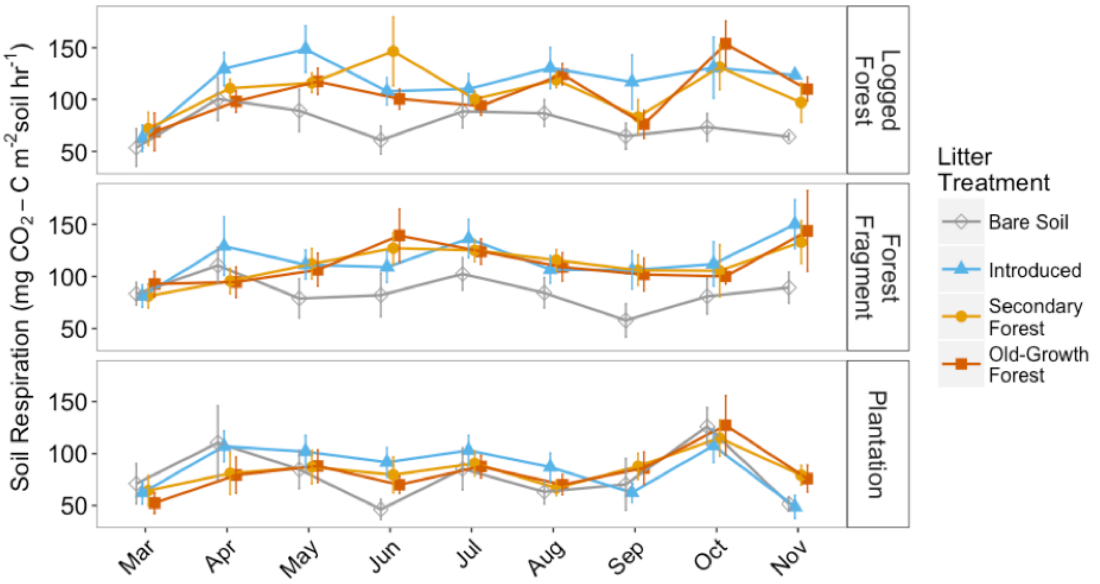


Figure 3.6 Soil respiration during nine months in three different habitat types representing different levels of forest degradation in Sabah, Malaysian Borneo, measurements were made over mesocosms with one of four litter treatments: bare soil control (grey diamond), oil palm fronds (blue triangles), secondary forest litter (orange circles) and primary forest litter (red squares). Means and standard errors are shown for n = 12 per time point from March until June and n = 6 per time point from September until October 2015.

Microbial biomass C (MBC) and N (MBN) were lowest in the plantation habitat (MBC 76±10 µg g^{-1}, MBN 10.8 ± 1.2 µg g^{-1}) followed by the logged forest (MBC: 106±11 µg g^{-1}, MBN: 20.9 ± 2 µg g^{-1}) and the forest fragments (MBC 140 ± 15 µg g^{-1}, MBN 26.7 ± 2.8 µg g^{-1}; see also Figure 3.7). There was a significant effect of habitat on both microbial C and N, whereby the plantation habitat had significantly lower microbial C ($F_{(2,70)}$ = 4.80, $p < 0.05$) and microbial N ($F_{(2,77)}$ = 19.11, $p < 0.05$) than the logged forest or forest

fragments; there was no effect of litter treatment. KCl-extractable N did not differ significantly between habitats or treatments.

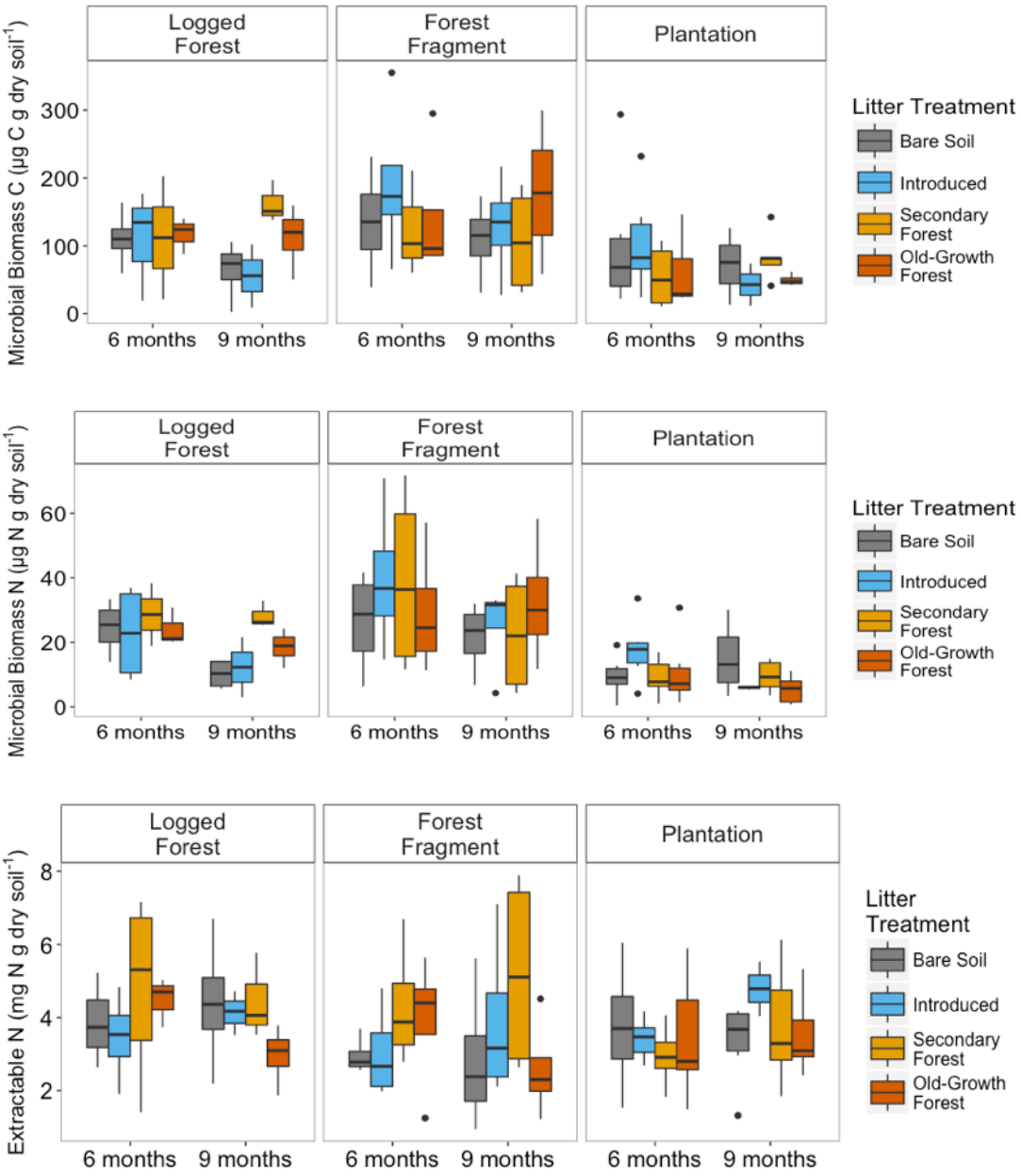


Figure 3.7 Soil microbial biomass C (top panels), soil microbial N (middle panels) and KCl extractable soil N (bottom panels) at 0-10 cm depth in three different habitat types representing different levels of forest degradation in Sabah, Malaysian Borneo. Samples were collected from mesocosms with different litter mixtures after six and nine months of decomposition (July and November 2015), where bare soil is grey, oil palm fronds is blue, secondary forest litter is yellow and old-growth forest litter is orange. Boxes and whiskers indicate interquartile ranges for *n* = 5 and black dots indicate outliers.

PLFA analysis revealed differences in soil microbial communities, with a clear separation of the oil palm habitat from the forest sites. Vector fitting to NMDS ordinations of plot-level data revealed that increased abundance of gram-positive

bacteria ($F_{2,11}$ = 8.67, p < 0.05). and reduced fungal biomass ($F_{2,11}$ = 3.19, p < 0.05; Figure 3.8) explained the separation of the oil palm habitat from the forested sites along the first ordination axis, whereas the variation among sites within habitat types was largely explained by soil P and pH.

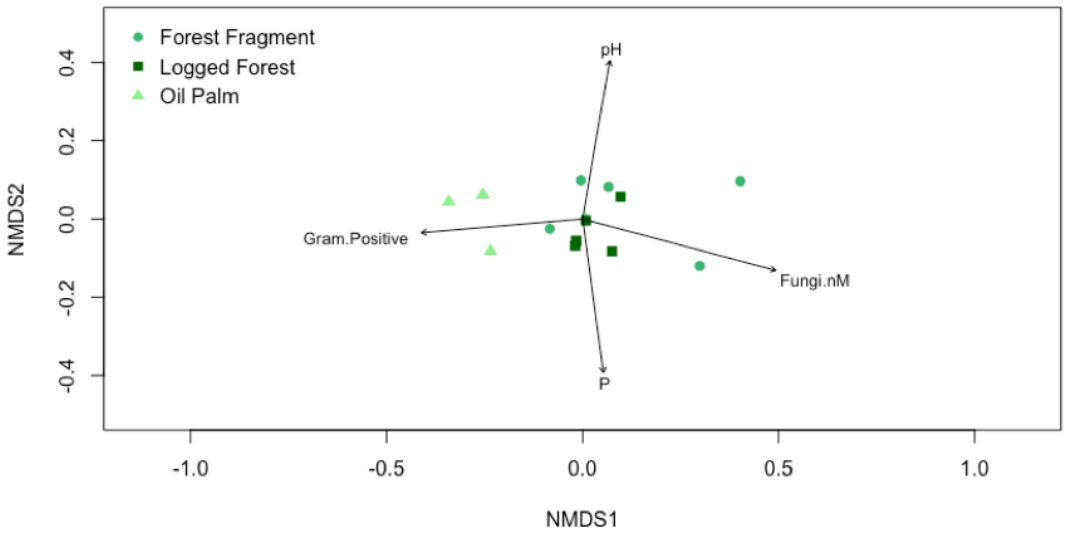


Figure 3.8 NMDS representation of the soil microbial and fungal community from PLFA analysis from soil: Samples collected from 0-10 cm in three tropical habitats representing different levels of forest degradation, logged forest (dark green squares), fragmented forest (medium green circles) and an oil palm plantation (light green triangles), during a litter decomposition experiment in the SAFE Project in Sabah, Malaysia in 2015. Ordinations were based on Bray–Curtis dissimilarities and significant correlations of community- weighted soil properties with ordination axes shown as arrows, where P is soil phosphorus, pH is soil pH, Gram.Positive is Gram Positive bacteria and Fungi.nM is fungal biomass.

3.5 Discussion

3.5.1 The importance of microclimate

All three habitats had similar soil properties (Table 3.1), which is notable because I expected lower soil C in the plantation as a result of soil disturbance during site preparation, which included terracing. The similarities in soil C content among the three habitats would suggest that any losses of soil C were incurred during logging events (Chazdon, 2014).

The lower soil respiration (Figure 3.6) and microbial biomass (Figure 3.7) observed in the plantation can be explained by the different microclimate provided by the oil palms compared to the forest habitats. The palms in the plantation were planted between six and nine meters apart so there the canopy was not closed, resulting in

higher soil temperature and much greater fluctuations in soil water content (Figure 3.4), which suggests that the soil in the plantations provided a less stable environment for micro-organisms (Zhang and Zak, 1995; Yang *et al.*, 2017). In addition, herbicide was applied every few months to prevent plant growth in the understorey and it is therefore possible that the higher levels of solar radiation combined with the lack of plant cover may have contributed to a decrease in soil respiration (Wang *et al.*, 2015). It is also possible that the herbicide hindered microbial activity (Perucci *et al.*, 2000). Finally, management practices are such that the palm fronds are piled at specific spots and not spread over the soil so that there is very litter organic matter addition to large areas of the plantation soils and my mesocosms were placed at least 1 m away from the palm tree trunks or the pile of fronds. It is important to emphasize that soil respiration in this context is a measure of microbial activity and not an index for greenhouse gas effects: higher soil respiration indicates greater growth and activity of plants and microbes, which can be used as a measure of soil health and function, whereas lower soil respiration generally means reduced microbial activity and restricted cycling of soil carbon (Ayres *et al.*, 2009). In the present study, there was no discernible increase in soil respiration or soil microbial biomass at the plantation sites when litter was added to mesocosms, which suggests that soil microbial community function has changed and the prevailing conditions in the plantation hinder the response of microbial decomposers to fresh litter inputs. This is confirmed by the PLFA analysis which showed that the gram positive bacteria, which are more tolerant to disturbances (Kirk *et al.*, 2004), were significantly higher in the oil palm habitat (Figure 3.8).

Whereas the lack of canopy cover explains the higher soil temperature in plantation sites (Figure 3.4), soil moisture did not differ from the forest habitats (Figure 3.4). Terracing increases bulk density and soil compaction, which reduces pore space and increases the clay content of the soil, this would result in the soils in the plantation being capable of retaining more water, even in drier conditions (Lal and Cummings, 1979; Comte, Colin, *et al.*, 2012). Soil moisture was measured at 0-5 cm depth, and observations in the field suggest that litter moisture content affected soil surface water content. The litter was very dry during the dry season and some mesocosms

were occasionally water-logged in the oil palm sites during the wet season. In future, litter moisture content could be calculated for each site at the time when respiration is being measured. In addition, the secondary roots of oil palms can extend up to 25 m horizontally away from the stem (Jourdan and Rey, 1997) at a soil depth of between 6 and 20 cm; these roots are responsible for 44 % of the root water acquisition (Ruer, 1969) and therefore they can provide long-term moisture to the topsoil even during the drier months.

The microclimate in the plantations likely also affected litter decomposition, as the overall mass loss was only 45% after nine months, compared to 60% mass loss after the same amount of time for the forest habitats, and more rapid decomposition of the oil palm litter compared to forest litter (Figure 3.5). Another key difference between oil palm plantations and forest habitats is the loss of faunal diversity in the former, especially invertebrate diversity (Fitzherbert *et al.*, 2008). This is important because invertebrates play an essential role in the decomposition of more complex leaf litter by degrading it and making more recalcitrant nutrients available to soil microbes (Barajas-Guzmán and Alvarez-Sánchez, 2003; Ashford *et al.*, 2013; García-Palacios *et al.*, 2016). As other studies have noted differences in the soil microbial composition of oil palm plantations compared to forested habitats, where specialised forest microbial communities were missing from oil palm plantations (Lee-Cruz *et al.*, 2013; van Straaten *et al.*, 2015). This would suggest that the soil microbes present in the oil palm plantation may not be capable of processing more complex and diverse forest leaf litter. However, microbial biomass was also lower in the plantation (Figure 3.7) and even the oil palm litter, which had the highest mass loss of the litter mixtures, decomposed more slowly in the plantation than in the forest habitats (Figure 3.5). Furthermore, the fungi species abundance from the PLFA analysis was similar for all three habitats but the fungi biomass was lower in the oil palm habitat (Figure 3.8) suggesting that the conversion to plantations caused both a reduction in microbial biomass and a shift in microbial community composition.

3.5.2 Litter properties and microbial activity

The positive correlation between decay rates and soil respiration shows that both variables are good indices of microbial activity. Furthermore, all litter treatments had higher respiration than the bare soil control (Figure 3.6) which demonstrates the importance of litter inputs for microbial activity (Vesterdal *et al.*, 2012; Sayer *et al.*, 2017). The plantation had significantly lower overall soil respiration than the forest habitats and, being a monoculture, had the lowest tree species diversity; whereas the two forested habitats had similar overall rates of soil respiration. This emphasizes the importance of tree diversity for soil ecosystem function, as previous studies have shown that not just the litter but the tree as a whole is important for soil microbes, and therefore soil C cycling (Salimon *et al.*, 2004; Both *et al.*, 2017).

The differences in soil respiration with litter treatments can also largely be attributed to oil palm fronds, as the secondary and old-growth forest litter decomposed and respired at very similar rates. First, respiration was higher than expected from oil palm fronds and the oil palm litter also had higher mass loss than the other litter treatments, suggesting that the microbes responded more to the oil palm litter. It is also interesting that respiration was higher from the oil palm litter than the other litter types in the plantation sites and the logged forest (Figure 3.6), which is likely a result of the palm frond litter providing more labile C to the soil microbes. The palm litter had much lower lignin content and L:N ratio (Table 3.2), which are recognised properties of more easily decomposable organic matter (Hättenschwiler *et al.*, 2010; Laird-Hopkins *et al.*, 2017). In addition, the oil palm fronds had higher P and K content; tropical forests are widely acknowledged to be limited by P availability (Hättenschwiler and Jørgensen, 2010; Camenzind *et al.*, 2018) and K has been found to promote cellulose decomposition (Kaspari *et al.*, 2008). Hence, the distinct properties of the oil palm litter, which was characterised by high content of labile C as well as P and K, could make it an attractive resource to microbial decomposers, and the low decay rates in the plantations can be attributed to microclimate and a decline in soil function.

Litter from the old-growth forest and the forest fragments had similar nutrient contents. Although the old-growth forest had slightly lower K and lignin content and slightly higher N content, the two forest litter types decomposed at similar rates. This would suggest that soil function in the degraded forests have been sustained and allow

for the decomposition of old-growth litter. This could be due to the high diversity of plant species present in both logged and fragmented habitats, which promotes soil microbial functional diversity despite the removal of key species such as dipterocarps (Saner *et al.*, 2009; Both *et al.*, 2017). However, this does not mean that the forest fragments and the logged forest have the same ecosystem functions and C cycling ability of an old-growth forest: a recent study comparing the SAFE logged forest and old-growth forest in the Maliau Basin, showed a clear reduction in decomposition rates in the logged forest regardless of the litter (Both *et al.*, 2017). For the present study this could mean that forest degradation may in fact have affected soil function and that this has a greater impact on decomposition processes than the diversity or species composition of the litter. Because I could not test decomposition rates in the old-growth forest, it was not possible for me to test this hypothesis.

Both forested habitats in this study are considered to be degraded, and as such it is likely that their ecosystem functioning has already changed substantially compared to old-growth forest. The logged forest and the forest fragments both had the same level of logging before the start of the experiment, and as tree identification was not possible at the time of the present study, I could not confirm that tree species composition in the logged forest sites differed from the forest fragments. Furthermore, the logging around the fragments had not taken place yet so the differences in forest degradation were probably not sufficient to detect changes in the ecosystem processes presented here. A study comparing soil respiration in different tropical ecosystems on the Malaysian peninsula (Adachi *et al.*, 2006) showed that soil C content of the top 10 cm in the old growth forest was significantly greater than in secondary forest or the oil palm plantation. Another study in Sabah carried out between Danum valley and a selectively logged forest at Maliau forest reserve also found that soil organic matter content was significantly higher in the old growth forest compared to the selectively logged forest (Saner *et al.*, 2012). In my experiment, the sites in the forest fragments and logged forest were a lot more varied in their levels of degradation (i.e., above ground plant composition) than planned as the sites had been chosen at random. Had I specifically chosen sites with different levels of disturbance I am confident I would have measured a difference in microbial activity.

3.6 Conclusion

Key litter properties explained the decay rates of different litter types with more easily degradable litter promoting higher microbial activity. Forest degradation and conversion to monoculture changes the local environment and microclimate as well as tree species diversity and composition, which accounts for the lower soil microbial activity seen in this study. The extremely low plant diversity in oil palm monocultures and common management practices, especially the lack of organic matter inputs to the soil, severely reduced microbial activity and ecosystem function. More sustainable practices to increase the amount of organic matter such as adding empty fruit bunches (Salmiyati et al., 2014) could be put in place to mitigate this. However, both degraded forest habitats were capable of processing litter from the old-growth forest, which is optimistic for future forest restoration projects to mitigate climate change.

CHAPTER 4 THE EFFECTS OF FOREST DEGRADATION ON SOIL CARBON DYNAMICS ALONG A DISTURBANCE GRADIENT IN BARRO COLORADO NATIONAL MONUMENT, PANAMA.

4.1 Abstract

Deforestation and land use change can affect the soils' ability to accumulate and store carbon (C). Tree cover in Panama, Central America, is dominated by secondary forests and plantations, with only 21% of the tree cover classified as intact forest. I set out to understand how changes in tree and plant composition in forested habitats at different levels of degradation affect soil carbon dynamics and litter decomposition.

I set up a mesocosm experiment using reciprocal litter treatments in three habitats along a forest recovery gradient to establish how different litter mixtures, representing varying degrees of forest degradation, affect soil C dynamics. I selected three habitats: a completely deforested area, a 40-year old secondary forest and an old-growth forest (>100 years old). I used natural leaf litter from the old-growth and secondary forest and an introduced single species, *Saccharum spontaneum* (L.) to differentiate between the effects of soil properties and litter type on soil respiration and decomposition rates.

Litter properties explained litter decomposition rates and microbial activity with the introduced single-species litter decomposing the fastest, regardless of the habitat. Litter from the old-growth and secondary forest sites decomposed at similar rates. Microclimate and soil properties of the different habitats explained the variation in

litter decomposition and microbial activity between habitats. The secondary forest and old growth forest had similar soil properties with equivalent levels of total soil C and N. Both microbial activity and litter decomposition were significantly lower in the modified deforested habitat suggesting that loss of tree cover caused a decrease in microbial activity and soil function.

Overall, my results suggest that mixed litter inputs are crucial for maintaining soil function, but that some soil processes in the regenerating secondary forest have recovered to old-growth forest levels after only 40 years.

4.2 Introduction

Soil is the largest terrestrial C pool containing an estimated 2344 Gt of organic C in the top 3 meters (Stockmann et al., 2013). It is estimated that a third of this pool is in the tropics of which approximately 16 to 20% of the soil organic C is in tropical evergreen forests (Dixon et al., 1994). Consequently, although tropical forests only cover c. 6% of the world's land, they are extremely important to the global soil C pool (Mayaux et al., 2005). From timber extraction to agricultural practices or urbanisation, tropical forests are being increasingly disturbed due to anthropogenic activities (Dent et al., 2013; Laurance, 2015). In Panama, 4.49 Mha (60%) of the land is covered by trees but only 937 kha (21%) is classified as intact forest; the rest of the tree cover is mostly timber plantations and secondary forest. Between 2001 and 2017, an estimated 352 kha (6% of the total tree cover) were deforested resulting in emissions of 33.8 Mt of CO_2 into the atmosphere. Secondary or degraded forests and agricultural land are now replacing old-growth forests as the dominant forest type in the tropics (FAO, 2016). This is a cause for concern as direct conversion from primary forest to agricultural land causes losses of 20% to 30% of SOC and secondary forests store an estimated 9% less C than primary forests (Don et al., 2011).

Deforestation causes a complete change in abiotic conditions; the removal of trees releases C from the soil in the form of CO_2 and hugely increases the amount of direct sunlight which in turn increases the variation in air and soil temperature as well as soil moisture (Dechert et al., 2004; Houghton, 2012). After complete deforestation, forest

regeneration generally starts with the emergence of grasses and shrubs which are adapted to growing in low soil nutrient and low shade environments. This is followed by secondary forest succession which starts with the implantation of pioneer tree species that are fast-growing, tolerant to sunlight and have high foliar nutrient concentrations that are better adapted to the drier microclimate (Swaine and Whitmore, 1988). By contrast, undisturbed old-growth tropical forests are generally characterized by slow-growing plant species that are more shade-tolerant and have greater belowground biomass and structural stability (Dent et al., 2013). In short, deforestation causes changes in the above-ground tree composition and as such also changes the composition of the leaf litter; this will have repercussions on soil C processes.

Decomposition of litter by soil micro-organisms provides nutrients for plant growth and influences C storage belowground (Bardgett et al., 2005). Lower soil microbial activity has been linked to decreases in sources of organic matter and decreases in aboveground plant diversity (Van Der Heijden et al., 2008). These interactions between plants and soil organisms are a key process of the soil C cycle. Changes in the properties of the litter could affect the ability of the soil microbes to decompose the litter. Litter decomposition rates are dependent on litter nutrient characteristics such as C, nitrogen or phosphorus as well as lignin content (Cornwell et al., 2008; Hättenschwiler et al., 2005; Laird-Hopkins et al., 2017). The main source of organic C in forests is litter, therefore changing the leaf litter input may alter the microbial composition or the efficiency of the microbial community because they have preferred C sources (Sayer, 2005).

Many decomposition studies have been carried out using litterbags and specific litter mixtures from chosen plant species. Most studies have used standardised representative litter mixtures which are accurate for temperate forests that have low species diversity, but this cannot account for the high diversity of a tropical forest. Many studies have used CO_2 efflux to assess microbial activity of different habitats but very few studies have measured CO_2 efflux changes over the decomposing litter in order to assess whether soil conditions or litter characteristics is the main driver of

litter decomposition (Ayres et al., 2009). If the litter provided is very different from the litter that the microbes are adapted to decompose, they might be less active and therefore the rate at which C is cycled will in turn decrease.

Due to their roles as C sinks, the accumulation and retention of C in soils is a key component of the biogeochemical cycle and is related to the quantity and quality of organic matter inputs (Cornwell et al., 2008; Pérez-Harguindeguy et al., 2000). Even small changes in the soil C pool could have important impacts on atmospheric CO_2 levels but such changes are complicated to measure. We can however measure changes in soil C dynamics with the aim of understanding and predicting future soil C storage. Understanding the functional interactions between the above and belowground soil food web that control soil C storage is essential to determine the links between changes in biodiversity and ecosystem function, such as C accumulation and retention during forest degradation.

To address the question on changes in tree and plant composition and their effects on soil C dynamics and litter decomposition, I set up a litter transplant mesocosm experiment under field conditions at the Smithsonian Tropical Research Institute Barro Colorado National Monument, in Panama. My mesocosm experiment used reciprocal litter treatments in three habitats along a forest recovery gradient to establish how different litter mixtures, representing varying degrees of forest degradation, affect soil C dynamics. I selected three habitats at different levels of degradation: a completely deforested area, a 40 year-old secondary succession forest and a 100 year-old old-growth forest. I used natural leaf litter from the old-growth and secondary forest and an introduced single species to represent the use of introduced foreign species for land-management purposes. Using natural leaf litter allowed me to account for the high species diversity present in tropical forests. Mesocosm experiments are a useful way of manipulating environmental conditions while retaining field environmental conditions and minimising disturbances (Laird-Hopkins et al., 2017). I aimed to test the following hypotheses:

1) Microbial activity will be different between habitats:

- There will be an inverse relationship between habitat disturbance and microbial activity where the higher the disturbance the lower the total soil respiration and the lower the litter decay rates.
- There will be an interactive effect of the habitat's soil properties and the litter treatment in each mesocosm on soil microbial activity and litter decomposition.
- Due to the level of disturbance and microclimate differences, such as C content and soil moisture and temperature, among habitats, total soil respiration will be lowest in the modified habitat and highest in the old growth forest.
- Microbial biomass will have an inverse relationship to the level of disturbance of the habitat with the lowest biomass being in the modified habitat and the highest in the old growth forest.

2) Litter traits will affect decomposition

- Litter decay rate will be negatively correlated with the complexity of the litter with the single species having a higher decay rate that the old forest litter mixtures regardless of the habitat in which it is decomposing.
- Microbial activity will be positively correlated to the decay rate with higher total soil respiration being linked to higher decay rates.
- Microbial biomass will increase with the diversity of the litter.

4.3 Methods

4.3.1 Site Description

I conducted my experiments within the Barro Colorado Nature Monument (BCNM, Figure 4.1) in Panama. BCNM (9°9'N, 79°51' W) is situated in and along the Panama Canal, it contains Barro Colorado island which was created during the damming of Gatùn lake in 1923 and five mainland peninsulas which were acquired in 1979 when the reserve was created.

Panama has a tropical climate with a wet season between May/June and November and a dry season between December and April. The weather conditions are similar to those of the island of Barro Colorado, *ca.* 5 km from the field sites. Between October 2016 and June 2017 (the duration of the experiment), the mean temperature was 26.9 $\pm$ 0.74 °C with a minimum of 22.8 °C in November 2016 and a maximum of 31 °C in October 2016 and received a mean rainfall of 231 $\pm$ 89.74 mm with a minimum of 8.6 mm in February 2017 and a maximum of 835.9 mm in November 2016. The experiment started in October 2016 during the wet season, the following dry season started in December 2016 and the following wet season in May 2017 (Datasets provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute, 2016 and 2017).

The forest in the monument is classified as a tropical evergreen lowland forest, which includes old-growth forest as well as large areas of secondary forest. The old-growth forest is over 100 years old, the dominant species included: *Leguminosae* and *Bombacaceae* (Denslow and Guzman, 2000), *Fabaceae* and *Malvaceae* (Dent et al., 2012) and *Oenocarpus mapora* (H. Karst). The secondary forest was re-established around 40 years ago on land that had been previously used by farmers for swidden agriculture (Dent et al., 2013); the dominant species included: *Cordia alliodora* (L.), *Spondias mombin* (L.), *Annona spraguei* (Saff.) and *Miconia argentea* (Sw.) (Dent et al., 2012). The peninsula is also home to a science field station which was built in the 1980s, that had been completely deforested and maintained as grassland ever since.

The soil at the study sites is classed as an oxisol with a pH of 4.5–5.0 with low phosphorus availability (Sayer, 2005; Turner et al., 2015).

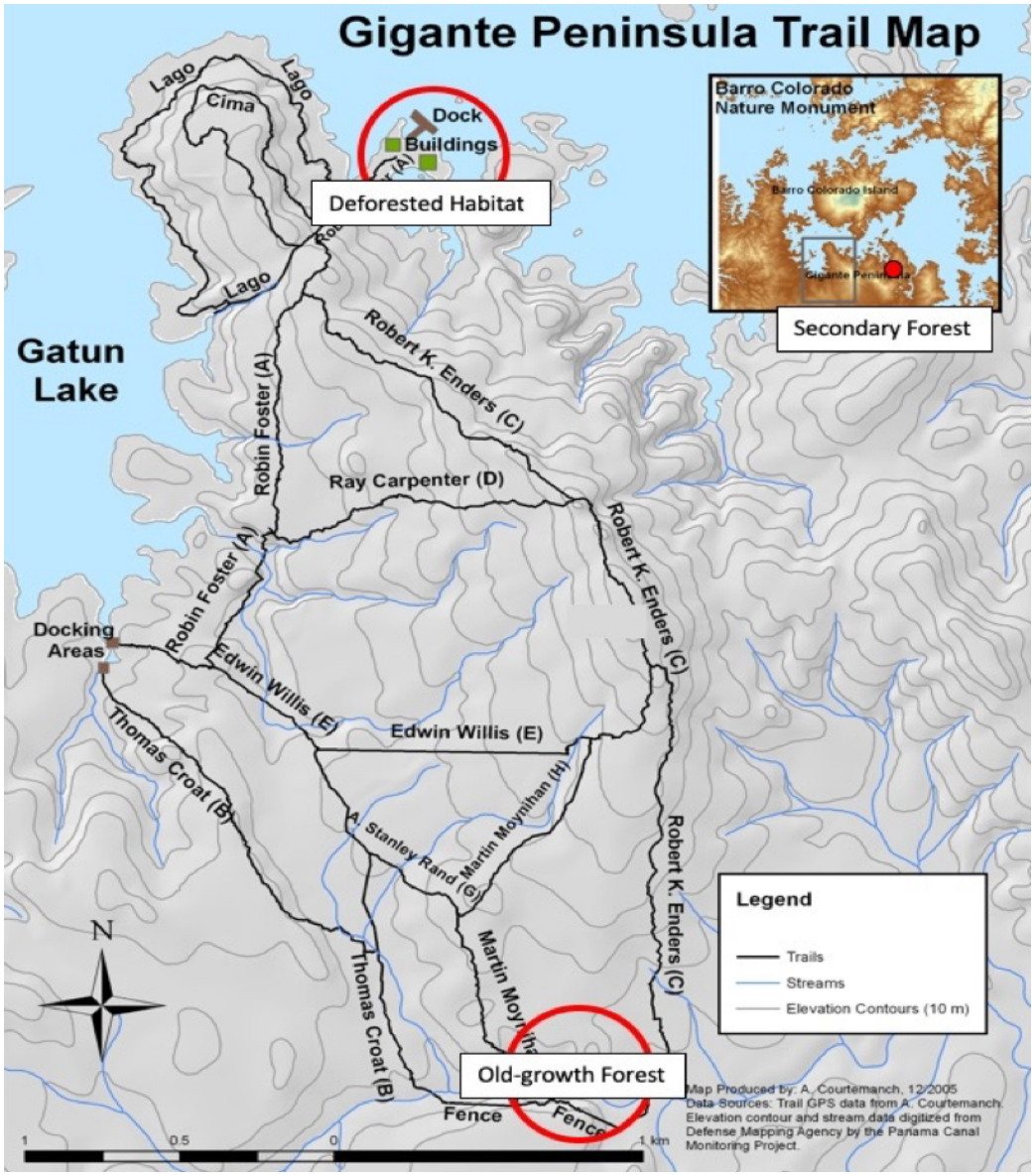


Figure 4.1 Map of the field experiment on Gigante Peninsula, Panama showing the location of the three habitats.

The present study was conducted entirely on the Gigante Peninsula, because it includes forest habitats at different levels (Denslow and Guzman, 2000) of disturbance on the same soil type. I chose three habitats: a pristine old-growth forest over 100 years old (Wright et al., 2011), a young secondary succession forest, and a deforested and converted modified habitat, grassland around a science field station. I chose six blocks approximately 600 m apart in each habitat for a total of 18 blocks. The areas within each habitat were selected such that the relief and aspect were consistent

across sites. All study sites had a slope of <5% and were situated at least 100 m away from waterways (Figure 4.1).

To determine the initial characteristics of the soil in each of the habitats, I collected three soil cores from all 18 blocks to a 10 cm depth using a 3.5 cm diameter punch corer in December 2016. All samples were stored in Ziplock^{TM} bags and shipped refrigerated to Lancaster University within one week of collection. On arrival, they were air-dried at 40°C for subsequent analyses of total C, nitrogen (N), phosphorus (P), potassium (K) and pH.

4.3.2 Experimental Design

To determine the relative influence of litter and soil properties on soil C dynamics at the sites along the forest disturbance gradient, I established a reciprocal litter transplant experiment (Figure 4.2) in mesocosms within three habitats, old-growth forest, secondary forest and a modified deforested habitat representing three increasing levels of forest disturbance in a similar set-up to the experiment carried out in the previous chapter in Malaysia (see section 3.2.1). I collected natural mixed litter from the old-growth and secondary forest sites, and to represent the modified habitat, I used the wild sugar cane *Saccharum spontaneum* (L.), native to the Indian subcontinent, that was originally introduced to stabilise the banks of the Panama Canal (Jones et al., 2004).

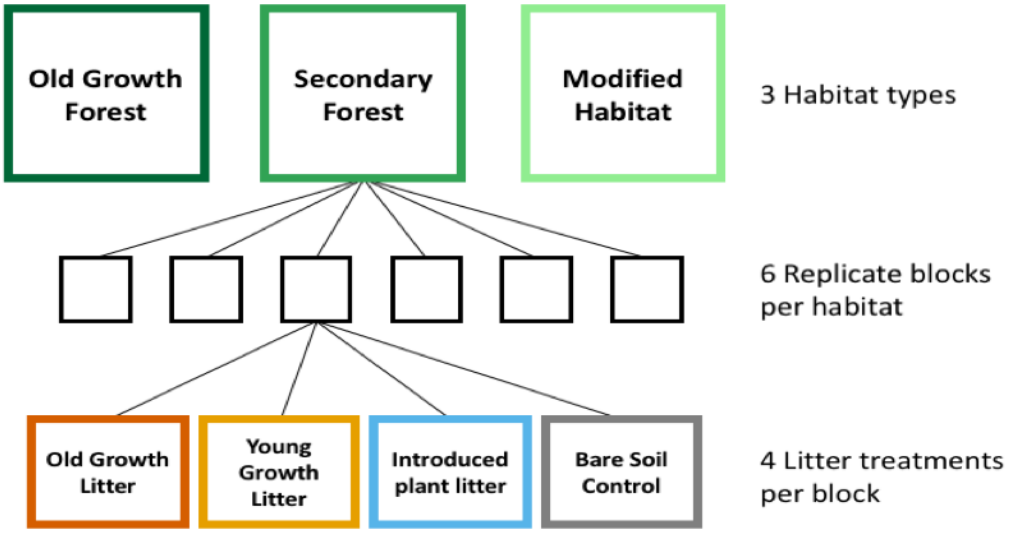


Figure 4.2 Design for the reciprocal litter transplant experiment in the Barro Colorado Natural Monument. The green squares represent the disturbance level of the three different habitats at three different levels of disturbance from least disturbed (dark green old growth forest) to most disturbed (lightest green, modified habitat). Within each habitat, I establish six replicate blocks of mesocosms, to which I applied four litter treatments: old-growth forest litter (red), secondary forest litter (orange), Saccharum spontaneum leaves (introduced species, blue) collected from Barro Colorado island, and a bare soil control (grey).

I placed two sets of replicate mesocosms in each of the 18 blocks so that each litter treatment would have two sets to allow for destructive sampling after six and nine months. The mesocosms were made of 20 cm diameter PVC pipes cut at a height of 14 to 15 cm and sunk into the ground to c. 4 cm depth so that height of each mesocosm above ground was 10 cm. The mesocosms were placed at least 1 m away from tree trunks and at least 0.5 m away from each other. To account for differences in basal soil and root respiration among blocks and sites, I also established two control mesocosms per block, in which all the litter was removed (bare soil). All other mesocosms received 16 g of litter from one of three treatments: old-growth, secondary, or introduced litter. To enable comparisons with previous studies, the amount of litter chosen was equivalent to the mean litter standing crop in Borneo which was conveniently the same as nearby forest sites on the Gigante Peninsula (Laird-Hopkins et al., 2017). The litter used for the old-growth and secondary forests had previously been collected from litter traps at each site in 2014 and 2015. The litter had been air-dried at 40°C on collection, chopped, and then stored in paper bags until 2016 when the present experiment was set up. To represent the disturbed site, I used leaves from the introduced *Saccharum spontaneum* (L.). (introduced litter) collected from the

northern part of Barro Colorado island and processed in the same manner as the other litters.

To assess litter decay rates, I prepared 108 litterbags of each litter mixture, using 16 g of litter in 1.4 mm square mosquito mesh bags of 18 cm^2 (equivalent to the surface inside the mesocosms); two bags per litter mixture were placed in each block no more than 1 m away from the mesocosms. One litterbag of each litter mixture was harvested from each block after 6 and 9 months to calculate litter decay rates

To summarise, the experiment comprised three habitats of ascending disturbance levels from an old growth forest to a secondary forest to a deforested modified habitat, each containing six replicate blocks of eight mesocosms with three litter treatments and a bare soil control for a total of 144 mesocosms. One block in the old growth forest had to be removed after three months due to a tree falling on the block.

4.3.3 Measurements of soil respiration and decomposition

To assess microbial activity in the decaying litter, I measured total soil respiration in the form of CO_2 efflux over each mesocosm once a month for nine months using a soil survey system with a 20 cm Survey Chamber attached to an infra-red gas analyser (Li-8100, LI-COR Biosciences, Lincoln, Nebraska, USA). I took measurements over the decaying litter to avoid disturbance to the soil surface. At the same time, I measured soil water content (SM150T soil moisture sensor, Delta-T Devices, Cambridge UK) at depth of 0-5 cm and soil temperature (at 0-10 cm depth) within 10 cm of each mesocosm (Fisher Scientific AG, Reinach Switzerland).

To measure nutrient release via litter leachate during the early stages of decomposition, I placed anion and cation exchange resins (PRS Probes®, WesternAG, Saskatchewan, Canada) beneath the litter on the soil surface inside of the mesocosms at the start of the experiment in October 2016. I collected the probes after 30 days in November 2016 removed soil particles with distilled water and a clean toothbrush and sent them to the manufacturer for analysis of total N, NO_3, NH_4, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, Cd.

Due to time and logistical constraints, soil and litter samples were collected from the mesocosms after six months and sent to Lancaster University but not analysed. Nine months after the start of the experiment, I collected the remaining litter from the second set of mesocosms and took two soil samples from each mesocosm to a depth of 10 cm using a 3.5 cm diameter punch corer. As there were limited facilities on-site, the cores were sealed in Ziplock™ bags and refrigerated at *c*. 5 °C for up to 7 days prior to further processing and analyses. The fresh soil samples were shipped to Lancaster University (UK) for analysis of microbial biomass C and N.

4.3.4 Laboratory analyses

Site soil and experiment litter characterisation

For the soil collected from each block in December 2016, I measured soil pH on the air-dried soil using 3 g dried soil in 9 ml deionised H_2O. The slurry was shaken by hand and left to settle for 30 min before measurement with a Seven Compact pH meter (Mettler Toledo® Seven Compact®, Leicester, UK).

I subsampled 5 g of air-dried soil and air-dried litter, ground each separate subsample using a ball mill (Mixer Mill 400, Retsch®, Haan, Germany) and analysed total C and N using 30 mg of ground soil or 15 mg of ground litter by combustion gas chromatography (Vario El III C/N analyser, Elementar, Stockport, UK). Sub samples of the dried soil from each block were analysed for total P and K and subsamples of the dried litter were analysed for B, Ca, Cu, Fe, MN, P, K, Na, S, Zn by the Scottish Agricultural Consultancy (Aberdeen, Scotland).

I also calculated fibre and lignin content of the litter using the dried and ground samples using the acid detergent fibre (ADF) and lignin (ADL) extraction method as described in Van Soest *et al.* (1991). One g of the ground litter was placed in a crucible with 1 g of celite. To determine cellulose content, the samples were first boiled with 100 ml of acid detergent solution and 4 drops of n-Octanol for 1 hour in a FOSS fibertec™ 8000 fibre analysis system (FOSS, Hilleroed Denmark). The samples were then rinsed with deionised water until acid-free, soaked in reagent-grade acetone and dried overnight at 105°C. The ADF was calculated by subtracting the weight of the

sample after extraction from the original sample weight. To determine lignin content, 25 ml of 72% H_2SO_4 was added to the ADF sample, the solution was stirred every hour for a 3 hour period. The crucibles were then rinsed with hot deionised H_2O, dried overnight at 105°C, and weighed before being ashed at 525°C for 3 hours. Finally, the crucibles were weighed once they had cooled to room temperature. ADL was calculated by dividing the final weight (corrected with blanks) from the initial weight.

Analyses of the soil and litter from the field experiment after nine months

I analysed soil microbial biomass C and N using the chloroform fumigation method fresh soil samples collected from the mesocosms that were sent to Lancaster University at the end of the experiment. Paired subsamples of the fresh soil were used for fumigation extraction (Jones and Willett, 2006; Vance et al., 1987). One subsample per pair was fumigated for 24 h with ethanol-free chloroform and both subsamples were extracted in 40 ml 0.5 M K_2SO_4, then centrifuged, filtered through Whatman 42® filter paper (GE Healthcare, Chicago, USA, or equivalent) that was pre-washed with 0.5 M K_2SO_4 solution. Total organic C (TOC) and total N in the extracts were analysed on a TOC-L combustion analyser coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Microbial biomass was calculated by subtracting the C or N concentrations in the fumigated from the unfumigated samples (correcting with blanks).

To calculate the decay rates of the litter from the field, I used the litterbags that were collected after six and nine months at the same time as the mesocosms were sampled. To clean the litterbags after removal from the field, I rinsed the bags under a continuous flow of water to remove as much soil as possible without damaging the leaves. All litter samples were rinsed for the same amount of time (3 minutes) and then oven-dried to constant weight at 40 °C. I calculated the percentage litter mass loss from the difference in weight between the initial litter sample (16 g) and the remaining litter and I calculated the decay rate according to Olson (1963, Eq 4.1):

$$\ln\left(\frac{X}{X_0}\right) = k\mathrm{t} \qquad \textbf{Eq 4.1}$$

Where: t is time in months since the bags were placed in the field, X is litter dry mass at collection, X_0 is the initial litter dry mass.

4.3.5 Data Analysis

I performed all statistical analysis using R version 3.4.2 (R Core Team, 2017). I used principal component analysis (PCA; *rda* function) to visualise the separation between habitats based on soil properties (total C, total N, C:N ratio, P, K and pH). I then used linear models to assess differences in individual soil properties among habitats and to assess differences in microbial biomass and KCl extractable N among habitats and litter treatments (*lm* function). I investigated the relationship between decomposition and mean soil respiration at three, six and nine months with Pearson's correlations, using the decay rates calculated from the litterbags collected after nine months.

I assessed the effects of habitat and litter treatment on soil temperature, moisture, respiration and response ratios as well as litter mass loss using nested linear mixed effects models with month and replicate block as random effects (lmer function in the lme4 package; Bates *et al.* 2017). Full models included habitat and litter treatment and their interaction as fixed effects, and significance was determined by sequentially dropping terms until a minimum adequate model was reached, using AIC and p-values to check for model improvement. The final models were compared to appropriate null models using likelihood ratio tests and the model fit was assessed using diagnostic plots (Crawley, 2007).

4.4 Results

4.4.1 Site Characteristics

There were small differences in soil nutrient contents and pH among the three habitats. The soil in the old growth forest, although not significantly different, had lower P content compared to the secondary forest and the modified habitat (Table 4.1. Soil pH was significantly lower in the modified habitat compared to the forest habitats. By contrast, total soil C and N were significantly lower in the modified habitat compared to the two forest habitats. Total C and total N, C:N and pH explained most of the differences between the three habitats.

Table 4.1 Soil nutrient content of each habitat used in the litter decomposition experiment in a lowland tropical forest in Panama. Mean values of pH, total soil carbon (C) content, total soil nitrogen (N) content, carbon to nitrogen (C:N) ratios, phosphorus (K) and potassium (P). Means and ± standard errors are shown for N = 6 composite soil samples, values marked with "a" are significantly different for $p < 0.05$.

Property\Habitat	Old growth forest	Secondary forest	Modified habitat
pH	6.01 (±0.11)	5.12 (±0.10)	5.42 (±0.29) [a]
Total C (% dry weight)	5.00 (±0.41)	5.67 (±0.23)	3.03 (±0.36) [a]
Total N (% dry weight)	0.53 (±0.03)	0.57 ± 0.03	0.04 (±0.04) [a]
C:N	9.32 (±0.25)	10.03 (±0.32)	10.04 (±1.14)
P ($mg.kg^{-1}$)	2.99 (±0.54)	4.89 (±0.78)	4.30 (±0.88)
K ($mg.kg^{-1}$)	726.69 (±167.66)	804.64 (±133.75)	748.48 (±143.59)

A principal components analysis of the four soil nutrients, C:N ratio and pH showed a clear separation of the modified habitat sites from the two forest habitats driven by total C and total N (Figure 4.3) whereas the old-growth and secondary forest sites were more similar. PC1 explained 33 % of the variation and loaded heavily on total C and N; PC2 explained 28 % of the variation and loaded heavily on C:N ratio and pH.

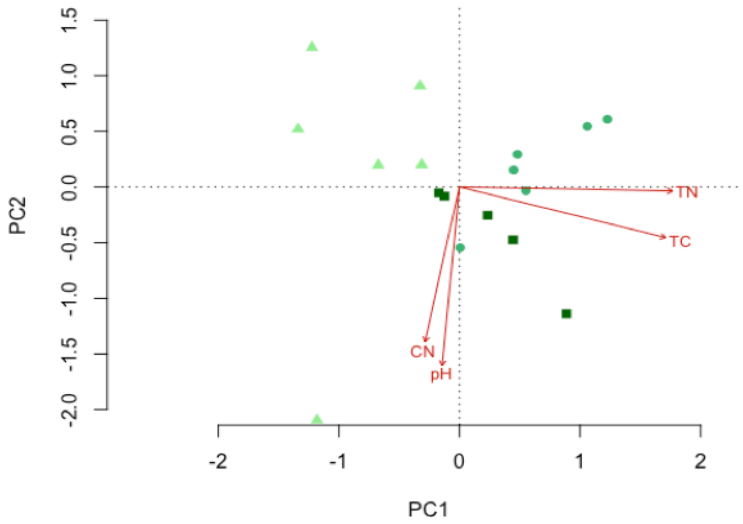


Figure 4.3 Principal components analysis of soil physical characteristics (Total C, total N, C:N, extractable P and extractable K) and pH of the three habitats used in a decomposition experiment in a lowland tropical evergreen forest in Panama where dark green squares in an old-growth forest, medium green circles is a secondary forest and light green forest is a deforested modified habitat. Arrows indicate the direction and degree of significant correlations between PCA axes and soil physical characteristics (n = 5).

The soil temperature of the modified habitat was significantly different when compared to the other two forest habitats ($\chi^2 = 163.43$, $p < 0.05$) with temperatures 1 to 2 °C higher than the forests from March until June. Soil moisture varied with seasonality, decreasing during the dry season and increasing during the wet season (Figure 4.4). Soil moisture was also significantly different for the modified habitat when compared to the forest habitats ($\chi^2 = 126.44$, $p < 0.05$) with a higher percentage soil moisture, up by 20% in June, during the wetter months (Figure 4.4).

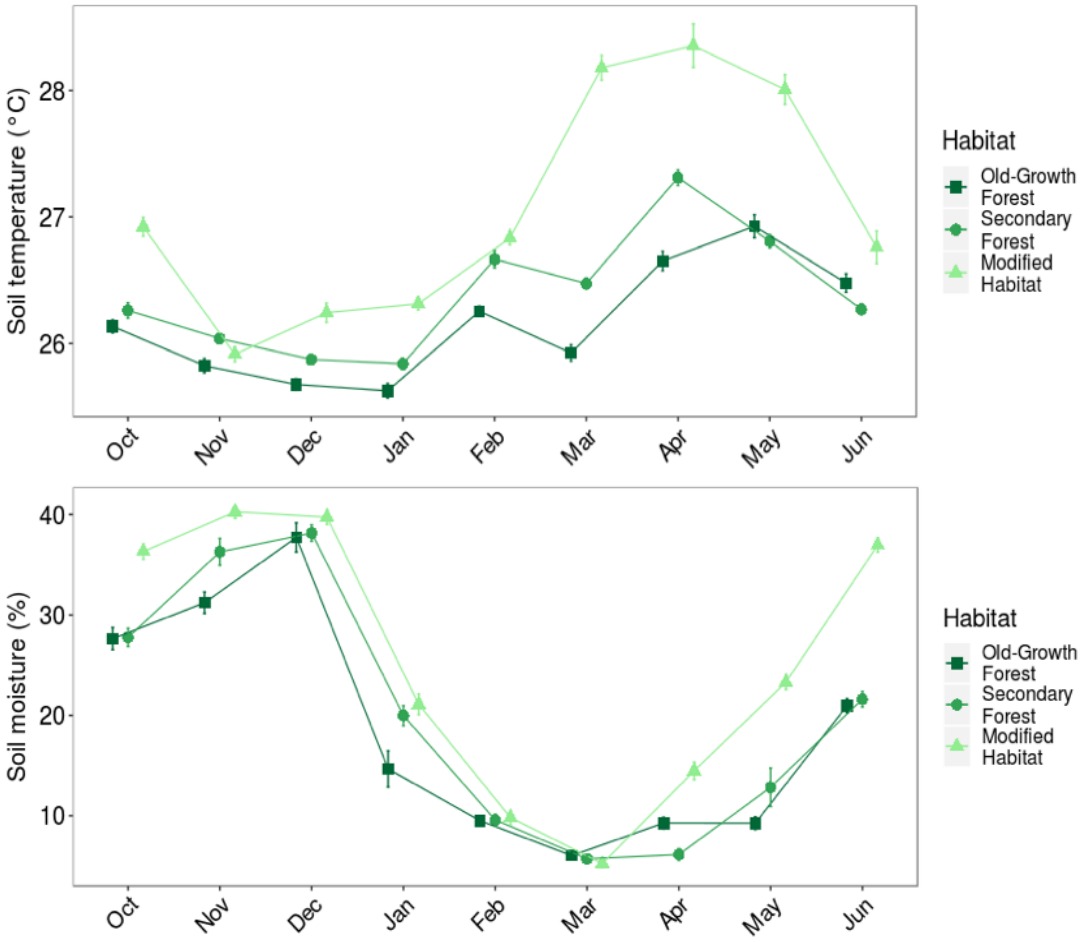


Figure 4.4 Soil temperature (top) and soil moisture (bottom) for each habitat used in a litter decomposition experiment in a lowland tropical forest in Panama. The measurements were taken for each mesocosm (n = 136 from October 2016 until March 2017 and n = 68 from April until June 2017) every month over 9 months. The habitats are as follows: Old growth forest (dark green squares), secondary forest (medium green circles) and a modified, deforested, habitat (light green triangles).

4.4.2 Litter decomposition

All three litter mixtures had similar nutrient content except *Saccharum spontaneum* had much higher macronutrient concentrations: double the concentration of P and four times the concentration of K potassium (Table 4.2). Old growth and young growth litter had similar nutrient contents. Young growth had the highest lignin:N (L:N) ratio and the introduced litter had the highest C:N ratio and the lowest lignin content and L:N ratio.

Table 4.2. Litter properties of the initial three litter mixtures used in a decomposition study in lowland tropical forest in Panama. The values shown are from one composite sample (N = 1) per litter type for carbon (C), nitrogen (N), phosphorus (P), potassium (K), C:N ratio, fibre, lignin, L:N ratio, calcium (Ca), magnesium (Mg), zinc (Zn), sulphur (S), sodium (Na), iron (Fe), boron (B) and manganese (Mn).

Property\Habitat	**Old-growth litter**	**Secondary forest litter**	**Introduced *saccharum spontaneum* litter**
C (% dry weight)	49.9	49.2	45.26
N (% dry weight)	1.8	1.3	1.15
P (% dry weight)	0.063	0.053	0.11
K (% dry weight)	0.26	0.34	1.38
C:N	27.4	37.2	39.2
Fibre (% dry weight)	33	32.34	22.02
Lignin (L) (% dry weight)	21	16.9	1.53
L:N	11.7	13	1.33
Ca (% dry weight)	1.56	1.28	0.38
Mg (% dry weight)	0.27	0.25	0.13
Zn ($mg.kg^{-1}$)	40.5	32	29.2
S (% dry weight)	0.16	0.17	0.13
Na (% dry weight)	0.022	0.039	<0.01
Fe ($mg.kg^{-1}$)	200	182	235
B ($mg.kg^{-1}$)	51.6	42.2	7.91
Mn ($mg.kg^{-1}$)	179	499	241

Overall, the litter decay rate was much lower in the modified habitat compared to the forest habitats regardless of the litter treatments. The introduced litter treatment had the highest litter mass loss at six and nine months (Figure 4.5) and it decomposed almost twice as fast as the secondary and old growth forest litter treatments, which decomposed at a similar rate. The model that best explained litter decay rates included habitat and treatment but not their interaction ($\chi^2 = 139.48$, $p < 0.0001$).

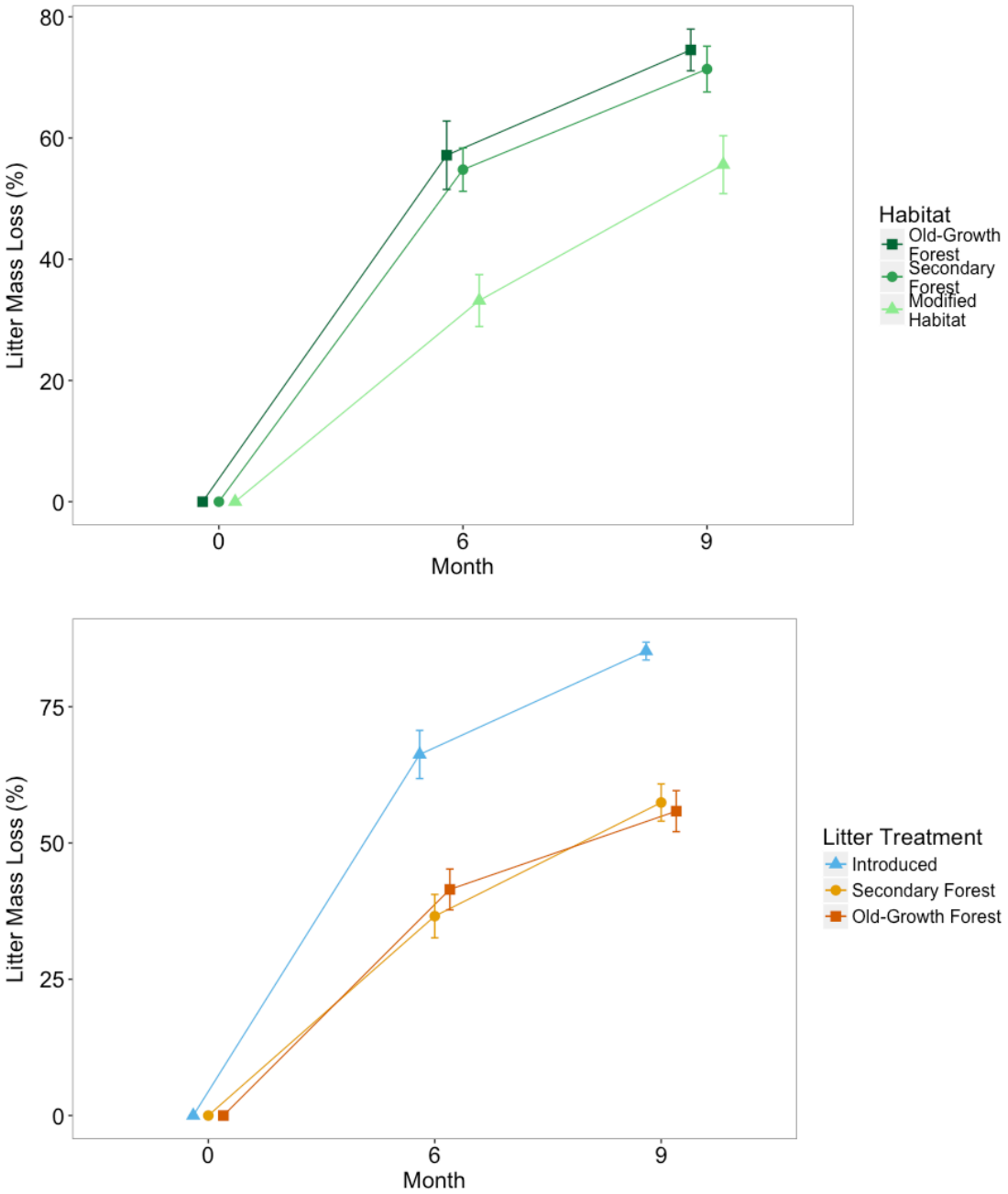


Figure 4.5 Litter mass loss of the litterbags placed in each habitat and collected after 6 and 9 months. The top graph shows the litter mass loss for each habitat with all treatments combined where old growth forest is in dark green squares, secondary forest is in medium green circles and a modified, deforested, habitat is in light green triangles. The bottom graph shows litter mass loss for each litter treatment regardless of habitat where the Introduced species litter, *saccharum spontaneum*, is in blue triangles, the secondary forest litter is in yellow circles and the old growth forest litter is in orange squares. The points have been aligned next to each other for clarity.

4.4.3 Total soil respiration

Total soil respiration (from then on referred to as soil respiration) showed a seasonal pattern in all habitat types, with a decrease in soil respiration during the dry months (February and March; Figure 4.6). The model that best explained the variation in soil respiration included litter treatment and habitat (χ^2 = 89.499, p < 0.001) with the lowest in the high-disturbance habitat regardless of litter treatment. However, the treatment effect was mostly explained by the difference between the bare soil and the treatments containing litter.

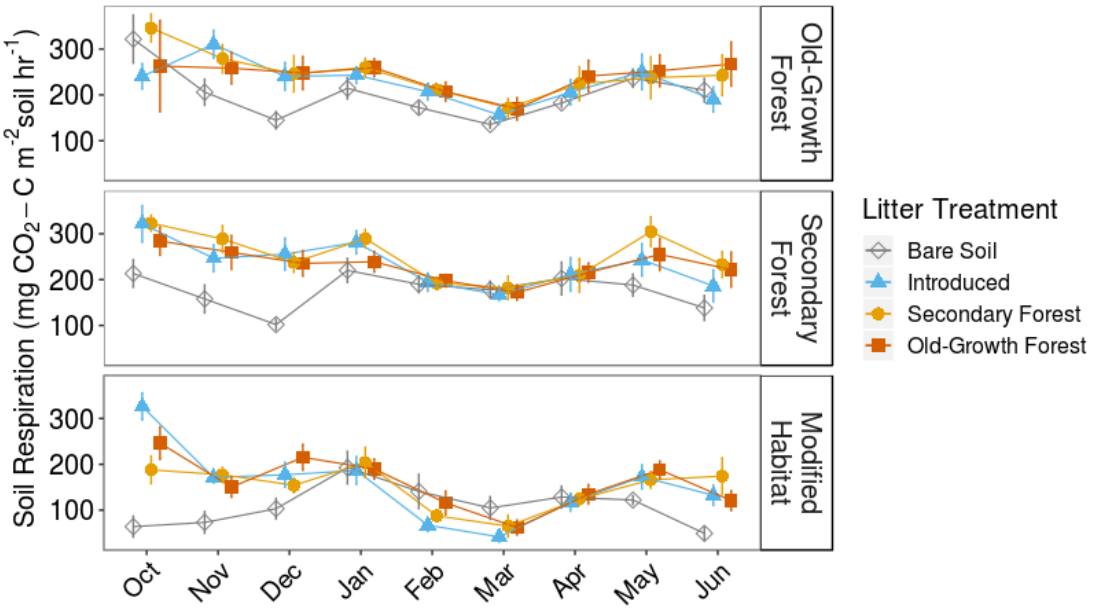


Figure 4.6 Total soil respiration from a litter decomposition experiment in a lowland tropical forest in Panama. The measurements were taken for each mesocosm (n = 136 from October 2016 until March 2017 and n = 68 from April until June 2017) every month over 9 months. The habitats are as follows: Old growth forest (top graph), secondary forest (middle graph) and a modified, deforested, habitat (bottom graph). The litter treatments in the mesocosm are represented as orange squares for old-growth forest, yellow circles for secondary forest, blue triangles for the introduced species and grey diamond is bare soil control.

When the bare soil treatment was removed from the model there was no difference in soil respiration between the litter treatments but the variation in fluxes between habitats remained the same, with much lower fluxes in the modified habitat and similar fluxes between the forest habitats (Figure 4.7). The model that best explained the variation in soil CO_2 efflux included only habitat (χ2 = 17.77, p < 0.001). Calculating and the response ratio by dividing the flux from each litter treatment with the flux of the bare soil, also showed no difference between litter treatments. In short, adding a

layer of litter increased microbial activity compared to bare soil, but the different litter mixtures did not significantly change microbial activity over the course of the experiment, regardless of the habitat.

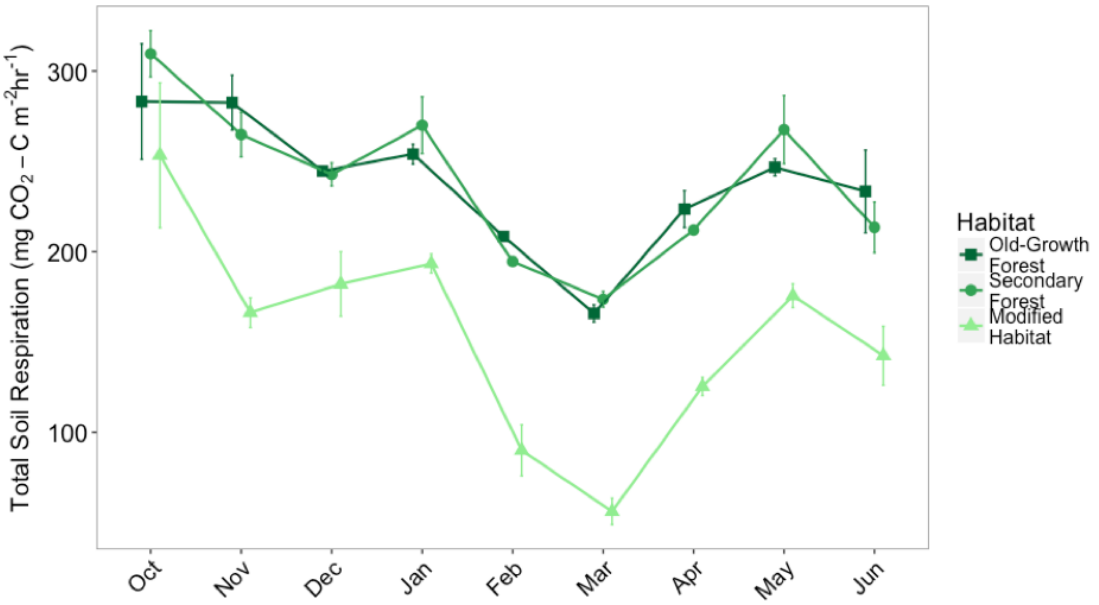


Figure 4.7 Total soil respiration measured over mesocosms in three different habitat types in a litter decomposition experiment in a lowland tropical evergreen forest in Panama over nine months. The measurements were taken for each mesocosm (n = 136 from October 2016 until March 2017 and n = 68 from April until June 2017) every month over 9 months. The habitats are represented as Old-growth forest in dark green squares, secondary forest in medium green circles and deforested modified habitat in light green triangles.

4.4.4 Relationship between soil microbial activity and litter

The secondary (MBC = 442 ± 18 µg/g dry soil, MBN = 69.4 ± 3.23 µg/g dry soil) and old growth forests (MBC = 336 ± 20 µg/g dry soil, MBN = 86.36 ± 4.72 µg/g dry soil) higher soil MBC and MBN (Figure 4.8) than the modified habitat which had much lower concentrations (MBC = 186 ± 15 µg/g, MBN = 33.1 ± 3.33 µg/g). The models that best explained soil MBC and MBN included habitat only (MBC: χ^2 = 27.99, p < 0.0001 and MBN: (χ^2 = 22.53, p < 0.0001) and there were no significant differences in microbial biomass between mesocosms with bare soil and mesocosms with litter in any habitat after nine months of decomposition in the field.

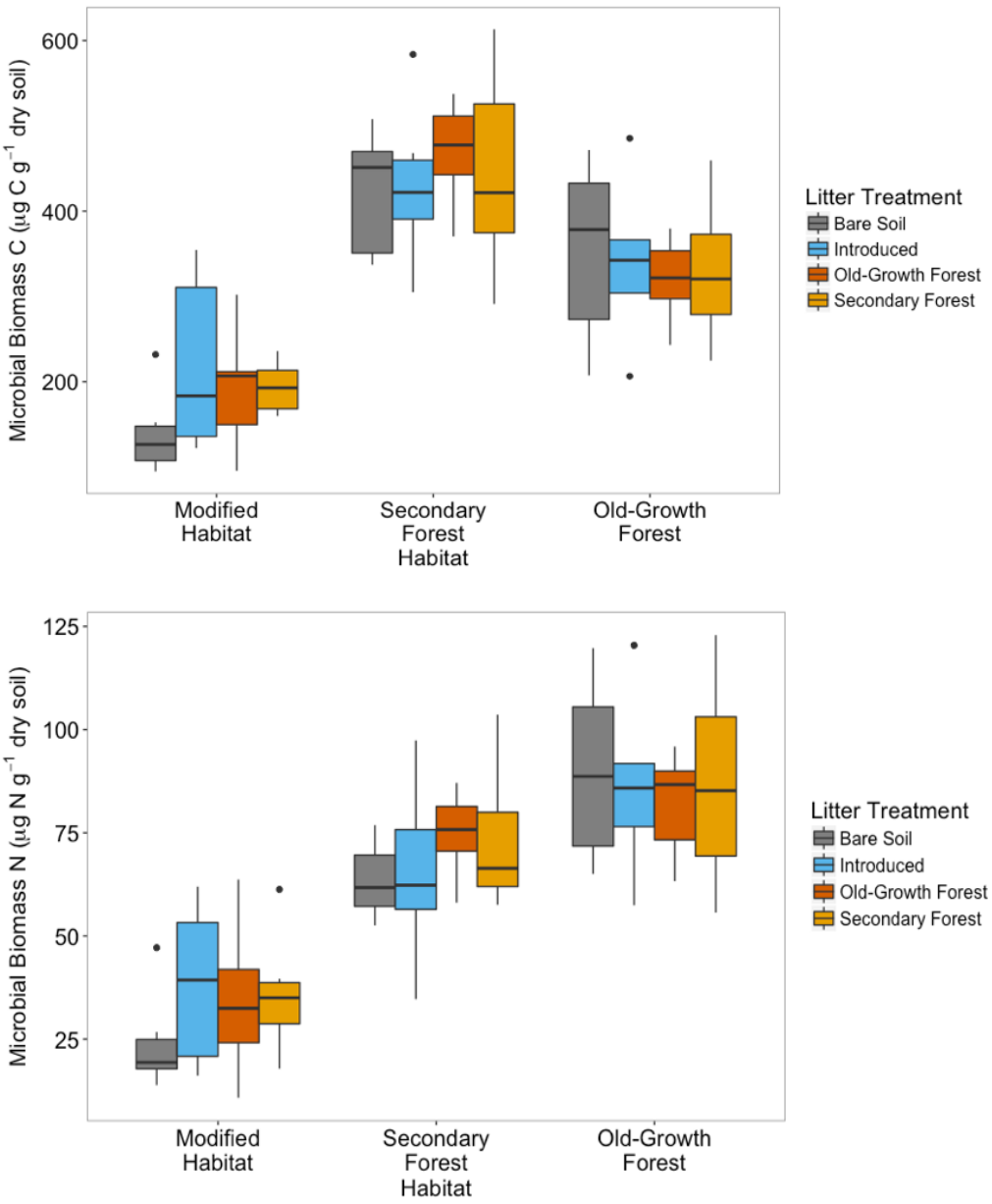


Figure 4.8 Soil (0-10 cm depth) microbial biomass carbon (top graph) and soil microbial nitrogen (bottom graph) in mesocosms with different litter mixtures after 9 months of decomposition. The treatments represent the litter treatments in the mesocosms for where, from left to right, the bare soil is in grey, the introduced species is in blue, the old-growth forest is in orange and the secondary forest is in yellow.

Higher mass loss is associated with higher soil respiration and both of these factors are affected by the type of litter and the habitat where higher disturbance and lack of diversity in the litter composition causes lower litter decomposition and therefore lower soil microbial activity. I ran correlations of soil respiration and decay rates at six months and nine months. There was a positive relationship between soil respiration and litter decomposition after six months; the model that best explained the relationship between litter decomposition and soil respiration after six months

included litter mass loss, habitat and treatment but not their interaction ($F_{(82,87)}$ = 12.22, $p < 0.0001$); after nine months there was no correlation.

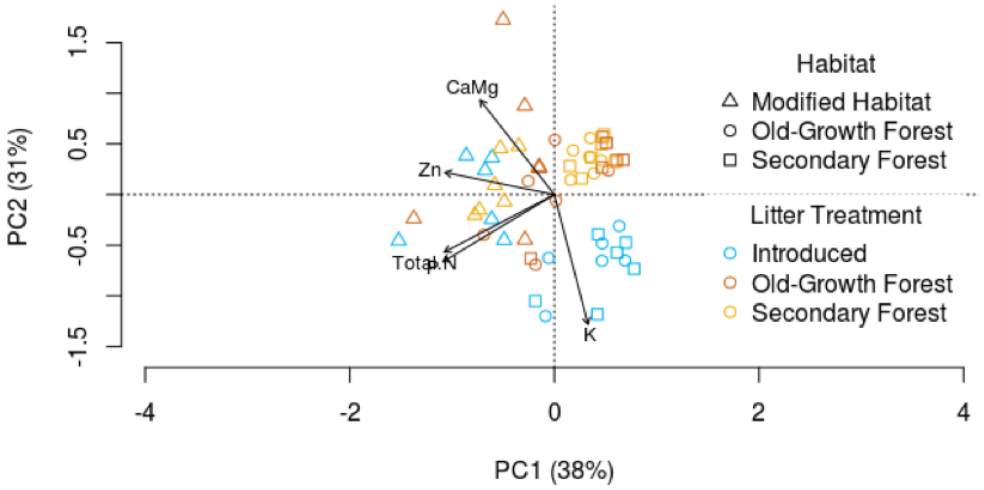


Figure 4.9 Principal components analysis showing differences in nutrient supply rates of resin probes placed between the litter and the soil during the first month of a litter decomposition experiment in a lowland tropical evergreen forest in Panama. Arrows indicate the direction and degree of significant correlations between PCA axes and nutrient supply rate (n = 3).

Furthermore, principal components analysis of the nutrient supply rates from the resin probes showed a differentiation between the introduced litter and the forest litters as well as between the modified habitat and the forest habitats (Figure 4.9).PC1 explained 38 % of the variation and loaded heavily on total N, P and Zn; PC2 explained 31% of the variation and loaded heavily on K, Ca and Mg. Mean overall soil respiration was positively correlated to PC1 ($r_{(49)}$ = 0.654, $p < 0.01$) and litter decay rates were positively correlated to PC1 ($r_{(44)}$ = 0.314, $p < 0.05$ and negatively correlated to PC2 ($r_{(44)}$ = 0.561, $p < 0.01$).

4.5 Discussion

4.5.1 The importance of microclimate

The influence of habitat on microbial activity, regardless of litter type, is a result of microclimate and the standing microbial biomass. Habitat type had the greatest effect on soil respiration and microbial biomass and the two forested habitats had similar soil respiration and microbial biomass (Figure 4.5 and 4.7). At nine months, nearly 80% of the litter had decomposed in the forested habitats but less than 60% had decomposed

in the disturbed habitat. The lower decay rate in the modified habitat can be explained by a combination of the prevailing microclimate, the lower soil nutrient content and lower soil microbial biomass. Tree cover in the disturbed habitat was sparse, and the lack of a closed canopy results in rapid desiccation of the litter on the soil surface (Powers, 2004). In my study, soil temperature in the disturbed habitat was much higher than in the forested habitats during the dry season, whereas soil water content was higher during the rainy season (Figure 4.4), indicating that both rapid desiccation and waterlogging may have slowed decomposition in the disturbed habitat. It is likely that the litter acted as a buffer for soil water at the surface (Sayer 2006), and the thicker litter layer in the forested habitats slowed the processes of soil drying and water saturation. The influence of litter moisture content could be assessed by collecting samples from the surrounding area each month when respiration was measured. A similar decline in decomposition in degraded tropical forests was also found in an experiment in China, in which open deforested land had significantly lower decomposition rates than old-growth and regenerating forests (Paudel et al., 2015). The lower microbial biomass (Figure 4.8) and lower nutrient availability (Table 4.1) of the modified habitat compared to the forest habitats is also likely to have directly limited decomposition processes. Litter decomposition rates are related to the abundance of organisms capable of decomposing the litter (Couteaux et al., 1995). Microbes are essential for decomposing leaf litter and therefore lower microbial biomass would result in lower litter decomposition (Singh and Gupta, 1977). Other studies have found that deforestation and the conversion to agriculture or grasslands significantly reduces soil C, nitrogen and microbial biomass which in turn alter decomposition processes (Dinesh et al., 2004, 2003; Gomez-Acata et al., 2014). The similar soil and litter properties in the secondary and primary forest indicate that key ecosystem functions in the regenerating secondary forest were able to recover within 40 years (Dent et al., 2013), even though it can take over a century before aboveground diversity and tree species composition resemble undisturbed forest (Martin et al., 2013). By contrast, the modified habitat had significantly lower soil respiration, microbial biomass and total C and N when compared to the forested habitats. The secondary forest and disturbed habitats are approximately the same age,

so the substantial differences between the forest and disturbed habitats are explained by the presence of trees and the ecosystem functions they support.

Litterfall from trees is particularly important for maintaining soil function (E. J. Sayer et al., 2006). In the disturbed habitat, the substantial increase in respiration in mesocosms with litter compared to bare soil, indicates that the very low aboveground litter inputs and resulting low soil C content may constrain microbial activity (García-Orenes et al., 2010; Reeves, 1997). For the modified habitat, the marked increase in microbial biomass in the mesocosms (Figure 4.8) with forest litter compared to bare soil is noteworthy because, in conjunction with the increased respiration in litter treatments in the disturbed habitat (Figure 4.6), it indicates that microbial activity in the disturbed habitat is strongly constrained by substrate availability. It is also possible that changes in arthropod diversity in modified habitats influences decomposition, as they are important for the breakdown of tougher litter, which the facilitates microbial decomposition (Handa et al., 2014; Laird-Hopkins et al., 2017; E.J. Sayer et al., 2006)

However, the lack of litter inputs is unlikely to be the sole reason for reduced microbial activity in the disturbed habitat, as litter manipulation studies in forests have found that soil respiration rates in litter removal plots did not differ significantly from control plots (with the litter intact; Bréchet et al., 2018; Moore et al., 2004; Sayer et al., 2007). The removal of trees does not only cause the removal of litterfall but also the loss of shading from the tree crown and therefore the loss of moisture and solar radiation protection as well as the loss of ecosystem functions supported by the root systems (Lopez-Sangil et al., 2017; Trumbore et al., 1995).

Litter decomposition and soil respiration

Although I found no significant effect of litter types on monthly soil respiration from the mesocosms, the differences in mean soil respiration among habitats were of a similar magnitude to the differences in decay rates, and there was a significant relationship between mean soil respiration and litter decay rates measured at six months. Root-rhizosphere respiration, the lateral movement of CO_2 through the soil, and seasonal changes in soil water content all contribute to variation in soil respiration

(Bréchet et al., 2018; Lopez-Sangil et al., 2017; Trumbore et al., 1995), making it hard to detect the influence of the discrete amounts of litter added to the mesocosms. Litter quality would only affect heterotrophic respiration, whereas I measured total soil CO_2 efflux, which also includes autotrophic respiration and previous work in old-growth forest at the study site demonstrated that root-rhizosphere respiration can represent up to *c*. 40% of total respiration (E.J. Sayer and Tanner, 2010). Nevertheless, litter decay explained 18% of the variation in mean soil respiration after six months, highlighting the importance of leaf litter as a resource for soil microbes (Leff et al., 2012; Sariyildiz and Anderson, 2003). Although the relationship between decay rates and soil respiration was no longer apparent after nine months, this can be explained by the extent of decomposition: I only added litter to the mesocosms at the start of the experiment and c. 60-80% of the litter had decomposed after nine months. The low mass and quality of the remaining litter substrate is therefore unlikely to have had a strong influence on total soil respiration.

The presence of leaf litter is more importance that the type of litter provided for microbial activity (Fanin et al., 2011), the significant decrease of soil respiration in the bare soil mesocosms compared to the mesocosms containing litter reflect this (Figure 4.6). Previous studies suggest that it is the environment as a whole provided by the presence of trees that is essential for microbial activity (Salimon et al., 2004; Schwendenmann et al., 2007; Sotta et al., 2006; Vasconcelos et al., 2008). The absence of trees in the modified habitat has reduced the amount of shade provided to the soil by the tree crown but also by the litterfall which have caused bigger soil temperature and moisture variations (Paudel et al., 2015).Deforestation causes a sudden loss of soil C and microbial biomass if given the chance to regrow, the forest soil can recover some of its soil function; if the habitat is maintained as a deforested system, the ecosystem functions remain missing.

4.5.2 litter properties and decay

The differences in decomposition rates are explained by general litter properties (regardless of habitat type). The higher decay rate of the introduced species litter compared to the decay rates of the forest litter mixtures can be explained by the lower

lignin and L:N ratio (by a magnitude of 10) as well as higher P and K (Figure 4.5; Bréchet et al., 2017; Coq et al., 2011; Hattenschwiler et al., 2005; He et al., 2016). The lignin content and the L:N ratio of litter are a good indicator of resource quality for microbial decomposers (Hättenschwiler et al., 2010; Ordoñez et al., 2009), and numerous studies demonstrate that litter with low lignin content and L:N ratios decomposes rapidly (Coq et al., 2011; Hirobe et al., 2004; Kalbitz et al., 2006). Furthermore, many ecosystem processes in lowland tropical forests are limited by P availability, and there is also evidence for K-limitation in the study area (Wright et al., 2011). Hence, the low lignin content and higher concentrations of limiting elements of the introduced litter result in much more rapid decomposition than the forest litter mixtures. Interestingly, the principal components analysis of nutrient availability measured by resin probes showed that K was a strong factor in explaining the separation of the introduced litter from the forest litter mixtures (Figure 4.9). A fertilization experiment in the study forest demonstrated that K addition promotes cellulose decomposition (Kaspari et al., 2008); hence my study provides further evidence for K-limitation of decomposition in the study forest. Conversely, the availability of Ca and Mg, which are linked to lower decay rates (Sayer et al., 2006), was higher in the forest litters; Ca and Mg make up the structure of the leaf skeleton which is the slowest decomposing part of the leaf (Hättenschwiler et al., 2010; Ordoñez et al., 2009).

The two forest mixtures decomposed at a similar rate in the two forest habitats even if the tree species composition of the secondary and old-growth forest differed this could suggest that some microbial communities can be interchangeable as long as their concentration is high enough. A reciprocal litter transplant of a single species pine litter with high lignin content and single-species broadleaf litter with a low lignin content showed that, although the microbial community differed between the pine and the broadleaf plantation, there was no effect of microbial communities on overall litter decomposition rates (He et al., 2016).

The similarity in decay rates and mass loss of the forest litters might also suggest that mixed litter is important, but species identity is not essential. A litter decomposition experiment using three species of pioneer litter and three species of old growth litter

in a 60-year old stand of the same study forest found that natural litter decomposed slower than the combination of its three dominant species and also differed from a representative mixture with litter from six species (Laird-Hopkins et al., 2017). This suggests that the decomposition of litter from dominant tree species is not necessarily representative of decomposition processes in highly diverse natural mixed litter, and that antagonistic and additive effects are both common and complex. In the case of highly diverse tropical forests, the abundance of tree species present in secondary forests and old-growth forests is high enough that even though the dominant tree species differ between the habitats the variety and abundance of less dominant species means that the overall litter quality is the same and therefore has the same functional characteristics. Despite the aboveground difference in tree composition, the overarching impact on soil C dynamics in this environment is a long-term reduction of tree cover.

4.6 Conclusion

Key litter properties explained the decay rates of different litter types regardless of the habitat, whereas forest disturbance changes the local environment, tree diversity, and microclimate, resulting in lower soil microbial activity. However, the lack of differences between forest habitats suggests that secondary regenerating forest had regained key soil functions after only 40 years. Hence, although the recovery trajectory of aboveground biomass, soil C, and tree diversity in secondary forests can take over 80 years (Martin et al., 2013) or as much as 1100 years (Douglas et al., 2018), the rapid regeneration of key ecosystem processes such as litter decomposition provides optimism for reforestation efforts to limit soil degradation in the tropics.

CHAPTER 5 SOIL CARBON DYNAMICS OF NATIVE AND NON-NATIVE TIMBER PLANTATIONS IN PANAMA.

5.1 Abstract

Tree species diversity affects decomposition processes and soil carbon (C) cycling via differences in litter traits. Mixing litter from species with distinct traits can have facilitative or antagonistic effects on the rates of mass loss, and soil microbial communities may preferentially decompose litter from tree species at the same site (the "Homefield Advantage"). Species choices and low diversity in tropical tree plantations is therefore likely to influence interactions between litter traits and soil microbes that underpin soil carbon dynamics and storage.

In Panama, new initiatives are assessing the viability of plantations with native trees after limited success with efforts to reforest agricultural land with teak (*Tectona grandis* L;f) plantations. One such initiative, the Agua Salud Project, compares the productivity of non-native teak to native timber trees; I used these trial plantations as an experimental platform to assess the influence of litter traits and soil properties on microbial processes involved in soil carbon cycling.

I established a litter transplant experiment using mesocosms and litterbags in plantations with the native species *Dalbergia retusa* and *Terminalia amazonia*, and in teak monocultures. I used single-species litter and all possible species combinations to assess non-additive effects of litter mixtures on decomposition and calculated the homefield advantage for single-species litter treatments. I measured soil respiration and litter decomposition as indicators of microbial activity, as well as soil properties and nutrient release from the decomposing litter.

Litter properties explained some of the variation in litter decomposition, but it was also affected by microclimate and the soil properties in the plantations. Microbial biomass and litter decomposition were much lower in *Tectona* than in the native plantations, but *Terminalia* litter had the lowest mass loss after 6 months, and there was a homefield disadvantage for soil respiration in mesocosms with *Terminalia* litter. Interestingly, although the *Dalbergia+Tectona* litter mixture decomposed slower than expected in the *Dalbergia* plantations, the litter mixture enhanced soil respiration in the *Tectona* plantation, which suggests an interaction between allelopathic chemicals in *Tectona* and the high nitrogen content of *Dalbergia*. Overall, my results indicate that tropical forest soils might be more resilient to change than expected, as the microbial communities are adapted to high plant species diversity; which leads me to be optimistic about restoring soil function in forest regeneration projects using native species.

5.2 Introduction

The Bonn challenge, launched in 2011, aims to reforest 150 million hectares of degraded land by 2020; it is particularly centred around reforesting degraded landscapes in and with rural communities (IUCN, 2018). In Central America, much of the eroding landscape is constituted of pasture lands and agricultural lands; many reforestation projects such as the Bonn challenge or REDD+ are encouraging small landowners to grow timber plantations. One of the most common species planted in the region is Teak (*Tectona grandis*) a tree that originates from South and Southeast Asia and as such is not native to the Americas. In Panama, Teak plantations represent 76 % of the timber plantations planted between 1992 and 2002. However, Teak does not grow particularly well on acidic clay soils of which Panama is mostly composed which could have dire consequences: economical for the landowners that have invested their land and money but also if the plantation does not thrive and the reforestation project fails the soil will carry on eroding.

Forests and trees play an essential physical and biological role in maintaining the soil C pool through the addition and cycling of organic C and other nutrients from decomposing leaf litter (Hättenschwiler et al., 2005; Swift, 2001). Many factors affect

soil C cycling within forested ecosystems, one that is especially important in tropical ecosystems is plant diversity. Plants have diverse functional traits which in turn affect the ability of the soil microbial communities which are a key part of soil C cycling to decompose the leaf litter provided by the different tree species. Different plants and therefore trees will produce litter of varying decomposability and will interact with the rhizosphere and cause microenvironmental changes which will in term affect microbial activity and therefore soil cycling (Fornara et al., 2009; Hättenschwiler et al., 2005; Hobbie, 1992).

Firstly, as mentioned earlier, plant diversity has been shown to have an effect on microbial activity and litter decomposition but this effect can be additive, when the interaction of many species will cause leaf litter to decompose faster and microbial activity to increase, or antagonistically, when the interaction of a species mixture will cause litter to decompose more slowly (Hättenschwiler and Jørgensen, 2010; Sayer et al., 2006; Xu et al., 2013). Secondly, it has been hypothesised that plants and microbial communities in their vicinity will have an affinity and therefore litter will decompose faster if it is close to its parent tree; this has been called the home-field advantage (Ayres et al., 2009; Freschet et al., 2012). Finally, other research suggests that some plant species release chemicals that will hinder other plants and microbial communities from growing in their vicinity; this has been named the “homeland security” hypothesis” (Cummings et al., 2013).

It is in this context that I set up an experiment to test whether there were benefits to using native timber species as opposed to teak for soil C cycling as part of a reforestation project in Panama. Understanding the interactions between aboveground plant inputs, decomposition processes and the soil food web that mediate soil C storage belowground is essential to determine how different timber species alter ecosystem function in afforestation projects.

To date, only few research projects have focused on functional properties of leaf litter, especially in tropical forests (Hättenschwiler & Jørgensen 2010; Fanin et al. 2011; Liang et al. 2016). These studies have demonstrated that the rate of decomposition is determined by the physical and chemical traits of leaf litter, which determine the

quality of substrate available to decomposer organisms (Berg et al., 1993, Perez-Harguindeguy et al., 2000). Decomposers preferentially break down high-quality litter first, which enables the transfer of nutrients to facilitate the decomposition of low-quality litter (Hättenschwiler et al., 2005) and as a result, the functional diversity of the litter governs the rate of decomposition.

It is important that we improve our understanding of soil processes during forest modification to understand what drives changes in C stocks and to determine why some soils are more affected by forest degradation than others.

To determine the effects of plant traits on decomposition processes, I established litter transplant experiments in monoculture plantations at Agua Salud, focussing on three species: Teak (*Tectona grandis*), Cocobolo (*Dalbergia retusa*) and Amarillo (*Terminalia amazonia*).

To measure differences in microbial activity, I devised a reciprocal litter transplant experiment using mesocosms which is a tried-and-tested method that can be used to explore how changes in forest plant composition affect decomposition and C dynamics via altered leaf litter inputs. In situ mesocosms allow for a greater degree of control than large-scale plots while maintaining natural field conditions and recent work demonstrates that rates of soil respiration and decomposition in response to experimental treatments in mesocosms are comparable to large-scale experiments (Laird-Hopkins et al., 2017; Szanser et al., 2011). I investigated the following Hypotheses:

1) Leaf properties will explain the variation in decomposition rates among species and litter mixtures.
2) Rates of decomposition will be greater for leaf litter mixtures than single-species litter as a result of trait complementarity.
3) Differences in soil microbial activity, microbial biomass C and N, and soil respiration among litter mixtures will be related to specific litter traits.
4) There will be a homefield advantage for the native litter species
5) *Tectona grandis* will suffer from homeland security and will have reduced microbial and decomposition activity

5.3 Methods

5.3.1 STUDY SITE AND LITTER MIXTURES

The Agua Salud (AS) Project is situated in the Panama Canal Watershed (9°13'N, 79°47'W, 330 m a.s.l), in Panama, Central America; it is a large-scale experiment studying ecosystem functions and services in varying land-use types ranging from forest to timber plantations to pastoral land and is administered by the Smithsonian Tropical Research Institute (Mayoral et al., 2017). The soils are classified as infertile oxisols (Turner and Engelbrecht, 2011). The study area has a tropical climate with a distinct wet and dry season; the dry season occurs generally between January and April (Sayer and Tanner, 2010). Agua Salud receives a mean of 2700 mm of rainfall per year and has a yearly mean daytime temperature of 32°C (Ogden et al., 2013).

Figure 5.1 Map of the Agua Salud Project in Panama. My mesocosm transplant experiment between three monoculture plantations took place within the red circle in 2017.

The present study was conducted within plantations at Agua Salud (Figure 5.1), which were established on sites formerly used as pastures that had reverted to young secondary forest for five years prior to the initial clearing and planting of the monocultures in 2008. The project planted five native timber species (monocultures

and mixtures) as well as teak, *Tectona grandis* (L.f), an introduced timber species from Southeast Asia. Each tree was fertilized at planting with 57 g of a complete nitrogen-phosphorus-potassium fertilizer (12:24:12 NPK) and organic material mixed with soil and 57 g of triple sulphate; the fertilizers were applied several centimetres from the roots (Mayoral et al., 2017). The native timber species were planted in plots of 42-m × 36.5-m with each plot containing 225 trees and the *Tectona* plantation was planted as one larger plantation of 1 km^2. The underlying vegetation between the trees was cut every three months except in the teak where the vegetation was not cut during the present experiment. The distance between sites did not exceed 2 km, and the sites were chosen to ensure similar slope, elevation and bedrock.

5.3.2 Experimental design

I established a transplant experiment in three different monoculture plantations with five replicate blocks in each plantation for a total of 15 blocks. The plantations were planted with *Tectona grandis* (TEC) an introduced species, *Dalbergia retusa* Hemsl. (DAL), a-nitrogen fixing slow-growing species, or *Terminalia amazonia* J.F.Gmel., Exell (TERM), a fast growing species (Mayoral et al., 2017). In each plantation, I established mesocosms made of PVC pipe (20 cm in diameter and 15 cm in height, Figure 5.3). The mesocosms were sunk into the ground so that the height above the soil was 10 cm and placed at least 1 m away from any tree trunk and at a distance of 0.5 m away from each other. The mesocosms were installed at least one month prior to the start of the experiment to allow the surrounding soil to recover from the disturbance. Each block consisted of two sets of eight mesocosms (16 per block) to allow destructive sampling after three and six months, making a total of 240 mesocosms.

I randomly assigned one of eight litter treatments to two of the mesocosms per block, representing single-species litter from the habitats and litter mixtures of all possible two- or three species combination of species mixtures. Hence, the treatments were: TEC, TERM, DAL, TEC+TERM, TEC+DAL, TERM+DAL, TEC+TERM+DAL and a bare soil control (CT; Figure 5.2).

Litter for the experiment was collected by hand, choosing freshly fallen leaves in each monoculture plot and air-drying them at 30°C. Each single species litter was chopped by hand to give pieces of *c*. 4 cm^2, and homogenised. Each mesocosm received 6 g of litter at the start of the experiment, using equal mass of each species in the litter mixtures; hence, the two-species mixtures contained 3 g of litter from each species and the three-species mixture contained 2 g of litter from each species. The weights for the litterbags were 1.5 g for each litter species in the two-species mixture and 1 g for the three species mixture for a total of 3 g. Subsamples of each litter type were ground for nutrient analysis (see below).

I started the experiment by placing 6 g of litter in each mesocosm. To simulate natural litterfall, I then added 1 g of litter every month over 6 months so that by the last measurement each mesocosm had received a total of 12 g of air-dried litter, which was equivalent to the mean litter standing crop of all three monocultures (*n* = 15).

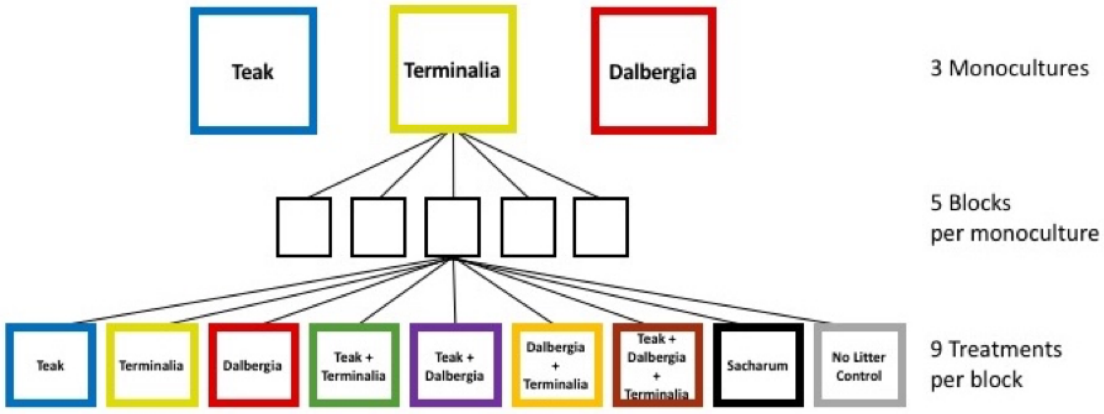


Figure 5.2 Design of the litter transplant mesocosm experiment. At the top are the three monoculture plantation, in each plantation are five replicate plots, in each plot are eight litter treatments starting with single litters then all the combinations of litter mixtures and a bare soil control treatment, where TEC is *Tectona grandis*, TERM is *Terminalia amazonia* and DAL is *Dalbergia retusa*.

I used litterbags to calculate litter decomposition after three and six months; I made 210 litterbags using 1.4-mm fibreglass mesh. Each bag was 8 cm^2 and contained 3 g of air-dried material, which was equivalent to half the surface area and half the amount of litter in the mesocosms. I placed two bags per species and mixture in each replicate block and collected one bag per litter type and block after three months and again after six months. After collection, I cleaned each litterbag for two minutes to remove as much soil as possible without losing litter material; the rinsed litter was then oven-

dried at 40°C for 48 hrs before being weighed separately to calculate percentage mass loss after three and six months.

Figure 5.3 Monoculture plantation of teak (*Tectona grandis*, lefy) and *Terminalia Amazonia* plantation showing the mesocosms (right) used in a mesocosm experiment at the Agua Salud project in Panama, Central America between January and July 2017.

5.3.3 Soil respiration and soil sampling

Soil respiration

In situ soil respiration was measured over the mesocosms using an infrared gas analyser (Li-8100; LI-COR Bioscience, Lincoln, USA) with a 20-cm diameter survey chamber by placing the chamber over the mesocosm after gently removing the mesh containing the leaf litter. At the same time, soil temperature was taken using a Fisherbrand® Traceable Thermometer (Fisherbrand, Hampton, USA) and moisture was measured using a SM150T soil moisture sensor (Delta-T Devices, Cambridge, UK) for each mesocosm soil moisture.

Soil sampling

All soil sampling was carried out to a depth of 10 cm using a 3 cm diameter punch corer. To characterise initial soil total C and N, phosphorus (P) and potassium (K) and

pH, I collected 5 soil cores from each plantation block for a total of 15 blocks (Figure 5.2) in December 2016. The soil samples were air-dried at 30°C then stored in polyethylene resealable bags and shipped to Lancaster University for chemical analysis.

After three and six months, I collected two soil cores inside one mesocosm per treatment and block so that five samples per treatment in each monoculture were collected. To determine soil microbial biomass C and N, the samples were stored in resealable plastic bags and shipped refrigerated to Lancaster University. The remaining subsamples were air-dried at 40°C and archived in accordance with DEFRA regulations.

5.3.4 Laboratory analysis

Soil and litter nutrients

Total C and N was analysed on ground air-dried soil and litter samples (Mixer Mill 400, Retsch®, Haan, Germany) by high temperature combustion gas chromatography (Vario El III C/N analyser; Elementar, Stockport, UK) at Lancaster University using 30 mg of soil and 15 mg of litter.

Air dried, ground soil and litter samples were sent to SAC Consulting (Aberdeen, Scotland) for soil extractable phosphorus (P) and potassium (K) and litter nutrient concentrations (P, K, Ca, Mg).

Soil microbial biomass by fumigation-extraction

I used paired 6 g subsamples of fresh soil to determine microbial biomass C and N using the modified fumigation-extraction method (Jones and Willett, 2006; Vance et al., 1987). One subsample per pair was fumigated with ethanol-free amylene-stabilised chloroform for 24h. Both the fumigated and non-fumigated samples were extracted with 40 ml 0.5 M K_2SO_4 then centrifuged and filtered through Whatman 42® (GE Healthcare, Chicago, USA) filter paper or equivalent, which was previously rinsed with K_2SO_4 solution. The extracts were then diluted nine times with deionised water before being analysed for total organic C and total N on a TOC-L combustion analyser coupled

with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Microbial biomass C and N was then calculated by subtracting the non-fumigated sample from the fumigated sample.

Nutrient release from decomposing litter

To measure nutrients release during litter decomposition, I placed ion exchange resin membranes (PRS Probes®, WesternAG, Saskatchewan, Canada) beneath the mesh baskets containing the litter in the mesocosms in April 2017, four months into the experiment. The PRS probes were placed in each litter treatment in three replicate blocks per plantation (n = 3 per litter treatment and plantation). After one month (exposure time 27-29 days), I collected the probes and stored them at 5°C before cleaning them thoroughly with pressurised deionised water and a small brush as instructed by the manufacturer. The cleaned probes were then sent to the manufacturer for analysis of available anions NO_3N and cations (NO_3, NH_4, P, K, Ca, Mg).

Litter fibre and lignin

To determine the fibre and lignin content of the litter, I used the acid detergent extraction described by Van Soest *et al.* (1991). The method has two steps; the first acid detergent fibre (ADF) step extracts all the fibre from the litter and the second acid detergent lignin (ADL) step extracts the lignin from the extracted fibre. Briefly, 1 g of dried and ground litter were placed in a crucible with 1 g of acetanilide. The litter and acetanilide mixtures were boiled with 100 ml of acid detergent solution for one hour in a FOSS fibertec™ 8000 fibre analysis system (FOSS, Hilleroed Denmark). The samples were then rinsed with warm distilled water until acid-free and rinsed with reagent-grade acetone. The samples were dried overnight at 105°C before being weighed. The total extracted fibre content (ADF) was calculated by subtracting the weight of the processed sample from the original sample weight (corrected with blanks). After weighing, the samples were soaked in 25 ml H_2SO_4 (72%) for three hours; the crucibles were rinsed with hot distilled water using the FOSS fibertec™ 8000 fibre analysis system (FOSS, Hilleroed Denmark) and dried at 105°C overnight before being placed in a furnace at 525°C for three hours. The samples were finally left to cool to room temperature in a desiccator before weighing. The lignin content (ADL) was calculated

by subtracting the weight of the sample at the final stage from the weight of total extracted fibre (ADF) with correction with blanks.

5.3.5 Data analysis

Litter decomposition rates

I calculated percentage mass loss (Eq 5.1) by subtracting the remaining litter after three or six months from the weight of the litter at the start of the experiment using the equation. I calculated the litter decay rates (Eq 5.2) for each treatment and block using the equation as defined in Olson (1963) where *t* is time in years, X_0 is the original weight and *X* is the weight after decomposition.

$$\ln\left(\frac{X}{X_0}\right) = kt \qquad \textbf{Eq 5.2}$$

Response ratio (Eq 5.3) of the soil respiration was calculated for each litter treatment in each replicate block at each month using the following equation:

$$R = \ln\left(\frac{L}{S}\right) \qquad \textbf{Eq 5.3}$$

Where R is the response ratio, L is the respiration from the mesocosm containing litter and S is the respiration from the bare soil mesocosm from the same plot.

Homefield Advantage

I calculated homefield advantage (HFA, Eq 5.4) of the litter decay rates and overall mean soil respiration of the single species litters after three and six months using the equation described by Ayres et al. (2009), which is the same equation used to calculate homefield advantage in sports, whereby HFA > 0 indicates that the litter decomposes faster in its home plantation, HFA = 0 indicates no HFA and HFA < 0 indicates that the litter decomposes slower in its home plantation:

$$HFA = (HDD - ADD - H)/(n - 2)$$

$$HDD = (D_{aA} - D_{bA}) + (D_{aA} - D_{cA})$$

$$ADD = (D_{aB} - D_{bB}) + (D_{aC} - D_{cC}) \qquad \textbf{Eq 5.4}$$

$$H = \frac{(HDDa + HDDb + HDDc)}{(n - 1)}$$

Where HFA is the additional decomposition at home, HDD is the home decomposition difference and ADD is the away from home decomposition difference. D is the measure of decomposition (decay or respiration rate), a, b, and c are the single-species litters, A, B, and C are the plantations for each respective litter species, n is the number of litter species, and H is the sum of all HDD divided by n - 1.

Additive effects of species mixtures

To calculate if there were any additive or antagonistic effects from the three different litters used in the experiment, I calculated the mean respiration and mass loss of two or three single species for the expected respiration and mass loss and compared it to its respective species mixture that had been placed in the field (observed mass loss and respiration).

5.3.6 Statistical analysis

All statistical analyses were conducted in R version 3.4.2 (R Core Team, 2018). Principal component analysis (PCA) was used to find if there was a separation between plantations on soil properties (total C, Total N, C:N ratio, P, K and pH) and between litter or plantation on the nutrient supply rates from the resin probes using the vegan package (Oksanen et al., 2015). Individual nutrients were fitted as vectors to indicate the properties that were driving differences between the grouping factors. I also used linear models to assess if there were differences in the soil properties between the plantations (lm function in the lme4 package, Bates et al., 2017).

The effects of plantation and litter on soil temperature, moisture, respiration and response ratios as well as litter mass loss were assessed using linear mixed effects models with time as a fixed effect and replicate blocks as a random effect (lmer function in the lme4 package; Bates et al. 2014) to account for the crossed experimental design with repeated measures. These models were then compared to null models that didn't include time as a fixed effect using likelihood ratio tests. AIC and p values were used to compare models and the model fit was assessed using diagnostic plots (Crawley, 2007). The effects of plantation and litter on microbial biomass C and N, nutrient supply from the resin probes and the effects of litter addition were assessed by using linear models (lm function in the lme4 package, Bates et al., 2014).To estimate if there was a HFA for litter mass loss or soil respiration I performed a t-test on each HFA score to see if it differed significantly from 0 (n = 5).

5.4 Results

5.4.1 Site and litter characteristics

Soil nutrient concentrations were similar for all three plantations but the *Dalbergia* plantation had a significantly lower soil C:N ratio ($F_{(2,12)}$ = 4.35, p = 0.04) and lower soil extractable K ($F_{(2,12)}$ = 21.42, p > 0.01) compared to the other two plantations, whereas total soil N was marginally lower in the *Tectona* plantation (p = 0.09; Table 5.1).

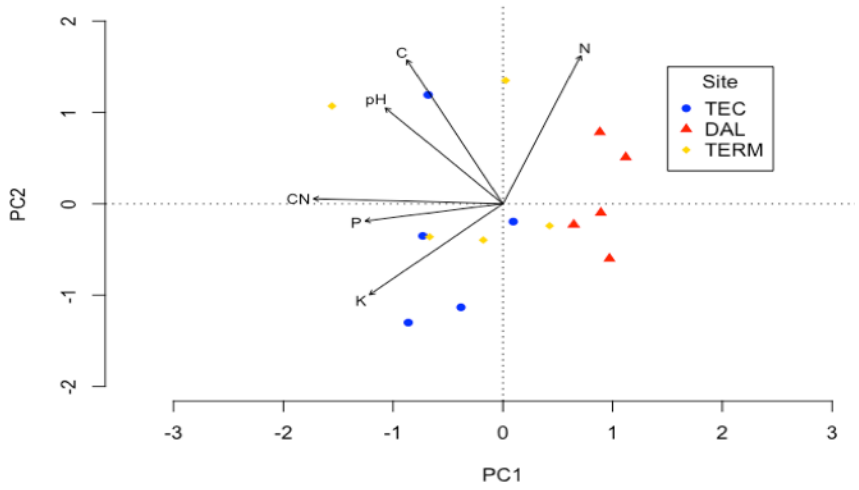


Figure 5.4 Principal components analysis of soil properties (C, N, P, K, C:N ratio and pH) measured in each of five blocks in three monoculture plantations: *Tectona grandis* (TEC, blue circles), *Dalbergia retusa* (DAL, red triangles) and *Terminalia amazonia* (TERM, yellow diamonds) at the start of a mesocosm experiment at the Agua Salud project in Panama, Central America.

Principal components analysis of the soil properties showed a separation of the *Dalbergia* plantations from the *Tectona* and *Terminalia* plantations, which were clustered together (Figure 5.4); the separation was explained by soil P and C:N ratios on the first PCA axis and by soil total C and N on the second axis. The first PCA axis explained 39% of the variation and soil C:N had the highest loading; the second axis explained 37% of the variation and soil C and N had the highest loading.

Table 5.1 Initial soil nutrient contents for three monoculture plantations used in a litter decomposition experiment in the Agua Salud project in Panama, showing soil pH, total soil carbon (C) content, total soil nitrogen (N) content, carbon to nitrogen (C:N) ratios, extractable phosphorus (P), and potassium (K). Means and ± standard errors are shown for n = 5 composite soil samples; different lower-case superscript letters indicate significant differences among plantations at $p < 0.05$ and different upper-case superscript letters indicate trends at $p < 0.1$.

Soil property\Plantation	*Tectona*	*Dalbergia*	*Terminalia*
Total C (%)	4.58 ± 0.20	4.38 ± 0.08	4.90 ± 0.23
Total N (%)	0.41 ± 0.02^{A}	0.45 ± 0.01^{B}	0.44 ± 0.02^{B}
C:N ratio	11.29 ± 0.13^{a}	9.69 ± 0.07^{b}	11.07 ± 0.32^{a}
P (mg kg^{-1})	3.35 ± 0.30	3.09 ± 0.17	3.75 ± 0.34
K (mg kg^{-1})	759 ± 72^{a}	501 ± 58^{b}	651 ± 55^{a}
pH	5.064 ± 0.08	4.93 ± 0.08	5.032 ± 0.06

Of the three single-species litters, DAL had the highest N content, the lowest C:N ratio and P content as well as a much lower lignin to N (L:N) ratio. TEC litter had the highest fibre and lignin content but the L:N ratio was similar to the TERM litter. TERM litter had the highest C:N ratio and nearly double the Ca content of the TEC and DAL litter (Table 5.2).

Table 5.2 Litter properties of the three species used in a decomposition study at the Agua Salud project in Panama; where TEC is *Tectona grandis* litter, Dal is *Dalbergia retusa* litter and TERM is *Terminalia amazonia* litter. The values shown are from one composite sample (N = 1) per litter type for carbon (C), nitrogen (N), phosphorus (P), potassium (K), C:N ratio, fibre, lignin, L:N ratio, calcium (Ca), magnesium (Mg), zinc (Zn), sulphur (S), sodium (Na), iron (Fe), boron (B) and manganese (Mn).

Litter nutrients\Litter type	TEC	DAL	TERM
Total C %	49.31	46.91	47.28
Total N	1.48	2.24	1.03
C:N ratio	33.2	20.9	46
P %	0.0894	0.0399	0.0568
K %	0.468	0.479	0.673
Fibre	29.9	22.8	24.5
Lignin (L)	13.6	8.1	10.2
L:N	10.18	3.86	10.29
Ca %	1.23	1.35	2.16
Mg %	0.36	0.322	0.215

The soil temperature in the *Terminalia* plantation was significantly lower than the *Tectona* and *Dalbergia* plantations (χ^2 = 3146.7, $p < 0.05$) from February until April. Soil moisture content varied seasonally, with very low values in the dry season and a steady increase in soil water content during the wet season from April until the end of the study (Figure 5.5). Soil moisture was consistently higher in the *Terminalia* plantation than the *Tectona* and *Dalbergia* plantations (χ^2 = 4899.9, $p < 0.05$; Figure 5.5).

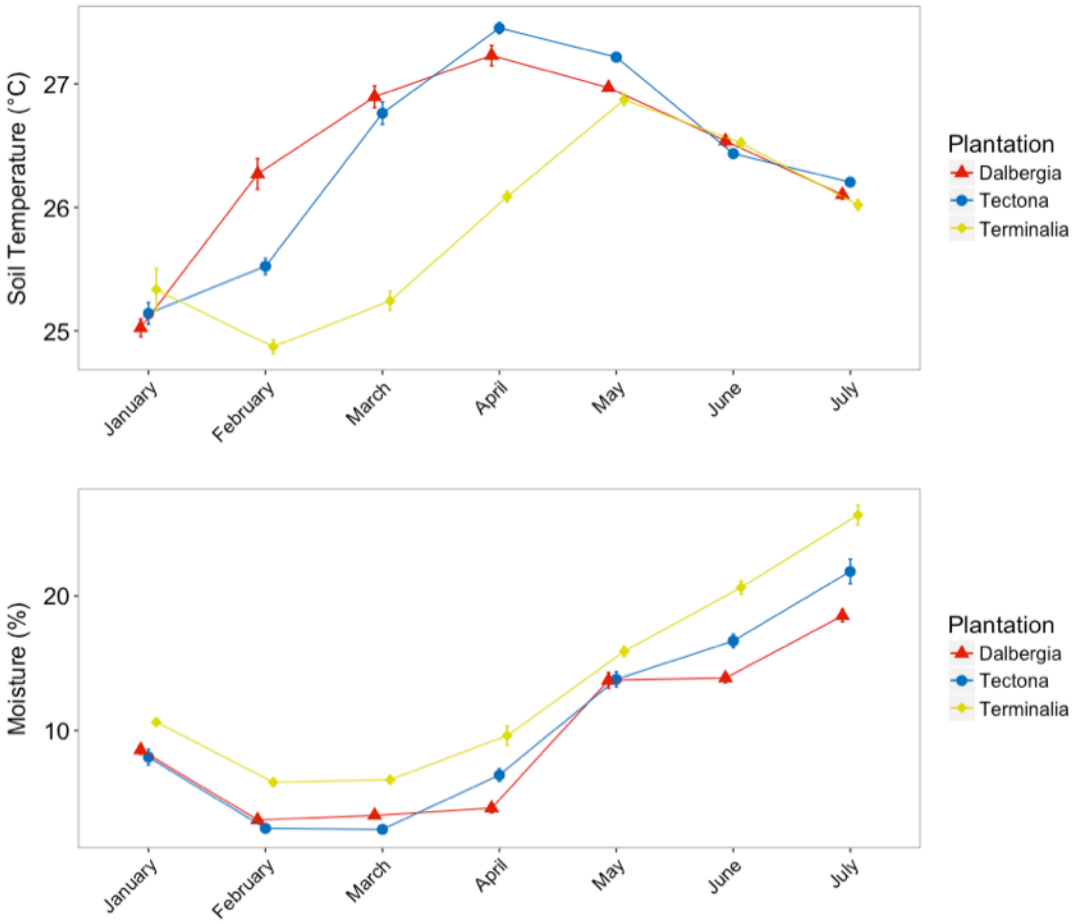


Figure 5.5 Soil temperature (top) and soil moisture (bottom) in monoculture plantations of *Tectona grandis* (blue circles), *Dalbergia retusa* (red triangles) and *Terminalia amazonia* (yellow diamonds) during a litter decomposition experiment in the Agua Salud project in Panama. The measurements were taken for each mesocosm (n = 14 per block from January until April 2017 and n = 7 per block from May until July 2017) every month for 7 months.

5.4.2 Single Species

Overall, soil respiration was lowest in the *Tectona* plantation followed by the *Dalbergia* plantation and was highest in the *Terminalia* plantation. The model that best explained soil respiration included plantation, litter treatment and their interaction (χ^2 = 1024.5, p < 0.0001), as respiration rates from mesocosms with TEC litter was slightly higher in the *Terminalia* plantation compared to the other two, but lower within the *Tectona* plantation when compared to the other litter types (Figure 5.6). However, it should be noted that there was no significant effect of plantation or litter treatment when the response ratios of soil respiration were analysed.

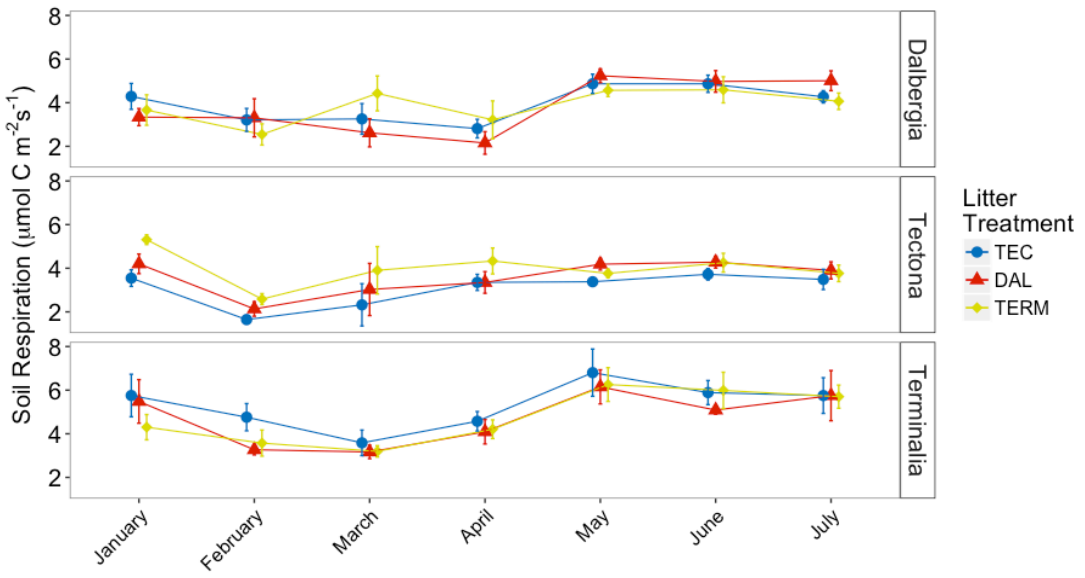


Figure 5.6 Soil respiration in a litter decomposition transplant experiment in monoculture plantations of *Tectona grandis*, *Dalbergia retusa* and *Terminalia amazonia* during a litter decomposition experiment where TEC (blue circles) is *Tectona grandis, DAL is Dalbergia retusa* (red triangles) and TERM (yellow diamonds) *Terminalia amazonia* in the Agua Salud project in Panama. The measurements were taken for each mesocosm (n = 10 per plantation from January until April 2017 and n = 5 per plantation from May until July 2017) every month for 7 months.

The *Tectona* plantation had the lowest soil microbial biomass C, regardless of litter treatment whereas the *Terminalia* and *Dalbergia* plantations had similar microbial biomass C. Litter treatment had no significant effect on microbial biomass C after six months of decomposition (Table 5.3) and the model that best explained microbial biomass C included plantation only ($\chi^2 = 710.57$, $p < 0.0001$). Microbial biomass N was highest in the *Tectona* plantation followed by *Dalbergia* and *Terminalia* plantations regardless of litter treatment. Litter treatment had no significant effect on microbial biomass N after six months of decomposition (Table 5.3) and the model that best explained microbial biomass N included plantation only ($\chi^2 = 444.12$, $p < 0.0001$).

Table 5.3 Soil microbial carbon (C) and nitrogen (N) for four litter treatments, DAL = *Dalbergia retusa,* TEC = *Tectona grandis* and TERM = *Terminalia amazonia*, placed in three monoculture plantations used in a litter decomposition experiment in the Agua Salud project in Panama. Means and ± standard errors are shown for n = 5 soil samples for each litter treatment; different lower-case superscript letters indicate significant differences among plantations at p <0.05.

Plantation	Litter Treatment	Microbial Biomass C (µg C/g dry weight soil)	Microbial Biomass N (µg N/g dry weight soil)
Tectona grandis[a]	bare soil	107.12 (± 7.34)	74.70 (± 4.82)
	TERM	116.29 (± 10.51)	82.5 (± 6.58)
	DAL	109.51 (± 4.42)	80.86 ± 2.75)
	TEC	109.47 (± 7.51)	82.95 ± 3.94)
Dalbergia retusa[b]	bare soil	282.42 (± 91.01)	63.34 (± 9.60)
	TERM	289.92 (± 72.98)	68.48 (± 7.26)
	DAL	302.41 (± 81.36)	68.16 (± 7.30)
	TEC	251.07 (± 69.16)	64.56 (± 14.83)
Terminalia amazonia[b]	bare soil	422.77 (± 53.55)	57.03 (± 3.55)
	TERM	324.88 (± 59.00)	38.83 (± 7.15)
	DAL	309.45 (± 95.63)	41.94 (± 11.01)
	TEC	249.79 (± 54.62)	37.40 (± 7.38)

After six months (three months of dry season and three months of wet season), DAL litter had the highest decay rate regardless of plantation and the TERM litter tended to have a lower decay rate, but all single-species litters decayed more slowly in the *Terminalia* plantation (Figure 5.5). Litter treatment ($F_{(2,32)}$ = 5.75, $p < 0.05$) and plantation ($F_{(2,32)}$ = 10.48, $p < 0.05$) both had a significant effect on litter decay rates.

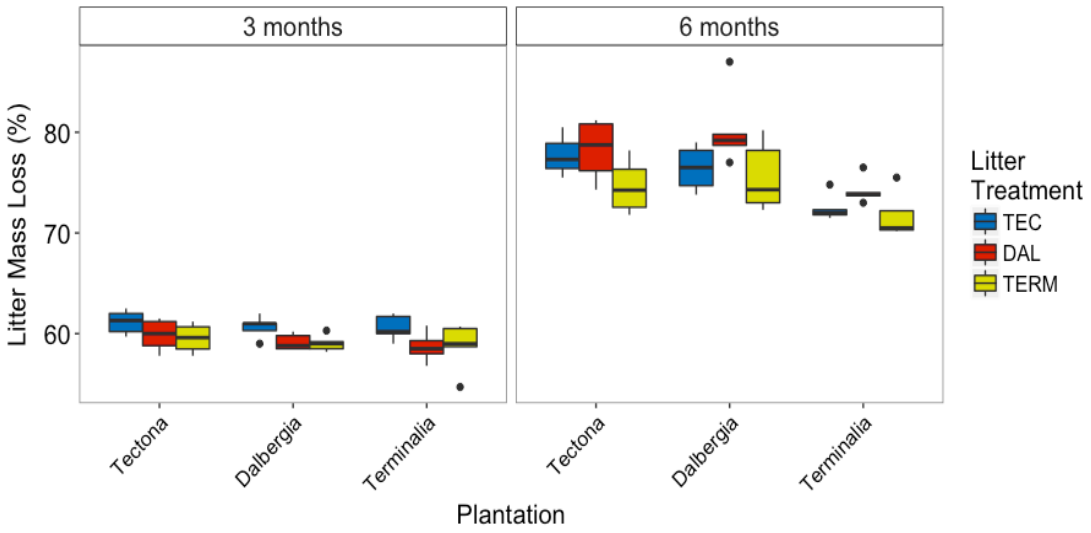


Figure 5.7 Litter mass loss in a litter decomposition transplant experiment in monoculture plantations of *Tectona. Grandis Dalbergia retusa* and *Terminalia amazonia* during a litter decomposition experiment in the Agua Salud project in Panama. The measurements were taken for each litter type, *Tectona. Grandis* (TEC, blue), *Dalbergia retusa* (DAL, red) and *Terminalia amazonia* (TERM, yellow) at each plantation (n = 5) after three months (April 2017) and six months (July 2017) of decomposing in the field.

A principal components analysis of the nutrients released from the decomposing litter using resin probes showed no separation of the different litter treatments or the plantations. Total N was highest in the *Tectona* plantation ($F_{(2,18)}$ = 8.20, $p < 0.05$); nitrate concentrations were below the detection limit of the instrument for all the litters in the *Terminalia* plantation, but the DAL litter had higher nitrate concentrations compared to TEC and TERM litter in the *Tectona* plantation ($F(2,8) = 12.75$, $p < 0.05$).

Table 5.4 Nutrient supply rate from resin probes placed between the soil and the leaf litter, *Tectona grandis* (TEC), *Dalbergia retusa* (DAL) and *Terminalia amazonia* (TERM), inside mesocosms for three monoculture plantations used in a litter decomposition experiment in the Agua Salud project in Panama. Showing total nitrogen (Total N), nitrate (NO_3), ammonium (NH_4), phosphorus (P)n potassium (K), calcium (Ca), and magnesium (Mg). Means and ± standard errors are shown for n = 3; different lower-case superscript letters indicate significant differences among plantations at $p < 0.1$, different upper-case superscript letters indicate differences between litter treatments $p < 0.01$ and an asterisk indicates significant differences for the interaction of plot and litter; b.d. indicates measurements below instrument detection limits.

		Total N	NO_3	NH_4 *	P	K	Ca	Mg [a]
Litter	**Plantation**	**(g 10 cm^{-2} 28 days^{-1})**						
TEC	Tectona	23.77 (±7.65)^{a}	9.69 (±3.65)^{B}	14.08 (±4.94	3.07 (±1.12)	83.93 (±12.66)	196.89 (±59.08)^{b}	73.41 (±19.50)^{b}
	Dalbergia	14.68 (±2.84)^{b}	4.73 (±2.22)^{B}	9.9 (±3.49)	3.98 (±2.76)	173.45 (±27.76)	127.01 (±17.19)^{b}	65.32 (±17.13)^{a}
	Terminalia	9.04 (±3.17)^{b}	b.d.	7.55 (±3.34)	1.70 (±0.74)	77.89 (±32.43)	155.91 (±53.99)^{a}	64.03 (±34.71)^{b}
DAL	Tectona	50.69 (±24.83)^{a}	44.62 (±22.96)^{A}	6.07 (±3.03)	1.11 (±0.22)	53.12 (±13.04)	87.75 (±7.79)^{b}	26.55 (±0.87)^{b}
	Dalbergia	8.65 (±0.04 ^{b}	2.31 (±0.35)^{A}	6.34 (±0.78)	1.22 (±0.17)	86.19 (±18.56)	88.13 (±26.56)^{b}	34.64 (±9.16)^{a}
	Terminalia	12.45 (±6.50)^{b}	b.d	17.45 (±7.93)	1.10 (±0.17)	86.76 (±26.57)	304.35 (±117.69)^{a}	122.56 (±51.16)^{a}
TERM	Tectona	39.77 (±8.55)^{a}	18.67 (±5.67)^{B}	21.11 (± 4.63)	1.66 (±0.35)	117.62 (±29.43)	239.57 (±69.73)^{b}	132.56 (±68.41)^{b}
	Dalbergia	10.73 (±2.24)^{b}	2.91 (±0.98)^{B}	7.81 (±1.62)	1.96 (±0.75)	135.28 (±28.08)	123.25 (±14.40)^{b}	46.09 (± 6.84)^{a}
	Terminalia	8.80 (±1.91)^{b}	b.d.	6.14 (±2.52)	1.83 (±0.22)	150.49 (±67.06)	380.40 (±104.97)^{a}	165.48 (±67.06)^{b}

5.4.3 Homefield Advantage

Although there was no evidence of HFA for mass loss (HFA was not significantly different from zero) after three and six months, there was evidence for HFA of soil respiration. Respiration in mesocosms with TERM litter was significantly lower in the home plantation after both three ($t_{(df = 4)} = -3.66$, $p < 0.05$) and six months ($t_{(df = 4)} = -4.15$, $p < 0.05$) and respiration in mesocosms with TEC litter was lower in the home plantation after six months ($t_{(df = 4)} = -4.12$, $p < 0.05$). Hence, there was a homefield disadvantage for TERM and TEC litter because soil respiration was lower in the home environment.

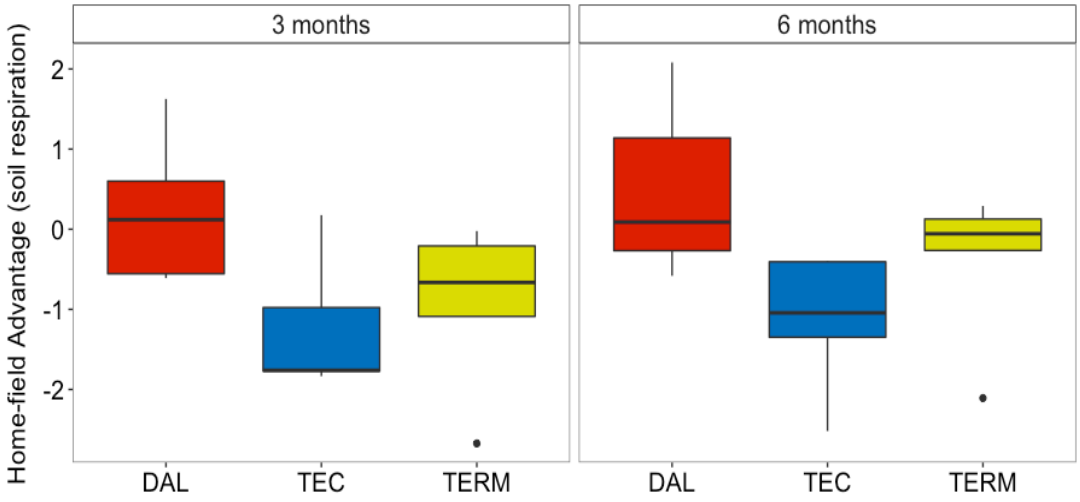


Figure 5.8 Homefield advantage of the mean of three (left) and six (right) months of soil respiration in a litter decomposition transplant experiment in monoculture plantations of *Tectona grandis* (tec) *Dalbergia retusa* (dal) and *Terminalia amazonia* (ter) during a litter decomposition experiment in the Agua Salud project in Panama. Home field advantage was calculated for each litter type, *Tectona. Grandis* (TEC, blue), *Dalbergia retusa* (DAL, red) and *Terminalia amazonia* (TERM, yellow) at each plantation (n = 5).

5.4.4 Non-Additive effects of species mixtures

There was no significant facilitative or antagonistic effect of mixed TEC+TERM, DAL+TERM or TEC+DAL+TERM litters on litter mass loss or soil respiration. However, after three months of decomposition in the field, the observed mass loss of the TEC+DAL mixed litter was lower than expected from the mean of the constituent single-species litterbags in all the plantations ($F_{(1,\ 45)}$ = 11.24, $p < 0.05$). After six months, the decay rate was lower for the mixed species litterbags in the *Dalbergia* plantation only (Figure 5.9) but there were no significant differences between observed and expected nutrient concentrations measured by resin probes for the TEC+DAL litter mixture.

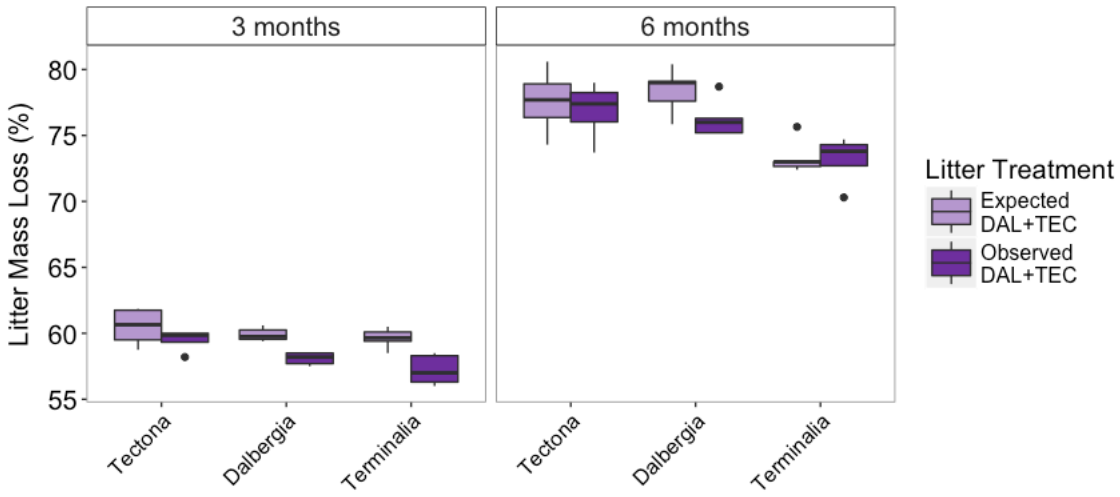


Figure 5.9 Litter mass loss from the mean of two single species (*Tectona grandis* and *Dalbergia retusa*, "Expected" in light purple) and of the mixed litterbag of the same species ("Observed" in dark purple) after three (left) and six (right) months in a litter decomposition transplant experiment in monoculture plantations of *Tectona grandis Dalbergia retusa* and *Terminalia amazonia* during a litter decomposition experiment in the Agua Salud project in Panama (n = 5).

Although decomposition of the TEC+DAL litter mixture was lower than expected, observed respiration from the TEC+DAL mesocosms was slightly but consistently higher than expected rates, but only in the *Tectona* plantation. Hence, the model that best explained the non-additive effect of litter mixtures on soil respiration included plantation and litter type as well as their interaction ($\chi^2 = 62.103$, $p < 0.05$, Figure 5.10).

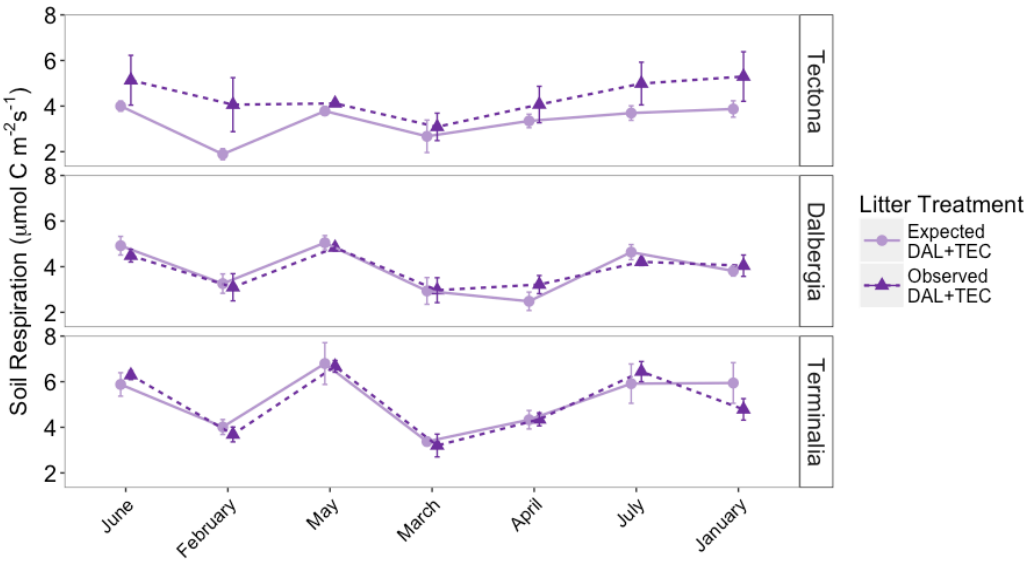


Figure 5.10 Soil respiration from the mean of two single species (*Tectona grandis* and *Dalbergia retusa*, "Expected" in light purple and of the mixed litterbag of the same species ("Observed" in dark purple) in a litter decomposition transplant experiment in monoculture plantations of *Dalbergia retusa, Tectona grandis* and *Terminalia amazonia* during a litter decomposition experiment in the Agua Salud project in Panama. The measurements were taken for each mesocosm (n = 10 per plantation from January until April 2017 and n = 5 per plantation from May until July 2017) every month for 7 months.

5.5 Discussion

5.5.1 Site differences?

Plantation type had a strong influence on rates of decomposition and soil respiration in this study. This can be partly attributed to differences in canopy cover, which resulted in a distinct microclimate at the soil surface among plantations. In particular, the *Terminalia* plantation had a closed canopy (Mayoral et al., 2017), which maintained a lower soil temperature and higher levels of soil moisture during the dry season (Figure 5.5). In addition, litter cover buffers temperature and moisture fluctuations on the forest floor (Sayer, 2006) and the litter standing crop was greater in the *Terminalia* plantation than in the *Tectona* or *Dalbergia* plantations. Hence, the microclimate at the forest floor is likely to have influenced litter decay rates, but there were also important differences in soil properties among plantations.

Although the *Tectona* and *Terminalia* plantations had similar soil nutrient content and pH, soil microbial biomass C was significantly lower in *Tectona* compared to the other plantations, whereas the *Terminalia* plantations had the highest microbial biomass C and soil respiration rates. *Tectona* trees are known to have allelopathic properties, releasing chemical compounds that restrict the growth of other plants and microbial communities, which could explain the lower microbial biomass (Hawkes et al., 2006; Jung et al., 2012; Leela and Arumugam, 2014) and soil respiration in the *Tectona* plantation (Figure 5.6). Furthermore, as extraradical mycorrhizal mycelium can comprise up to one third of the microbial biomass and *Tectona* is not native to Panama, it is possible that its symbiotic mycorrhizal fungi might not be present (Ajeesh et al., 2017). Hence, the allelopathic properties of the *Tectona* may have restricted native microbial communities, which were not replaced by new ones (Hawkes et al., 2006; Högberg and Högberg, 2002).

Litter decomposition was slowest in the *Terminalia* plantation (Figure 5.7), regardless of litter type; this is surprising, because high microbial biomass C (Table 5.3) and soil respiration rates (Figure 5.6) could suggest greater microbial activity. However, I found no correlation between litter decomposition and soil respiration because I removed the litter from the mesocosms before taking respiration measurements. It is therefore likely that the high respiration rates in the *Terminalia* plantations were largely root-rhizosphere respiration (Binkley et al., 2006; King et al., 2001; Trumbore et al., 1995; Yi et al., 2007). The rapid growth of *Terminalia* would explain higher rates of root-rhizosphere respiration, but it is nonetheless intriguing that decomposition rates at the plantation were low, because the microclimate was more favourable to decomposition during the dry season.

5.5.2 Leaf properties explained some variation in litter decomposition

Litter quality is often defined in terms of the nutrient and structural C content of the leaves (Laird-Hopkins et al., 2017). In my study, DAL litter had the lowest L:N ratio, which is often related to rapid decomposition (Bréchet et al., 2017) and I therefore expected to measure the most rapid mass loss in DAL litter. However, the greatest

mass loss during the first three months of the experiment was observed in TEC litter (Figure 5.7). It is possible that this could be a methodological artefact. *Tectona* leaves are large and had to be cut for use in the litterbags and mesocosms, whereas the leaves of the other two species were much smaller and did not receive the same amount of damage from chopping. Cutting leaves is a method used to simulate herbivory and has been found to affect leaf functional traits (Hjältén, 2008). By contrast, the lower rates of decomposition of the TER litter is likely explained by the greater leaf toughness of mature *Terminalia* leaves compared to mature *Dalbergia* leaves measured at the Agua Salud project (Paul et al., 2012; Pérez-Harguindeguy et al., 2000).

As expected, after six months of decomposition, the DAL litter had the highest decay rate (Figure 5.7). The slower decomposition rates at three months could be explained by dry season conditions and the limited access invertebrates had into the mesocosms. Initial decomposition by invertebrate decomposers can be important to start the decomposition process by damaging intact leaves and making more complex C compounds available (Hattenschwiler et al., 2010; Wardle et al., 2002). Another study carried out at the Agua Salud project (Paul et al., 2012) found that the *Dalbergia* leaves had the lowest herbivory rates of all the species planted in the experiment and therefore reduced comminution of DAL litter by invertebrates could have slowed initial decomposition. The stoichiometric needs of the soil microbes to decompose litter may also have played a role in the initial stages of decomposition. *Dalbergia* is an N-fixing species and had the highest soil and litter N content of the study species. High N concentrations can result in P-limitation of decomposition (Hobbie and Vitousek, 2000) and hence, decomposition could have been hindered by the low concentrations of P and high concentrations of N in the *Dalbergia* plantations and litter (Table 5.1 and Table 5.2). This is supported by the results of the resin probes, showing lower release of P from the DAL litter (Table 5.4), which could indicate immobilisation of P in the N-rich DAL litter.

5.5.3 Homefield advantage and homeland security

I expected the two native species litters to have a greater homefield advantage than the TEC litter because the microbial decomposer communities would be better adapted to litter from native trees species (Ayres et al., 2009). Instead, I measured a negative HFA (home-field disadvantage) for the TEC litter, which could be explained by the lower microbial biomass in the *Tectona* plantation and by the allopathic compounds in *Tectona* leaves (Homeland security theory; Cummings et al., 2013).

It should also be noted that there was a trend towards a positive HFA for both decomposition and soil respiration for the DAL litter (Figure **5.8**), although the result was not statistically significant. This suggests that there may be a specialisation of microbial and/or invertebrate communities in the *Dalbergia* plantations; especially as the soil properties from the *Dalbergia* plantation differed from the other two plantations (Figure 5.4) and arthropods are essential to litter decomposition and have been known to specialise to specific litter (Hättenschwiler and Gasser, 2005; Laird-Hopkins et al., 2017).

5.5.4 Additive and antagonistic effects of litter mixtures

Given the relatively minor differences in litter nutrient and fibre content (Table 5.2), the lack of strong non-additive effects is perhaps not surprising. I found no facilitative or antagonistic effects for the DAL + TERM, the TEC + TERM, or the three species mixtures, which is likely due to the high decay rates of all three litters (Gartner and Cardon, 2004; Hattenschwiler and Gasser, 2005). Nonetheless, the antagonistic effects on mass loss of the DAL+TEC litter mixture in the *Dalbergia* plantations is likely explained by the high resistance to herbivory of the *Dalbergia* leaves and the release of allelopathic compounds from TEC leaves (Leela and Arumugam, 2014). This could also explain why the antagonistic effect was strongest in the early stages of decay. However, it is noteworthy that the non-additive effect of the TEC+DAL litter mixture on soil respiration was positive in the *Tectona* plantation, which could indicate that leachates from the high-quality DAL litter stimulated microbial activity in the underlying soil. It would seem that there is a mismatch between the decay processes in the litter and the microbial activity in the underlying mineral soil. It is likely that the

microbial activity is more affected by the litter leachate rather than the litter itself; although the resin probes did not reveal differences in nutrient release that could explain these results, they were installed during the rainy season after four months, and it is therefore likely they did not capture the first flush of nutrients from the freshly decomposing litter (Joly et al., 2016).

5.6 Conclusion

The decomposition of litter and associated microbial activity are influenced by a multitude of different factors, including microclimate, litter traits, and soil properties. My study demonstrates that phenomena such as non-additive effects and the home-field advantage of decomposition may be much more complex in tropical forests compared to temperate regions because the soils and microbial decomposer communities are adapted to very high species diversity, which could mitigate facilitative or antagonistic effects of different litter types and mixtures, even in monoculture plantations. It is nonetheless striking that some of the largest differences were found in the *Tectona* plantations and for TEC litter, which indicates that microbial processes are being modified by this non-native species. It is further noteworthy that although the different litter types had a variable influence on soil respiration rates, there was no clear link between decomposition rates and soil microbial activity stimulated by litter leachates. Further research should focus on identifying how different tree species influence soil microbial community composition via litter leachates and whether non-additive effects and the home-field advantage of litter decomposition can be better detected with greater differences in litter traits and tree functional types.

CHAPTER 6 GENERAL DISCUSSION

Understanding the effect of forest modification on soil carbon (C) in the tropics is vital if we are to remediate anthropogenic actions such as land degradation and climate change. However, it is often difficult to study the multitude of processes involved in plant-soil interactions at the ecosystem scale due to many factors including experiment length, cost and, in the case of tropical forests, accessibility to sites. The overarching aim of this thesis was to explore the effect of forest modification in tropical ecosystems by investigating soil C dynamics and plant-soil interactions using litter transplant experiments. This chapter summarises and discusses the key findings of this thesis, their implications, and conclusions for future work.

6.1 Summary

In Chapter 3, I focused on the effects of forest degradation due to the expansion of oil palm cultivation in Sabah Borneo. I assessed soil C dynamics in logged forests, forest fragments and oil palm plantations as they are likely to be the dominant land cover types in the future (Global Forest Watch, 2018) I found that decomposition processes and microbial activity were similar in both forest habitats but were greatly reduced in the oil palm plantations due to changes in microclimate, reduced litter diversity, and the lack of litter inputs from the palm trees, which were the main source of organic matter. This suggests that diverse litter inputs are essential to maintaining the processes underpinning soil C and nutrient cycling. However, even though the loss of key species in human-modified forests has not influenced overall decay rates of litter, this does not necessarily mean that these degraded forests retain the same soil C turnover capabilities as an old-growth forest and further work is required to establish whether there is a threshold of diversity loss that compromises soil function. Although the remote location of the study sites and the difference in soil type of the nearest primary forest precluded a direct comparison between degraded and old-growth forest, I was able to incorporate this aspect my subsequent study in Panama, Central America. Greater sustainability of oil palm management could be achieved by adding

organic residue to improve soil function. Although this is already recommended by the Roundtable for Sustainable Palm Oil (RSPO), the measure was not applied in the plantations included in my study. Intercropping and the application of oil palm fruit bunches between the palms have already shown improvement in soil function and biodiversity at other sites in Southeast Asia (Ashraf et al., 2018; Tao et al., 2018, 2017).

In Chapter 4, I assessed the effects of forest modification on soil C dynamics along a gradient of forest regeneration in the Barro Colorado Nature Monument (BCNM) in Panama, comparing a managed forest clearing to a regenerating secondary forest and an old-growth forest using the same experimental setup as Chapter 3. My key finding from this study was that the soil in the secondary forest had similar soil respiration and decomposition rates to the old-growth forest, which suggests that soil ecosystem function in the secondary forest has been largely restored with the increase in tree cover and diversity. Both forested habitats had similar surface soil C content and microbial biomass, which were both greater than in the deforested habitat. My measurements of decomposition and soil respiration further indicated that microbial activity in the secondary forest had recovered to levels equivalent with old-growth forest after 40 years of regeneration. Future work focussing on the influence of changing plant species composition during the early stages of secondary succession could therefore provide valuable information for the targeted restoration of soil function after land abandonment.

The parallel experiments along forest disturbance gradients in Chapters 3 and 4 also allow me to compare and contrast changes in ecosystem function with forest modification in the old-world (Borneo) and neo-tropics (BCNM). My intention in conducting studies on both continents was to establish whether there are general patterns in tropical forest ecosystem responses to disturbance. The local climate differed slightly over the duration of the experiments. Borneo had a higher mean soil water content (29.6 ± 0.29 %) than BCNM (22.16 ± 0.44) but soil temperature was higher in BCNM (26.4 ±0.02 °C) than in Borneo (24.16 ±0.04 °C), which will have some influence on the comparison of soil respiration and decomposition rates. However, the most notable difference between the sites were in the in-soil properties; the forest habitats in BCNM had more than double the soil C content compared to the forest

habitats in Borneo and the soils in Panama also had higher N and K concentrations compared to Borneo. The differences in soil properties could explain why microbial biomass C and soil respiration in the BCNM forest habitats were twice those in Borneo, as soil organic matter content is important for sustaining microbial biomass and activity (Xu et al. 2018). This is of particular interest because soil respiration was positively correlated to litter decay in both countries. Mass loss after nine months was greater in BCNM (with 80 %) than in Borneo (60%), which emphasizes the links between soil C content, microbial biomass and C turnover in tropical forests. In both experiments, the modified habitat, which had the lowest litter input and plant diversity, had lower microbial biomass C concentration and soil respiration than their forested counterparts. Hence, regardless of geographical location and initial soil C content, a reduction in litter inputs from management practices caused a similar decrease in microbial activity. However, whereas microbial biomass in the deforested habitat in BCNM increased substantially in response to litter inputs, there was no corresponding increase in the oil palm plantation in Borneo, which could suggest that the management practices of oil palm monocultures, particularly the removal of palm fronds and herbaceous vegetation, have greatly reduced soil function and recovery capacity. Therefore, litter inputs are essential to preserve soil function and forest restoration can be beneficial to the recovery of soil C cycling.

Having established the importance of diverse litter inputs for key soil processes by comparing natural forest litter and single-species introduced litter, I set up a targeted study to assess the effects of litter mixtures in more detail. In Chapter 5, I focused on the effects of specific tree species and their foliar traits to determine how they influence soil C dynamics. I tested how litter mixtures and litter origin (home vs. away) affect decomposition processes and microbial activity. To assess how plant functional traits, influence microbial activity, I compared the introduced timber tree species *Tectona grandis* to two native species that are also used for commercial timber: the slow-growing legume, *Dalbergia retusa,* which has the potential to fix nitrogen, and the fast-growing species *Terminalia amazonia.* I predicted that the difference in growth rates and functional types would translate to differences in leaf and litter properties, which would in turn affect the rates of decomposition and soil respiration.

Although I found clear evidence for the influence of measured leaf properties on litter decomposition and soil respiration, the full picture was much more complex. Other factors such as the amount of herbivory and the release of allelopathic chemicals also potentially influenced microbial activity and litter decomposition, such that the results from the tests of litter mixtures and litter origin were highly variable. Overall, there was no clear decline in soil function in any of the monocultures, which suggests that tropical forest soils are more resilient than expected and forest restoration projects using native tree species would be beneficial for maintaining or restoring soil processes. My results also demonstrated that further work is needed to assess the potential influence of arthropod activity on decomposition, as forest degradation and forest management can influence the diversity of invertebrates (Laird-Hopkins et al., 2017; Sayer et al., 2010), which is likely to have knock-on effects of decomposition and soil C dynamics (Powers et al. 2014). The potential influence of herbivory on litter decomposition, e.g. via grass inputs or foliar damage, presents an intriguing new line of inquiry.

6.2 Limitations and Opportunities

Doing research in tropical forests often involves remote sites and limited access to fully equipped soil biology laboratories compared to e.g. doing research in the UK. During the course of my work, the assistance of collaborators was essential in procuring materials and chemicals that were needed for my research. However, the reliance on project partners and external providers while working in a remote location also had implications for my work, for example when I was unable to carry out analyses due to long delays in sample transport, lab access, and shipping equipment to countries such as Panama or Borneo. Although these difficulties reduced the number of measurements and analyses, I was able to carry out, the simplicity of the mesocosm experiment design helped me overcome other potential limitations and allowed me to conduct robust experiments under challenging conditions.

Mesocosms are an efficient and cost-effective method for carrying out experiments in the field while minimising possible disturbances to the environment that often occur in large-scale experiments (Ayres et al., 2009; Laird-Hopkins et al., 2017; Szanser et al.,

2011). Limiting disturbance was essential in the undertaking of the studies presented in this thesis, as some of my work was carried out in environments in which minimal disturbances were required. In the Agua Salud project, I had to ensure that my work would not affect other experiments and studies taking place at the same site, and the use of the mesocosms allowed me to do this. Furthermore, carrying the PVC pipes to remote sites did not require any special transport or equipment which was very advantageous for setting up plots that were hard to access. Importantly, the mesocosms also allowed me to assess the links between litter decomposition and soil respiration, which can both be used as a measure of soil microbial activity.

Using mesocosms for litter transplant experiments reduces the amount of litter required for the experimental treatments. It is nonetheless important to add sufficient litter that the effects of the litter treatments can be distinguished from the influence of the underlying mineral soil, especially in studies involving soil CO_2 efflux measurements. Based on my personal observations in the field, such experiments would ideally incorporate sufficient litterfall at the start of the study to represent the surrounding litter standing crop. In the first two experiments in Borneo and Panama (Chapters 3 and 4) I used 16 g of litter, which was equivalent to the existing litter standing crop, and took soil respiration measurements over the litter. In the third experiment in Agua Salud (Chapter 5), I was unable to collect sufficient single-species litter and I therefore only initially added 6 g of litter to the mesocosms, which was half the weight of the mean standing crop which could explain why I found no clear influence of the litter treatments on soil respiration.

Overall, the mesocosms represented a viable option to apply experimental treatments in remote locations in the field with a high degree of control. The mesocosms can also be used to pursue other lines of enquiry identified from my findings, such as using targeted arthropod exclusions to determine the importance of arthropod diversity for decomposition (Laird-Hopkins et al. 2017) and soil respiration or applying experimental grass additions or leaf damage treatments to assess the influence of herbivory.

6.3 Conclusion

Human-modified tropical forests remain important ecosystems for C storage and their protection and responsible management is essential for climate change mitigation. A diverse and rich source of organic matter is essential for conserving soil C and should be taken into account for sustainable management of commercial plantations. For example, the addition of organic residues from oil palm or intercropping with another cash crop. The work presented in this thesis demonstrates the complexity of assessing the role of plant-soil interactions in highly diverse tropical forests, where hundreds of plant species can influence soil properties and processes within a few hectares. Whereas reduced plant diversity in degraded or regenerating forests had no clear impact upon the soil processes I investigated, diversity loss and litter inputs in commercial monocultures affected soil function to varying degrees, and the consequences for soil C storage will depend on the species being cultivated and the management of the site. My findings highlight the need for further research on the linkages among tree species, arthropod activity, and microbial communities to understand the full impact of forest modification on soil function, and to assess management options for sustainable tropical plantations. Plant-soil interactions are an essential and complex part of the forest C cycle and there is much work still to be done to fully understand the intricacies of interactions between above- and belowground communities in tropical forests.

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