Linking tree community functional change with soil carbon dynamics during secondary succession in a naturally regenerating tropical forest in Panama

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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, Lindsay Banin, Daisy Dent and Ute Skiba

This thesis is 40974 words and therefore does not exceed the permitted maximum

Abstract

Naturally regenerating tropical forests are increasingly important for their role in the global carbon (C) balance, particularly due to their ability to rapidly sequester large amounts of C in aboveground biomass during forest regrowth. Over half of all C in tropical forests is stored belowground, yet in contrast to the predictable pattern aboveground, there is no clear pattern of soil C accumulation with time during forest regrowth, and we are therefore currently unable to predict and increase soil C sequestration during tropical forest regrowth. Soil C turnover and storage depends on the input of plant-derived organic matter which is likely to be affected by shifts in tree community resource-use strategy (functional group) during secondary succession from light-demanding to shade-tolerant species, and the corresponding reduction in litter quality. As tree community composition can differ between forest stands of similar ages, I hypothesised that tree community functional composition would better predict soil C dynamics during secondary tropical forest succession than stand age and specifically, that differences in litter quality between shade-tolerant and light-demanding tree species would influence rates of soil C turnover via litter decay rates and changes to the soil microbial community. The body of work presented in this thesis provides compelling evidence in support of my overarching hypothesis that soil C accumulation is more closely related to tree functional composition than forest age. My studies highlight some of the potential pathways by which tree community composition can influence soil C storage via plant-derived organic matter inputs representing substrate for the soil microbial community. Overall, the research presented in this thesis demonstrates that tree functional composition could be one of the main factors determining belowground C storage and therefore, my work represents an important first step towards using tree functional groups to predict soil C accumulation in secondary tropical forests.

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1 Introduction

1.1 The new normal - regenerating tropical forests

The world's natural ecosystems are changing. Human activity has radically altered the natural environment to such an extent that undisturbed 'pristine' or 'intact' ecosystems are becoming a rarity and human-modified landscapes, in a myriad of conditions now dominate much of the planet (Foley *et al.*, 2005). As the human population and patterns of consumption continues to expand, and the drivers of environmental change persist, human modified landscapes will become increasingly important as the primary providers of global ecosystem services (ESS). Tropical forests in particular are being modified by human activities at an alarming rate (Chazdon and Guariguata, 2016; FAO, 2018).

To understand how changes to natural ecosystems might affect the cycling of matter and energy and the wider implications of this, there is a pressing need to advance scientific knowledge on ecosystem functioning in a changing world. Global carbon (C) cycling and its role in climate change, is high on the agenda as a research priority, most notably in the globally extensive and rapidly changing tropical forest ecosystems (FAO, 2018). A comprehensive, mechanistic understanding of C cycling and storage as tropical forests undergo change is essential to improve model predictions (Box 1), inform policies and practices to halt the release of carbon dioxide (CO_2) and help mitigate the effects of climate change through C sequestration.

Box 1. Emissions from land use change (ELUC) represent one of the key areas of uncertainty in the global C budget, which is currently estimated at 1.5 ±0.7 Gt C yr^{-1} (Friedlingstein *et al.*, 2019), and a significant of this is occurring in the tropics. ELUC uses land-use and land-use change data, bookkeeping models and dynamic global vegetation models (DGVMs) to estimate the net CO_2 flux (emissions and removals) from land use, land use change, and forestry, which includes deforestation, afforestation, logging and forest degradation, shifting cultivation, and regrowth of forests following wood harvest or abandonment of agriculture (Friedlingstein *et al.*, 2019). The high uncertainty for ELUC is due to a range of factors including inconsistencies between the bookkeeping models and the DGVMs, difficulties in quantifying some of the processes in DGVMs, and uncertainties in above- and belowground C stocks and fluxes (Friedlingstein *et al.*, 2019). Therefore, improving our understanding of C fluxes between tropical forests and the atmosphere during land-use change is essential in order to reduce uncertainty and improve global C models which in turn can better inform policies and practices to halt the release of CO_2 and help mitigate the effects of climate change.

1.2 Growing importance of secondary tropical forests for global carbon balance

Although tropical forests occupy a small fraction of total land surface, they play a leading role in helping maintain life on Earth: they are estimated to support around half of all species and are key players in climate regulation, most notably for their crucial contribution to global terrestrial C storage and dynamics (Pan *et al.*, 2011; Townsend *et al.*, 2011; Martin, Bullock and Newton, 2013; Anderson-Teixeira *et al.*, 2016). Often described as the 'lungs of the planet', tropical forests function as the largest component of the terrestrial C sink, as they are responsible for around *c*. 40% of net primary production (NPP; Cleveland *et al.*, 2011) and store *c*. 45% of terrestrial C (Anderson-Teixeira *et al.*, 2016), sequestering around half of global anthropogenic CO_2 emissions (Yin, Wu and Li, 2018). They are also the largest natural source of CO_2 (Sayer *et al.*, 2011) returning C to the atmosphere via the decomposition or burning of organic matter. Hence, tropical forests play a key role in the global C cycle via major bidirectional transfer of CO_2 with the atmosphere, and until recently, tropical forest ecosystems represented the most important terrestrial C sink.

Over the last two decades, human activities have turned tropical forest regions from a C sink into a source of CO_2 emissions (Malhi, 2010). Deforestation and land-use change such as logging and agricultural expansion continues to decimate vast areas of intact tropical forest annually

(Chazdon and Guariguata, 2016; FAO, 2018), which not only has devastating consequences for biodiversity (Barlow *et al.*, 2016) but has also led to growing uncertainties regarding the C balance of tropical forests. Tropical deforestation and land-use change is the second largest source of anthropogenic greenhouse gas emissions after fossil fuel use (FAO, 2018) with an estimated 2.6 Gt CO_2 emitted between 2010-2014 due to the expansion of croplands, pastures and forestry plantations (Pendrill *et al.*, 2019). Furthermore, recent research suggests that the ability of remaining intact tropical forests to sequester C is in decline (Hubau *et al.*, 2020), while CO_2 release from microbial activity is increasing (Bond-lamberty *et al.*, 2018), raising the concern that tropical forests may now be functioning as a net C source rather than a sink (Baccini *et al.*, 2017).

As a consequence of wide-spread tropical deforestation and land-use change, over half of all remaining tropical forest is now classed as secondary, degraded or regenerating forest (FAO, 2015; Poorter *et al.*, 2016). Agricultural abandonment, as part the common practice of swidden agriculture, along with recovery from selective logging, has created an extensive mosaic of naturally regenerating 'secondary' or 'regrowth' forest throughout the tropics, which is expected to expand in the future (Chazdon, 2014). As such, secondary regrowth forests have received growing attention and are increasingly looked towards as critical providers of tropical forest ecosystem services for both biodiversity conservation (Chazdon *et al.*, 2009) and climate regulation (Poorter *et al.*, 2016).

Naturally regenerating tropical forests can rapidly accumulate atmospheric CO_2 in aboveground biomass (Pan *et al.*, 2011) which is estimated to reach 90% of intact forests after *c.* 66 years with an average biomass recovery after 20 years of 122 megagrams per hectare (Poorter *et al.*, 2016). The net uptake of C by secondary regrowth forests (3.05 Mg C y^{-1}) is 11 times that of old-growth (Poorter *et al.*, 2016). As such, natural regeneration of tropical forests is widely considered to be an effective low-cost mechanism to sequester large amounts of CO_2 from the atmosphere (Pan *et al.*, 2011; Chazdon, 2016), and therefore represents a significant climate change mitigation strategy (Chazdon and Guariguata, 2016; Schwartz *et al.*, 2017; Lewis *et al.*, 2019; Romijn *et al.*, 2019; Mackey *et al.*, 2020).

The C sequestration potential of naturally regenerating tropical forests can be an important motivating factor to reach national targets for forest restoration (Poorter *et al.*, 2016) for example though initiatives such as The Bonn Challenge (https://www.bonnchallenge.org), and Initiative 20 × 20 (https://initiative20x20.org). To date, nearly 300 Mha of degraded land has

been committed for forest restoration in the tropics and subtropics through local and global initiatives (Lewis *et al.*, 2019). However, the majority of this area has been earmarked for commercial plantations, which support local economies but are estimated to sequester around 40 times less C than natural forests (Lewis *et al.*, 2019). Along with the protection of remaining old growth forests in the fight against rising levels of atmospheric CO_2 (Pan *et al.*, 2011; Mackey *et al.*, 2020), these findings further emphasize the significant potential of naturally regenerating tropical forests as a whole. This highlights the need for reliable predictions of C accumulation during forest regrowth to encourage greater inclusion of naturally regenerating forest in restoration pledges and policies and for modelling future C and climate change scenarios.

Whilst evidence of rapid tree regrowth following disturbance highlights the huge C sequestration potential of naturally regenerating tropical forests aboveground, we know relatively little about the changes, interactions and processes involved in the storage and cycling of C in tropical forest soils during secondary forest regrowth (Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013).

Soil carbon dynamics in secondary tropical forests

Soils make up the largest C pool in the terrestrial biosphere (Yang, Luo and Finzi, 2011) and account for over half of the C stock in tropical forests (Don, Schumacher and Freibauer, 2011). Tropical forest soils are an important part of the global C balance, but our understanding of soil C dynamics during secondary forest regrowth is hampered by the complexity of biogeochemical processes and interactions, the lack of studies in tropical regions, and inconsistent patterns of soil C losses and gains during forest disturbance and recovery (Yang, Luo and Finzi, 2011; Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017).

In contrast to the relationship between forest stand age (years since last disturbance event) and aboveground biomass C accumulation, patterns of C loss and gain in soils during tropical forest regrowth are less clear. For example, several studies, including meta-analyses and syntheses, have reported either a weak relationship, no significant change, or contrasting results for changes in soil C during tropical forest regrowth as a function of forest age (Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017). Additionally, a global synthesis (including studies from other climate zones) concluded that the majority of individual studies showed no significant relationship

between soil C dynamics and forest fallow age (Yang, Luo and Finzi, 2011). These results suggest that forest stand age is of limited use for predicting soil C dynamics in secondary tropical forests and that other factors may better explain patterns in soil C during forest regrowth.

Although the cycling and storage of C in tropical forest soils is partly determined by climate, soil characteristics and land-use history (Marín-Spiotta and Sharma, 2013), changes in tree functional groups and the quality and quantity of plant-derived organic matter during forest regrowth are expected to have a significant impact on soil C dynamics (Laird-Hopkins *et al.*, 2017; Kerdraon *et al.*, 2019). Plant litter decomposition plays a fundamental role in soil C storage (Sayer, 2006; Prescott, 2010; Sayer and Tanner, 2010). During decomposition, C and nutrients are sequentially broken down and made available to plants, beginning with the more labile and soluble compounds within litter (e.g. sugars) and eventually the most recalcitrant forms (e.g. lignin) (Kutsch, Bahn and Heinemeyer, 2009). Plant-derived C compounds are either mineralised and returned to the atmosphere as CO_2 or immobilised in the soil matrix (Kutsch, Bahn and Heinemeyer, 2009). The balance between soil C storage and release during decomposition could eventually determine whether a forest functions as a C source or sink and hence even slight changes affecting the decomposition of organic matter can have a significant effect on C dynamics (Sayer *et al.*, 2011).

Changes in organic matter quality, i.e. dominant C forms and nutrient concentrations, are expected to have important implications for soil C turnover by influencing the abundance, structure, and activity of the soil microbial community. Microbial biomass is a key driver of biogeochemical processes and is strongly linked to substrate availability (Kutsch, Bahn and Heinemeyer, 2009), whereby total microbial biomass tends to increase with increasing soil organic matter content (Yao *et al.*, 2000). Recent research suggests that labile C compounds are used more efficiently and thus stimulate the turnover of microbial biomass, resulting in the production of increasingly stable C compounds (Cotrufo, Wallenstein and Boot, 2013). The formation of stable soil organic matter may also be partially explained by differences in soil microbial structure. For example, the metabolization of easily degradable compounds from nutrient-rich organic matter is generally associated with small-bodied, fast-growing microorganisms termed zymogenous or r-strategists (e.g. Gram-negative bacteria), which have high turnover rates. By contrast, degradation of more complex structural compounds generally found in nutrient-poor organic matter requires the activity of larger, slow-growing microorganisms termed autochtonous or K-strategists (e.g. fungi and Gram-positive bacteria),

which have slower turnover rates (Kutsch, Bahn and Heinemeyer, 2009; Zhou *et al.*, 2017). Hence, soil carbon storage may be largely determined by microbial community composition, which in turn is shaped by the quality of plant litter inputs.

Tree community functional changes during succession and influence on soil carbon dynamics

The quality and quantity of organic matter inputs to the soil is largely determined by the functional composition of the plant community (De Deyn, Cornelissen and Bardgett, 2008), which changes during secondary succession (Chazdon, 2014). In tropical forests, light-demanding tree species that invest in fast growth are characterised as having 'high quality', easily decomposable leaves, which have high nutrient concentrations (e.g. N, P), high specific leaf area (SLA) and low content of structural compounds such as lignin (Wright *et al.*, 2004; Chazdon, 2014). Conversely, shade-tolerant trees preferentially invest in structure, defence and longevity, resulting in leaves characterised as 'low quality', typically with low nutrient concentrations, high fibre and lignin content and greater concentrations of foliar defence compounds such as tannins and phenols (Wright *et al.*, 2004; Ostertag *et al.*, 2008). Hence, changes in the functional composition of tree communities during secondary forest succession could greatly influence the turnover and storage of organic matter in the soil.

Changes in tree species composition and functional groups during secondary succession are largely driven by changes in the availability of resources (light, water, and nutrients) as forests mature. During early succession, high light levels allow fast-growing 'pioneer' species to out-compete slower-growing species to dominate the canopy. However, as light becomes more limited in later succession there is a progression towards a dominance of shade-tolerant species (Dent, DeWalt and Denslow, 2012; Chazdon, 2014; Whitfeld *et al.*, 2014). The shift from light-demanding to shade-tolerant species can also be characterised as a shift in the resource use strategy of the dominant tree species, from 'acquisitive' to 'conservative' along a fast-slow plant economic spectrum (Reich, 2014), which is reflected in a set of coordinated leaf functional traits (Conti and Díaz, 2013) resulting in 'high' and 'low' litter quality respectively (Wright *et al.*, 2004; Ostertag *et al.*, 2008; Chazdon, 2014). Hence, the shift from light-demanding to shade-tolerant tree functional groups during secondary succession and the accompanying reduction in litter quality is expected to influence rates of soil C turnover via changes to litter decomposition rates and microbial respiration.

However, the trajectory of tree community composition can be influenced by a multitude of factors, including soil physicochemical properties and former land-use (Chazdon, 2014) and as such, the functional characteristics of dominant tree species may differ within and among secondary forest stands of the same age (Norden *et al.*, 2015; Boukili and Chazdon, 2017). Consequently, characterising tree communities in secondary tropical forests by functional groups rather than age (or time since last disturbance) may represent a better approach to reveal relationships between above- and belowground C dynamics during seconday succession.

Thesis objectives

The body of work presented in this thesis aims to address some of the challenges involved in predicting soil C accumulation during secondary forest succession by investigating the relationship between tree functional assemblages and soil C dynamics. The present thesis comprises five chapters: this introduction to the thesis (Chapter 1), three original pieces of research (Chapters 2-4) and an overall discussion of the work (Chapter 5).

My studies assessed changes in tree functional groups, soil C, decomposition processes and soil microbial communities along a chronosequence of naturally regenerating moist tropical forest located within Barro Colorado Nature Monument (BCNM) in Panama:

In **Chapter 2** I test the hypothesis that tree functional groups have a stronger influence on soil C than forest age by assessing the relationship between the relative influence of shade-tolerance tree species on soil C (concentrations and stocks) across five forest age classes. I demonstrate that surface soil C content is strongly related to tree community shade-tolerance, suggesting that high-quality plant inputs may play a key role in soil C accumulation during secondary succession.

In **Chapter 3** I test the hypothesis that the shift from light-demanding to shade-tolerant tree species during secondary succession is reflected in changes in C turnover and litter decay rates. I conducted an *in situ* decomposition experiment across an age gradient of naturally regenerating tropical forest, and measured litter decay and soil respiration rates to represent soil C dynamics. I demonstrate that litter from light-demanding species decomposes more rapidly than that of shade-tolerant species and the temporal response of soil respiration rates reflect differences in litter decay rates.

In **Chapter 4** I test the hypothesis that dominant tree functional groups influence the abundance, structure and activity of soil microbial communities. My study assessed the relationships between soil microbial metrics, tree community shade-tolerance, and litter decomposition or soil respiration rates as proxies for soil C turnover across an age gradient of naturally regenerating tropical forest. I demonstrate clear links between tree community shade-tolerance, soil microbial biomass and soil microbial community structure, which influence decomposition and soil respiration.

2 Soil carbon in a regenerating tropical forest is related to tree functional composition, rather than stand age

2.1 Abstract

Regenerating tropical forests are increasingly important for their role in the global carbon (C) budget. Carbon stocks in aboveground biomass can recover to old-growth forest levels within 60-100 years, but more than half of all C in tropical forests is stored belowground, and our understanding of C accumulation in soils during tropical forest recovery is limited. Importantly, soil C accumulation does not necessarily reflect patterns in aboveground biomass C accrual during forest regrowth, as factors related to past land-use, species composition, and soil characteristics may also be important controls of soil C accumulation during tropical forest recovery. In this study, I assessed the relationship between soil C, forest age, and stand characteristics during secondary forest succession across an age-gradient of between 40 to 120Y naturally regenerating secondary forest (SF) and old-growth (OG) tropical forest stands in Panama. Using tree census data and light response classes (a proxy measure of shade tolerance and function) I assessed the relationship between the relative dominance of tree functional groups and soil C accumulation. As expected, soil C decreased with depth in all stands and there was no significant relationship between soil C and increasing stand age and no clear influence of past land-use. However, soil C decreased with increasing stand basal area and there was a strong relationship between tree functional groups and soil C content at 10-cm depth, whereby soil C increased with the increasing relative influence of light-demanding species. The accumulation of belowground C is more strongly linked to tree species composition than forest age or soil characteristics in these forests. The faster turnover of nutrients through the decomposition of generally more nutrient rich organic matter (leaf litter) could result in a greater build-up of C in the surface layer of soils in stands with a higher proportion of light demanding species. These findings help improve our understanding of above-belowground relationships during tropical forest secondary succession and crucially, the C sequestration potential of recovering and restored tropical forest.

2.2 Introduction

Naturally regenerating tropical forests

Tropical forests are critically important ecosystems for the rich biodiversity they support and as key providers of wider ecosystem services, in particular for their crucial contribution to global terrestrial carbon (C) storage and dynamics (Pan *et al.*, 2011; Townsend *et al.*, 2011; Martin, Bullock and Newton, 2013; Anderson-Teixeira *et al.*, 2016). Human activity, such as logging and agricultural practices, continues to decimate vast areas of primary (intact) tropical forest annually (FAO, 2018). Over half of all remaining tropical forest is now classed as secondary, degraded or regenerating (FAO, 2015; Poorter *et al.*, 2016), increasing the global importance of these forests as the dominant provider of key tropical forest ecosystem services (Chazdon, 2014).

Tropical forest regeneration, through natural regrowth, afforestation or restoration activities, can rapidly sequester large amounts of atmospheric carbon dioxide (CO_2) in aboveground biomass. Following the cessation of agricultural practices such as crop production and pasture, naturally regenerating forests generally follow a predictable pattern of aboveground biomass recovery (Powers and Marín-Spiotta, 2017), characterised by rapid accumulation in the early stages of stand development until canopy closure, followed by gradual saturation, and then often a slight decline due to tree mortality in later stages of succession (Yang, Luo and Finzi, 2011; Zhu *et al.*, 2018). For example, in a multi-site chronosequence study in the Neotropics, naturally regenerating tropical forests recovered 90% of old-growth biomass values after *c.* 66 years, with an average biomass recovery after 20 years of 122 megagrams per hectare (equivalent to a net uptake of 3.05 Mg of carbon per year) which is 11 times that of old-growth forests (Poorter *et al.*, 2016). These results reinforce the importance of regenerating tropical forests for their contribution in the global C cycle.

Although evidence of rapid tree regrowth following disturbance highlights the huge C sequestration potential of regenerating tropical forests, estimates suggest as much as 60% of the total tropical forest C stock is stored belowground in soils (Don, Schumacher and Freibauer, 2011). In contrast to our understanding of aboveground C recovery during secondary succession, we know relatively little about the changes, interactions and processes involved in the storage and cycling of C in tropical forest soils during secondary forest regrowth (Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013).

Controls of soil carbon storage and cycling during tropical forest regrowth

Soil C does not follow a predictable pattern of loss and recovery with land-use change in tropical forests. Several meta-analyses and syntheses, which bring evidence together for multiple field studies in the tropics, have reported either a weak relationship, no significant change, or contrasting results for changes in soil C during forest regrowth as a function of forest age (Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017). Similarly, a global synthesis (including studies from other climate zones) concluded that the majority of individual studies showed no significant relationship between soil C dynamics and forest fallow age (Yang, Luo and Finzi, 2011). Multiple factors (and their interactions) affect soil C dynamics during forest recovery, including climate (Marín-Spiotta and Sharma, 2013), soil and vegetation characteristics (Laganière, Angers and Paré, 2010), the extent of the initial disturbance, and the type, duration and intensity of previous land-use (Laganière, Angers and Paré, 2010). The relative influence of many of these factors remain poorly understood (Laganière, Angers and Paré, 2010; Li, Niu and Luo, 2012), particularly in tropical forests, where the astonishing diversity of plant species and potential variability in tree functional change between different chronosequences may mask underlying mechanisms.

Vegetation characteristics during succession

As soil C dynamics are largely driven by input from plant material (from above and belowground biomass and exudates) the changing characteristics of forest vegetation are expected to have an important influence on soil C cycling in regenerating tropical forests (Laird-Hopkins *et al.*, 2017). Although a number of factors can influence aboveground biomass recovery (Johnson *et al.*, 2000) and the successional trajectory of plant communities following human disturbance (Marìn-Spiotta *et al.*, 2008; Arroyo-Rodríguez *et al.*, 2017), under 'optimal' conditions where disturbance to soil and seedbank are minimal and forests can naturally regenerate, there are predictable changes in aboveground structural and functional characteristics during secondary succession (Chazdon, 2014).

Basal area change

Changes in basal area during forest succession could influence soil C storage, as the aboveground biomass of trees is generally reflected belowground in their root systems, and greater inputs of organic matter from larger root systems (necromass and exudates) as well as crowns, could contribute to soil C stocks. Although there is a general increase in tree basal area (BA) during the early stages of succession, basal area does not increase linearly with stand age, as 'tree packing' saturates and sometimes eventually declines with time. Although this 'intermediate peak theory' (Denslow and Guzman, 2000) has sometimes been shown to be scale-dependent where larger, landscape-scale studies continue to see an increase in stand BA with time (Mascaro *et al.*, 2012), BA is suggested to be a more useful predictor of successional changes during forest regrowth than forest age (Lohbeck *et al.*, 2012) and might therefore also be a good predictor of soil C stocks.

Functional change

The trajectory of tree species and functional composition during secondary forest succession is largely driven by competition among species for changing resources (light, nutrients and water), as fast-growing, light-demanding trees with 'acquisitive' growth strategies ('pioneer' species) are gradually replaced by more slow-growing, shade-tolerant trees with 'conservative' growth strategies (Chazdon, 2014; Whitfeld *et al.*, 2014). This shift in tree community shade tolerance and the associated changes in the physical and chemical properties of organic matter input to forest soils may significantly alter soil C and nutrient cycling throughout secondary succession through for example, the decreasing nitrogen (N) content in litterfall (Davidson *et al.*, 2007). Acquisitive growth strategists prioritise investment in light capture and rapid growth; generally, have a larger specific leaf area (SLA), higher foliar nutrient concentrations (lower C:N) and lower investment in foliar defence (leaf toughness). Conversely, conservative growth strategists invest more in structure and defence, resulting in slower litter decomposition. As a result of these differences in plant functional traits, we might therefore expect the rates of litter decomposition and C cycling to be faster in forests with a comparatively greater proportion of light-demanding species (more common in early succession) than in (typically older) forests with comparatively more shade-tolerant species, and that soil C would accumulate over time as forests mature.

Influence of land use and soil characteristics on carbon accumulation during succession

Former land use is an important predictor for initial soil C loss. For example, disturbance from ploughing in the conversion of forest to cropland causes greater initial soil C loss than land which maintains vegetative cover in the conversion to pasture (Don, Schumacher and Freibauer, 2011; Li, Niu and Luo, 2012). Highly disturbed soils therefore also have the greatest rate of soil C gain during recovery following the cessation of agriculture and forest regrowth (Laganière, Angers and Paré, 2010). Soil physical and chemical characteristics can also affect the accumulation and cycling of soil C; for example, soil pH can strongly affect the abundance and diversity of soil bacteria (Fierer and Jackson, 2006; Rousk *et al.*, 2010; Zhou, Wang and Luo, 2018), which play an essential role in soil C and nutrient dynamics through the decomposition of organic matter. An increase in soil N content is strongly correlated with soil C content and has been linked with the long-term sustainability of soil C sequestration (Yang, Luo and Finzi, 2011) and changes in soil N dynamics have been shown to be related to changes in vegetation during forest recovery (Amazonas *et al.*, 2011), however, soil N was not shown to increase with forest age in the SF stands on BCNM (Jones *et al.*, 2019).

Given the many potential influences on soil C accumulation during secondary succession, examining above-belowground processes and interactions during the recovery of naturally regenerating tropical forest provides an excellent opportunity to improve our understanding of belowground C dynamics during tropical forest regrowth and assess the wider potential of forest restoration activities to help mitigate atmospheric CO_2 concentrations thought soil C sequestration.

I investigated the relationship between functional changes in vegetation during secondary succession and patterns in soil C using an existing chronosequence of naturally regenerating moist tropical forest located within Barro Colorado Nature Monument (BCNM) in central Panama. The chronosequence provides an exceptional opportunity to study soil C recovery over a wide age range of recovering secondary tropical forest (40 – 120 years; Rozendaal *et al.*, 2019) and old growth forest, for which extensive aboveground plant census data is available. Successional and functional patterns in aboveground communities have previously been characterised by Dent, DeWalt and Denslow (2012); their results revealed that tree community

shade tolerance increased with stand age for three size classes (seedling, sapling and adult trees) along the chronosequence. However, the relationship between changes in aboveground functional groups during succession and soil C has not yet been characterised.

Given that soil C during secondary forest succession is likely to be strongly influenced by historic soil disturbance and soil characteristics, as well as the quantity and quality of plant inputs, I hypothesised that stand basal area and the proportion of different tree functional groups would have a greater influence on soil C content and stocks than stand age *per se*. Specifically, I hypothesised that:

1) Soil C content and stocks will increase with stand basal area

2) Soil C content and stocks will increase with greater dominance of shade-tolerant trees

3) Soil C content and stocks will increase with increasing soil pH and be greater in stands that were used only for pasture or were undisturbed than those that were ploughed for agricultural purposes.

To test my hypotheses, I measured soil C concentrations, estimated soil C stocks, and assessed tree functional groups across 10 forest stands in five age classes, taking into account differences in former land-use and soil properties.

2.3 Methods

Chronosequence description

The chronosequence stands are located throughout the Barro Colorado National Monument (BCNM) which comprises the 1500-ha Barro Colorado Island (BCI) and five surrounding mainland peninsulas (Figure 2.1). The climate is classified as moist tropical with a distinct dry season from January to April with a mean annual temperature of around 27°C and an average annual rainfall of 2600 mm, of which 90% falls in the rainy season (Windsor, 1990).

The chronosequence was chosen because it is considered to be largely representative of forest recovery following the wide-spread practice of 'swidden' agriculture in the tropics (Denslow and Guzman, 2000; Dent, DeWalt and Denslow, 2012), where deforestation for agriculture and subsequent farm abandonment over time has created a mosaic of old-growth (OG) and naturally regenerating secondary forests (SF). Past land-use within the chronosequence included pasture for cattle, fruit and vegetable production, and plantations (Denslow and Guzman, 2000; Leigh, Rand and Winsor 1983; Table 2.1). More recent human disturbance on the peninsulas of the BCNM form an extensive age gradient of tropical forest with 10 defined chronosequence stands comprising two replicate age categories which, at the time of the present study, were estimated at 40, 60, 90 and 120 years since agricultural abandonment, and two undisturbed OG stands (Dent, DeWalt and Denslow, 2012). Stand ages were determined using a combination of early publications and accounts; aerial photographs; and interviews with long-term residents, scientists, farmers and forest guards (Dalling and Denslow, 1998; Denslow and Guzman, 2000; Dewalt, Schnitzer and Denslow, 2000; Mascaro *et al.*, 2012). The age estimates for the stands are considered to be approximate to within 10 years of accuracy (Dalling and Denslow, 1998; Denslow and Guzman, 2000; Mascaro *et al.*, 2012). Stands were established on level terrain, running parallel to slopes and avoiding creeks and trails (Denslow and Guzman, 2000). Each stand has an area of at least 5 ha and there has been no further disturbance to stands since agricultural abandonment. (Denslow and Guzman, 2000; Dewalt, Schnitzer and Denslow, 2000; Dent, DeWalt and Denslow, 2012; Mascaro *et al.*, 2012). The wider study area provides data on species composition, plant traits and forest dynamics (Leigh, Rand and Windsor, 1983; Leigh. *et al.*, 2004; Kattge *et al.*, 2011; Condit, Chisholm and Hubbell, 2012). Species richness is quite constant across stands of different ages and attains levels of OG forest within 20 years of succession (Denslow and Guzman, 2000; Dent, DeWalt and Denslow, 2012). Tree species composition between stands of the same age class is more variable in the

youngest forest stands and becomes more similar with stand age (DeWalt, Maliakal and Denslow, 2003; Dent, DeWalt and Denslow, 2012). In contrast to species richness, functional diversity (characterised as community-level shade tolerance from basal area weighed mean) increases with stand age and converges with that of OG forest over time (Dent, DeWalt and Denslow, 2012).

The BCNM chronosequence overlies volcanic (andesite) and sedimentary (volcanic and marine derived) geological units, which have weathered to form a variety of soil types (Baillie *et al.*, 2007). Although the most significant difference in soil type is between those derived principally from volcanic composition (which weather to produce mainly kanditic and oxidic clays) and those from the sedimentary 'Caimito marine' facies (which produces more smectitic clays; Baillie *et al.*, 2007), a number of studies report little variation in soil chemical properties across soil types (Yavitt and Kelman Wieder, 1988; Yavitt, 2000; Barthold, Stallard and Elsenbeer, 2008). Soils on BCI are reported to be rich in nitrogen (N; Yavitt and Wieder, 1988) and have a high cation exchange capacity (CEC) despite differences in derived parent material (Baillie *et al.*, 2007). However, Yavitt (2000) found that volcanic derived soils on BCI have slightly but significantly higher phosphorous (P) concentrations than those from sedimentary rocks.

Soil C concentrations and stocks on BCI decrease significantly with depth but no significant variation was observed between soil types (Grimm *et al.*, 2008). Across all soil types, the upper 10 cm contains the highest SOC stocks but the standard deviation was high (Grimm *et al.*, 2008). Soil texture is also positively correlated with SOC, with soils of higher clay content generally containing higher SOC concentrations and clay content rather than mineralogy is thought to be more important for stabilizing SOC in these soils (Grimm *et al.*, 2008). Topography has a strong influence on SOC stocks in the topsoil with the highest values found at the foot of slopes and the lowest on the mid-slope locations, but this pattern was not observed at depth indicating that the influence of erosion on SOC is limited to the soil surface (Grimm *et al.*, 2008). It is unclear whether soil-forming processes and/or past land use changes influence the distribution of SOC in the topsoil, but Grimm et al (2008) suggest that present-day biomass input is of more importance as differences in past land use were only weakly related to SOC in the upper 30 cm of soil.

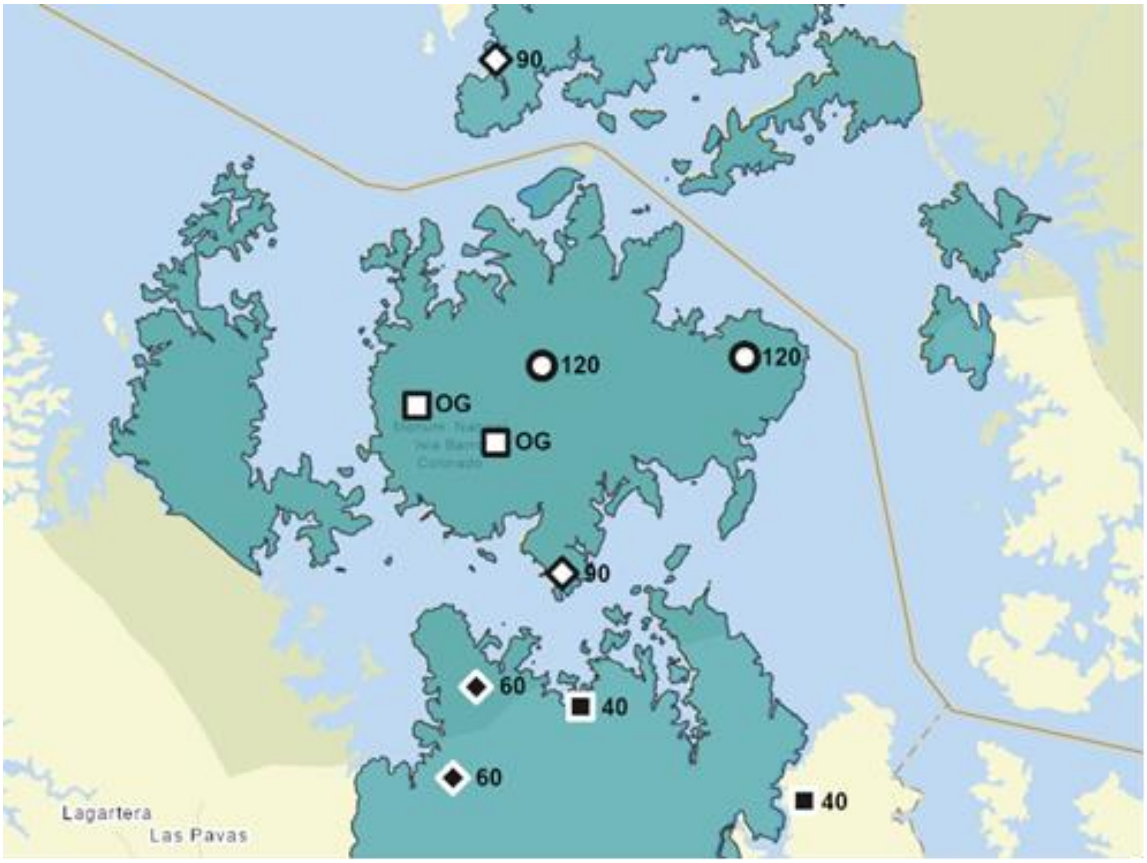


Figure 2.1 Map of the Barro Colorado Nature Monument (BCNM), showing the approximate location of 10 forest stands, two in each of five age classes; 40, 60, 90 and 120 year old secondary forest (SF), and two old-growth (OG) stands, in a chronosequence of naturally regenerating tropical forest in Panama, Central America.

Table 2.1 Stand and soil characteristics for a chronosequence of 10 forest stands in Panama, Central America Subscript numbers indicate the data source, where 1 = Denslow and Guzman (2000) and 2 = The geological composition of the Panama Canal Watershed (STRI GIS Laboratory).

Stand name$_1$	Age$_1$ (years)	Geology$_2$	Land-use$_1$	pH$_1$	Standing litter$_1$	Soil bulk density$_1$
PED	40	Tb	Pasture/swidden	5.88	417	0.57
SAI	40	Tb	Pasture/swidden	5.92	548	0.53
END	60	Tb	Plantation	6.52	507	0.51
FOS	60	Tb	Pasture/swidden	5.70	394	0.46

BOH	**90**	Tbo	Pasture/swidden/agriculture	6.62	451	0.48
POA	**90**	Tcv/Tcm	Swidden	5.46	692	0.57
BAR	**120**	Tcv	Pasture	6.28	383	0.59
PER	**120**	Tbo	Pasture	6.24	535	0.56
ARM	**OG**	Tb	Old growth	5.98	549	0.50
ZET	**OG**	Tcv	Old growth	5.88	776	0.48

Characterising successional and functional community change in forest stands

To test the influence of variation in tree functional groups on soil C along a successional gradient, I derived several stand-level measures of aboveground successional status to compare chronosequence stands via a site-specific literature review and from tree census data (D.Dent, unpublished data). I used the simple metric of forest stand age as well as the extensive forest census data available for the chronosequence, and functional data based on recognised changes in structural and functional attributes during forest regrowth. Chazdon (2014) characterises forest successional stages through three main criteria: total aboveground biomass or basal area; forest age or size structure of tree populations; and species composition. I used tree basal area as an indicator of aboveground biomass (Chazdon, 2014) which is shown to quickly recover during tropical forest regrowth (Poorter *et al.*, 2016) but the pattern of biomass accumulation during secondary succession can vary depending on factors such as site and the landscape-scale considered (Denslow and Guzman, 2000; Mascaro *et al.*, 2012). I also use a well-supported general description of aboveground functional community change during tropical forest secondary succession to characterise forest stands, where competition between species for changing resource availability (e.g. nutrients and light) during secondary succession often results in a predictable pattern of functional community change, characterised by contrasting life-history strategies and plant functional traits (Table 2.1). This shift in tree functional composition occurs when 'acquisitive growth strategists' (fast-growing, light-demanding, gap specialists) or 'pioneer' species, common in the early stages of forest regrowth, are gradually replaced by 'conservative growth strategists' (slow-growing, shade-tolerant, old-growth specialists) or 'climax' species, as light levels decrease in the later stages of succession

(Zhang, Zang and Qi, 2008; Chazdon, 2014; Ghazoul and Chazdon, 2017) resulting in the establishment of a more shade-tolerant community (Dent, DeWalt and Denslow, 2012).

Table 2.2 General changes in foliar traits during succession in tropical forest, modified from Chazdon (2014)

Foliar traits	*Change with successional age*
Leaf nitrogen content	High → Low
SLA (specific leaf area per mass)	High → Low
Leaf LMA (leaf mass per unit area)	Low → High
Leaf density	Low → High
Leaf toughness	Low → High

Forest stand age and basal area

To investigate patterns in soil C as a function of time, I adjusted the chronosequence stand ages from the original estimates by Denslow and Guzman (2000) to include the time since publication, and rounded them to nearest decade to obtain age classes. I calculated stand basal area from census data collected in 2011 (D. Dent, unpublished data). Stem diameter at breast height (1.3-m; DBH) for each individual >10 cm DBH was converted to stem basal area (m^2; Eq. 1) and the sum of all stems in each stand was then divided by the total area of the stand covered in the census to obtain mean stand basal area (Eq. 2).

$$Stem\ basal\ area\ (m^2) = \left(\frac{DBH(cm)}{200}\right)^2 * \pi \qquad \textbf{Eq. 1}$$

$$Stand\ basal\ area\ \left(\frac{m^2}{ha}\right) = \frac{\sum\ stem\ basal\ area}{area\ of\ stand\ (ha)} \qquad \textbf{Eq. 2}$$

Tree community shade-tolerance

Following the findings of Dent, DeWalt and Denslow (2012), I used tree community shade tolerance to characterise changes in aboveground functional diversity during succession: where shade-tolerant species become increasingly dominant in the later stages of succession as the

canopy closes and the reduction in light affects sapling recruitment. I assigned each species to a light requirement category as detailed in Comita *et al.* (2007), which classifies species as gap specialist (G), intermediate (I), or shade-tolerant (S). I also used species growth response to increasing light, calculated from a species-specific light requirement index developed by Rüger et al. (2009; Box 1) as a robust metric of species shade tolerance.

Box 1. Species growth responses to increasing light

Ruger et at (2009) calculated species light effect values using data on tree sapling recruitment over two census intervals and available light from yearly censuses of canopy density from a tropical forest plot on Barro Colorado Island. They employed a hierarchical Bayesian approach to quantify the light dependence of recruitment in 263 woody species using a power function (linear log–log relationship) to model the light effect on recruitment. Different responses were expressed by the light effect parameter *b* which ranged from 0.6 to 3.3. Comparison between both census intervals showed that most species (38%) had a '**decelerating**' response to increasing light ($b<1$), 21% had an '**accelerating**' response ($b>1$), 3% had a '**negative**' response ($b<0$) and the remaining 38% of species showed different types of light response between census interval or had no recruits.

Table 2.3 Growth response categories for tree species along an age-gradient of lowland tropical forest sites in Panama Central America, based on tree sapling recruitment with increasing light (modified from Ruger et al. 2009).

Growth response	***Description***
Accelerating (>1)	Sapling recruitment increases with increasing light and the increase is higher at higher light levels
Decelerating (<1)	Sapling recruitment increases with increasing light, but the increase is lower at higher light levels
Negative (<0)	Sapling recruitment is higher at lower light and decreases with increasing light levels.

To compare community shade tolerance across the chronosequence, I calculated the relative influence (RI) of each growth response category using tree census data (D. Dent, unpublished

data). I assigned mean light effect values from two census intervals to each species in the chronosequence and classified them as accelerating (ACC), decelerating (DEC), and negative (NEG); species without light effect values were classified as 'unknown' (Table 2.2). I then calculated Importance Values (IV; %) for each tree species in the chronosequence based on the number of individuals (frequency; Eq. 3) and the sum of the basal area (dominance; Eq. 4). I chose to express the IV as mean measure of frequency and dominance (Eq. 5), as species with large numbers of individuals as well as those with a large total biomass are considered similarly important for determining ecosystem processes (Lohbeck *et al.*, 2012). Species frequency was calculated from the count of one stem per individual (regardless of number of stems per individual) to account for the limited spatial spread of root systems and possible constraints on canopy size of multi-stemmed individuals compared to that of an equal number of single-stemmed individuals. Species dominance was calculated from the sum basal area of all stems of each individual tree.

$$Relative\ frequency\ (\%) = \left(\frac{Number\ of\ individuals\ of\ a\ species}{Total\ number\ of\ individuals\ per\ stand}\right) * 100 \quad \text{Eq. 3}$$

$$Relative\ dominance\ (\%) = \left(\frac{\sum of\ basal\ area\ of\ a\ species}{Total\ basal\ area\ per\ stand}\right) * 100 \quad \text{Eq. 4}$$

$$Importance\ value\ (\%) = \frac{Relative\ frequency + Relative\ dominance}{2} \quad \text{Eq. 5}$$

Finally, I used the species IVs for each growth response category to calculate the relative influence (RI) of ACC, DEC and NEG species per stand, whereby the summed IVs for each growth response category were expressed as a proportion of the total per stand, allowing me to compare community shade tolerance across the chronosequence stands. Of the total 277 tree species across the chronosequence, light effect data were available for 200 species. Therefore *c.* 72 % of species were assigned to growth response categories (DEC = 129 species, ACC = 60 species, NEG = 11 species, unassigned = 77 species) which amounted to *c.* 88 % of the total proportion of species expressed as RI (DEC = 60 %, ACC = 24 %, NEG = 4 %, unknown = 12 %).

Stand and soil characterisation

Land-use history

To test the influence of past land-use on soil C, I used the chronosequence stand descriptions from Denslow and Guzman (2000) and divided stands into 'high' or 'low' soil disturbance categories based on presumed initial soil disturbance (and therefore C loss) during land conversion. The ten chronosequence stands split equally into the two categories; low soil disturbance consisted of OG stands and stands used only for pasture; high soil disturbance included stands used for swidden agriculture and plantations.

Soil sampling

To assess the relationship between functional changes in vegetation during secondary succession and patterns in soil C in each of the ten chronosequence stands, I established four (20 m x 10 m) sampling blocks, spaced at 40 m intervals along a 160 m transect. I collected soils in each of the 40 sampling blocks between May and June 2016 at three sampling points (at 5, 10 and 15 m along the transect section within the sampling block). I collected three cores per sampling point by first carefully removing the surface litter and then sampling the mineral soil in 10 cm increments from 0-30 cm depth using a 4.8-cm diameter soil corer and bulked them per depth interval resulting in 12 composite samples per stand (four blocks x three depths = 120 total samples). Samples were stored at 4˚C within four hours of collection, sieved to <2 mm and subsampled in preparation for analyses within 48 hours of collection.

Laboratory analyses

To determine gravimetric soil water content, I oven-dried subsamples (20 g fresh weight) from the 0-10 cm and 10-20 cm depth increments at 105˚C for 48 hours. I measured soil pH on a 1:3 mixture of fresh soil and deionised water using bench pH meter (STARTER 2100, OHAUS, New Jersey, USA) within 48 hours of collection. To determine percentage soil C and N content, I ground a subsample of homogenized, air-dried soil from each of the three depth increments per stand using a ball mill (Mixer Mill 400, Retsch®, Haan, Germany), and total carbon and

nitrogen was analysed by high temperature combustion gas chromatography (Vario El III C/N analyser, Elementar, Stockport, UK).

Estimation of soil C and N stocks

Soil C stocks are expected to provide a more accurate comparison between stands as they take in to account differences in soil bulk density (BD), which can vary with soil type and land-use history and which generally increases with depth. I used mean soil bulk density values as measured by Jones *et al.* (2019) to calculate soil C stocks at the 0-10 and 10-20 cm depth increments using the following equation:

$$C_{stock} = D \times Bd \times C_{conc} \qquad \text{Eq.6}$$

where C_{conc} is percentage C, Bd is stand mean soil bulk density, C stock is the stock of carbon (Mg ha^{-1}) and D is the depth of sample (cm).

Data analysis

I used linear mixed effects models (lme4 package; Bates *et al.*, 2015) to assess the effect of each explanatory variable (age class, stand basal area, land-use history and tree functional groups) on soil C concentration (%) and stocks (Mg ha^{-1}) using R version 3.5.2 (R Core Team, 2018). The relationship between stand-level characteristics (basal area, land-use history and tree functional groups) and soil C was assessed using mean values of soil C per stand and depth with stand included as a random effect to account for the non-independence of values. All other variables were assessed using mean values per sampling block and depth, with block nested within stand as random effects. Soil C content was log-transformed prior to analysis to correct for non-normal distribution of data. As soil C decreases strongly with depth and is a clear predictor of variation in soil C, I included depth in both full and null models and tested the interaction between each explanatory variable and soil depth. The significance of each term in the model was determined by sequentially dropping terms and comparison to appropriate null models using likelihood ratio tests. Results are reported as significant at $p < 0.05$ and non-significant trends are reported at $p < 0.09$; for linear mixed effects models χ^2 and p values are given for the comparison between the final model and the corresponding null model.

2.4 Results

Soil carbon across an age gradient of naturally regenerating tropical forest

Soil carbon content (%)

Stand mean soil C concentrations at 0-10 cm depth were 4.74 ± 0.15%. Variability in mean soil C content between the two replicate forest stands within each age class was low (Figure 2.2a), and soil C declined strongly with depth in all age classes (Figure 2.2b; Table 2.2). Soil C content varied significantly among forest age classes and there was a significant interaction between age class and depth (χ^2 = 75.76, p < 0.001) but there was no clear pattern of increasing soil C content with forest stand age at any depth increment (Figure 2.2b; Table 2.2). The OG stands had the smallest decrease in soil C with depth, but only at the 20-30 cm depth increment was mean soil C content slightly (but not significantly) higher in the OG than in the SF stands (Figure 2.2b; Table 2.2).

Soil carbon stocks (Mg ha^{-1})

Mean soil C stocks at 0-10 cm were 56.96 ± 11.92 Mg ha^{-1}. The variation in soil C stocks with forest age was roughly similar to that of C content but showed greater variation between replicate stands in each age class (Figure 2.2c; Appendix A:Table S2.2), lower values in the 0-10 cm in the 60Y stand and generally lower variation between the 0-10 and 10-20 cm depth increments (Figure 2.2d; Appendix A:Table S2.2). There was a significant interaction between forest age class and soil depth (but this was not as strong as for soil C content). Forest age was a significant predictor for soil C stocks (χ^2 = 39.25, p = <0.001) but there was no clear pattern of increasing soil C stocks with forest age.

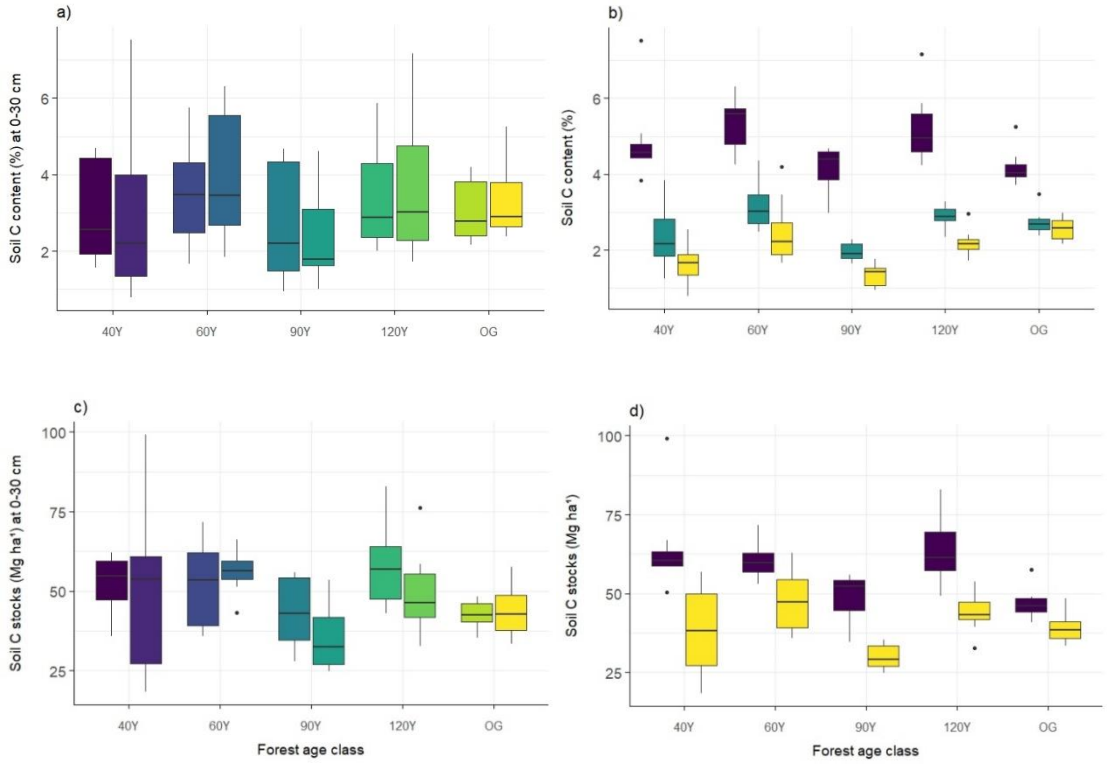


Figure 2.2 Soil carbon content at: a) 0-30 cm depth in each of the ten chronosequence stands, grouped by age category, and b) mean values per age class from three depth increments (dark blue = 0-10 cm, green = 10-20 cm, yellow = 20-30 cm). And soil carbon stock estimates (Mg ha^{-1}) at: c) 0-30 cm depth in each of the ten chronosequence stands, grouped by age category, and d) mean values per age class from two depth increments (dark blue = 0-10 cm, yellow = 10-20 cm) across an age gradient of lowland tropical forest sites in Panama, Central America. Boxes denote the 25th and 75th percentiles and median lines are given for n = 4 (a and c) and n = 8 (b and d from two stands per age class), whiskers indicate values up to 1.5x the interquartile range, and dots indicate outliers.

Soil carbon with increasing stand basal area (m^2 ha)

Stand basal area (BA) differed significantly among forest age classes ($f_{4,25}$ = 60.47, p = <0.05; Table Appendix A: Table S2.1) but did not increase with increasing stand age. The highest BA was in the intermediate age class (90Y stands).

In contrast to my first hypothesis, there was a negative relationship between soil C and stand basal area (BA) (Figure 2.3). Soil C decreased with both increasing stand BA and depth but there was no interaction. The negative relationship with stand BA was significant for both soil C content (χ^2 = 4.60, p = 0.032) and soil C stocks (χ^2 = 5.41, p= 0.020).

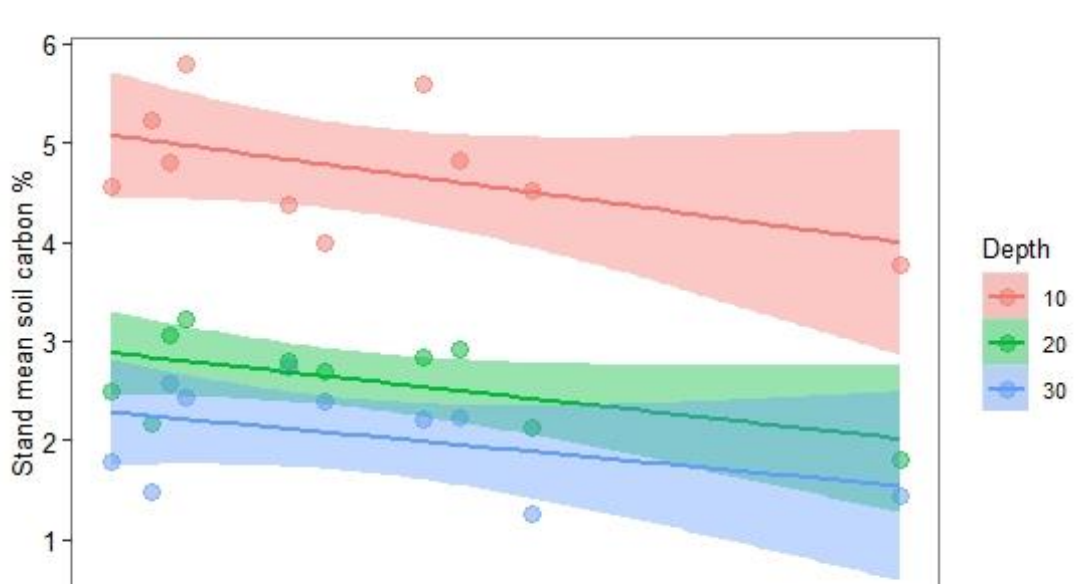


Figure 2.3 Stand mean soil carbon concentration with increasing stand basal area (m^2 ha^{-1}) in a chronosequence of lowland tropical forest Panama, Central America, showing soil carbon concentrations at three depth increments (0-10 cm, 10-20 cm, 20-30 cm) Points represent mean for *n* = 4, shaded area denotes 95% confidence interval from linear model (*lm* function) prediction.

Relationships between soil carbon and the relative influence of tree functional groups

The relative proportions of shade tolerant and light demanding tree species varied among forest age classes and differed significantly between the SF and OG forest stands. There was a general pattern of increasing DEC species, and decreasing ACC species with increasing forest age across the chronosequence in all but the 120Y stands where the RI of both ACC and DEC species was more similar to the 60Y stands than either the 90Y or OG stands (Appendix A Table S2.1). The RI of NEG species was low in all stands (>7%), and although there was no clear pattern with increasing stand age, the RI of NEG species was lowest in the 40Y and highest in the OG age category (Appendix A Table S2.1). The proportions of species not assigned to a growth response category was inconsistent across stands and age categories, the RI of 'unknown' species per stand ranged from 4.3 % - 21.6 % but there was no trend with forest stand age. There were clear differences in soil C (concentration and stocks) with the increasing relative influence (RI) of contrasting tree functional groups, and this relationship varied with depth (Figures 2.4 and 2.5). The effect of tree functional groups on both measures of soil C was

apparent only at the 0-10 cm depth increment but not at greater depths, resulting in a significant interaction term between functional group and depth in all models.

Increasing RI of accelerating species

At 0-10 cm, soil C increased significantly with the increasing RI of ACC species in forest stands for both C content (χ^2= 8.88, p= 0.031; Figure 2.4a) and stocks (χ^2= 14.68, p= <0.001; Figure 2.5a). The inclusion of soil pH did not significantly improve the models.

Increasing RI of decelerating species

In contrast, soil C decreased with the increasing RI of DEC species across the chronosequence at the 0-10 cm depth increment. The negative relationship between the RI of DEC species and soil C was significant for both C content (χ^2= 17.12, p= <0.001; Figure 2.4b) and C stocks (χ^2= 10.63, p = 0.005; Figure 2.5b). Soil pH did not significantly improve the models.

Increasing RI of decelerating: accelerating species

The opposing relationships between soil C and the RI of ACC and DEC species resulted in a significant negative relationship between the ratio of DEC:ACC species in stands and both soil C content (χ^2= 12.55, p= 0.006; Figure 2.4c); and C stocks (χ^2 = 9.83, p= 0.007; Figure 2.5c). In the case of soil C content, the inclusion of pH as a fixed effect significantly improved the model (χ^2= 3.99, p= 0.046).

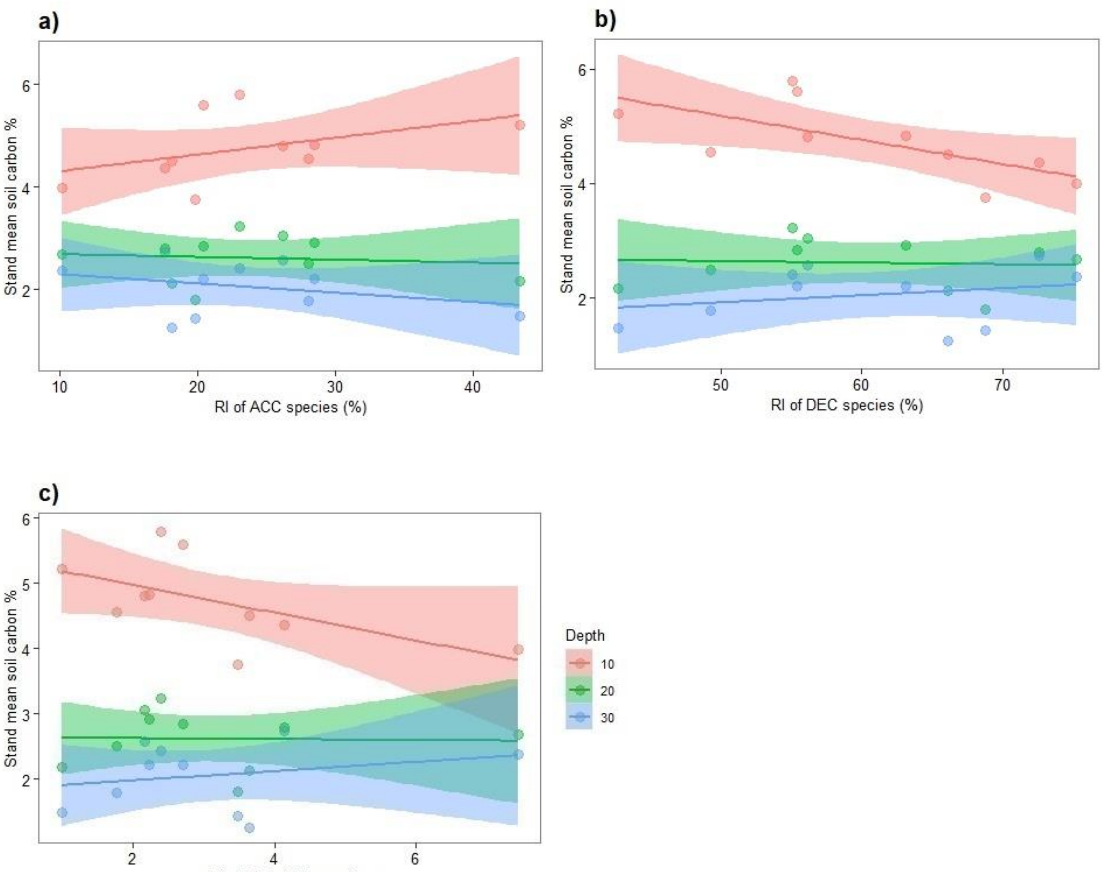


Figure 2.4 Stand mean soil carbon concentration with an increasing relative influence of: (a) accelerating species, (b) decelerating species, and (c) the ratio between decelerating and accelerating species, at 0-10 cm, 10-20 cm and 20-30 cm soil depth increments in a chronosequence of lowland tropical forest in Panama, Central America. Points represent mean for *n* = 4, shaded area denotes 95% confidence interval from linear model (*lm* function) prediction.

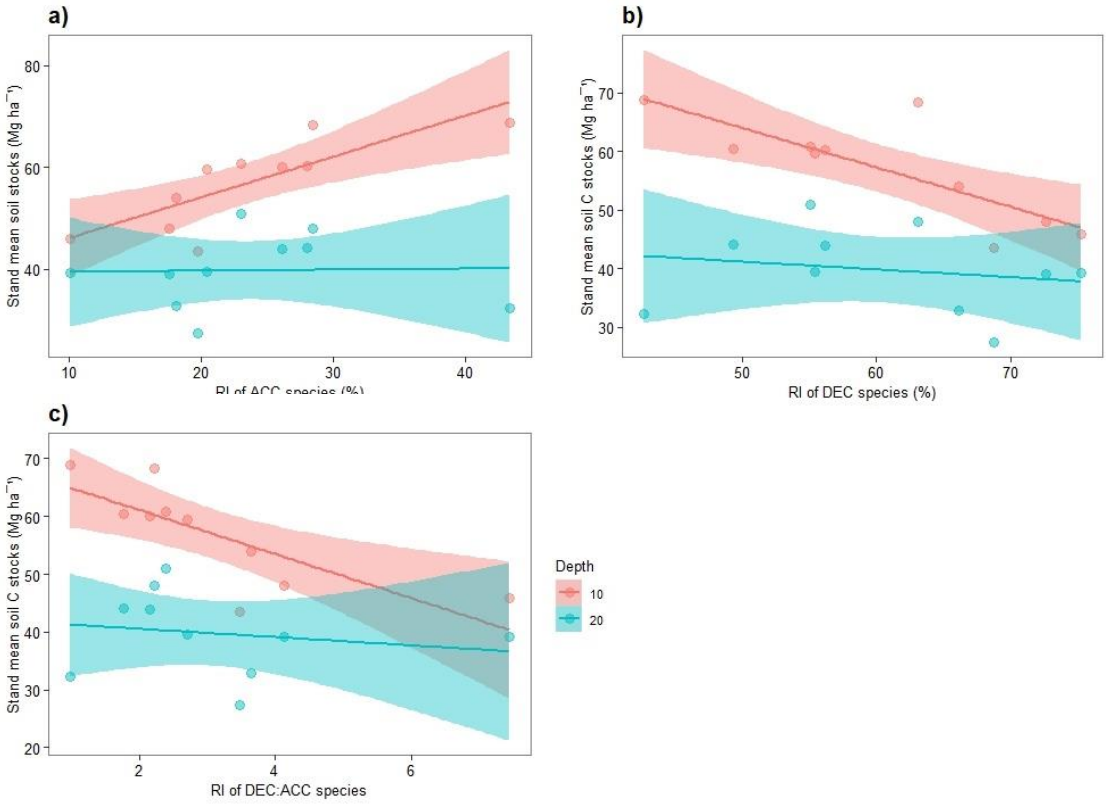


Figure 2.5 Estimates of stand mean soil carbon stocks (Mg ha^{-1}) at 0-10 and 10-20 cm depth with the increasing relative influence (RI) of a) ACC species, b) DEC species and, c) DEC:ACC species in 10 stands of a chronosequence of naturally regenerating lowland tropical forest in Panama, Central America. Points represent mean for *n* = 4, shaded area denotes 95% confidence interval from linear model (*lm* function) prediction.

Soil carbon and variation in soil characteristics and land-use history

I found no relationship between soil C content (%) or C stocks (Mg ha^{-1}) and land-use history at any depth increment using the broad classifications of past land-use and inferred soil disturbance. Soil C increased with increasing soil pH across the full 0-30 cm sample depth and although depth explained most of the variation in soil C content, the inclusion of pH, (without interaction) significantly improved the model (χ^2 = 6.02, p = 0.014, Figure 2.6). However, the relationship between soil C stocks and pH was not significant and the relationship was not significant at separate depth intervals. As expected, there was a strong correlation between soil C and N content but there was no consistent variation in soil characteristics with increasing stand age (Table 2.3).

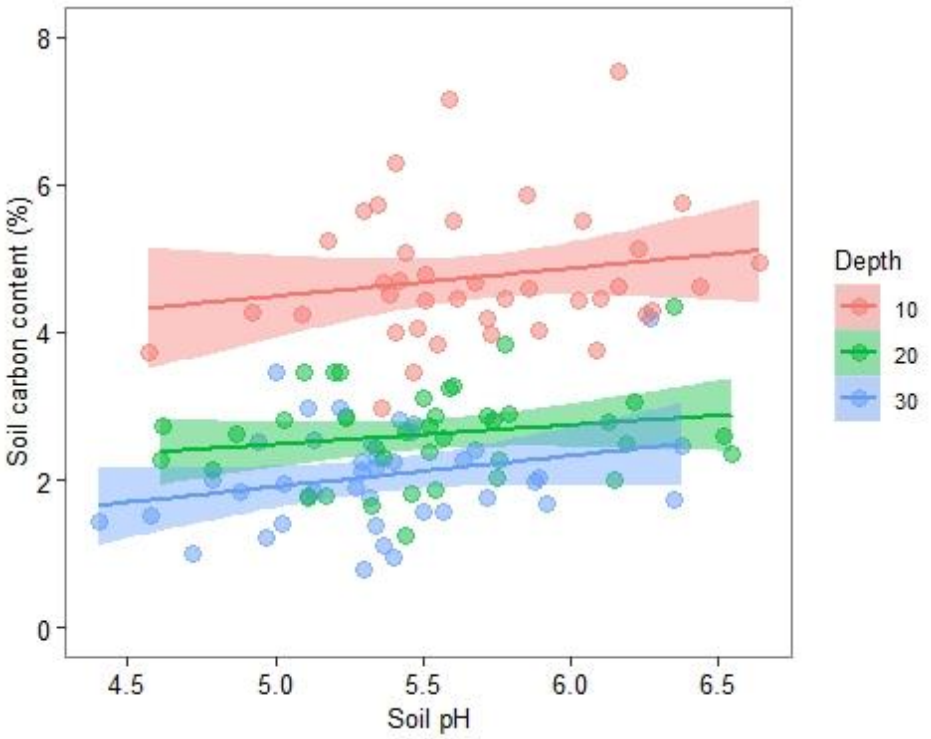


Figure 2.6 Soil carbon content along an increasing soil pH gradient from 10 stands in a chronosequence of naturally regenerating lowland tropical forest in Panama, Central America, at 0-10 cm, 10-20 cm and 20-30 cm depth increments. Points represent mean for *n* = 4, shaded area denotes 95% confidence interval from linear model (*lm* function) prediction.

Soil characteristics among forest age classes

Nitrogen

Soil N content (%) varied significantly among forest age classes (χ^2 = 34.21, *p* = <0.001); it was lowest in the two 90Y stands and highest in the 60Y stands, but the relationship between forest age and soil N was not unidirectional. There was a significant interaction between forest age class and soil depth, and variation among stand age classes increased with increasing depth. N content varied among age classes at the 10-20cm ($f_{4,5}$ = 5.36, *p* = 0.047; Table 2.3) and 20-30 cm ($f_{4,5}$ = 7.99, *p* = 0.0213; Table 2.3) depth increments and was higher in the OG stands than the SF stands at 20-30 cm depth. Soil N stocks also varied significantly among forest age classes at the 0-20 cm depth but not when tested at separate depths.

C:N

The ratio of soil C to N content varied significantly among forest age classes across the full depth range of 0-30 cm and increased significantly with increasing soil depth. The 90Y stands had the highest C:N at each depth increment.

pH

Soil pH varied among stands, explained by significantly higher pH in one of the 60Y stands but there was no significant variation between forest age classes at the full 0-30 cm depth range, or at separate depth increments.

Table 2.4 Mean soil values per age class for: C and N concentration (%), C:N ratio and pH plus standard error at three depth intervals (0-10, 10-20 and 20-30 cm) sampled from 4 blocks in each of two replicate stands in five age classes, in a chronosequence of naturally regenerating tropical forest in Panama, Central America. Means and standard errors are given for n = 8 (4 x replicate blocks per stand, 2 x stands per age class). Different super-script letters indicate significant differences among forest age classes at $p < 0.05$ determined by ANOVAS with Turkey post-hoc comparisons and correction for multiple comparisons.

Forest age class		40Y	60Y	90Y	120Y	OG
Soil C % With depth (cm)	0-10	4.89^{ab} (± 0.29)	5.30^{a} (± 0.73)	4.14^{b} (± 0.22)	5.22^{ab} (± 0.34)	4.18^{b} (± 0.17)
	10-20	2.34^{b} (± 0.29)	3.14^{a} (± 0.22)	1.96^{b} (± 0.09)	2.88^{a} (± 0.10)	2.74^{a} (± 0.12)
	20-30	1.63^{b} (± 0.19)	2.50^{a} (± 0.31)	1.34^{b} (± 0.10)	2.21^{a} (± 0.13)	2.56^{a} (± 0.10)
Soil N % with depth (cm)	0-10	0.40^{ab} (± 0.04)	0.46^{a} (± 0.02)	0.33^{b} (± 0.07)	0.43^{a} (± 0.02)	0.39^{ab} (± 0.02)
	10-20	0.15^{ab} (± 0.03)	0.23^{a} (± 0.02)	0.10^{b} (± 0.01)	0.22^{a} (± 0.01)	0.23^{a} (± 0.02)
	20-30	0.09^{b} (± 0.01)	0.17^{a} (± 0.04)	0.05^{b} (± 0.01)	0.13^{a} (± 0.02)	0.21^{a} (± 0.01)
Soil C:N with depth (cm)	0-10	12.45^{ab} (± 0.48)	11.54^{ab} (± 0.28)	12.96^{a} (± 0.58)	12.11^{ab} (± 0.34)	10.78^{b} (± 0.37)
	10-20	17.26^{ab} (± 2.06)	13.72^{b} (± 0.60)	23.52^{a} (± 3.29)	13.66^{b} (± 0.75)	12.00^{b} (± 0.65)
	20-30	19.65^{ab} (± 1.73)	17.64^{b} (± 2.84)	25.97^{a} (± 1.90)	18.20^{ab} (± 2.08)	12.46^{b} (± 0.48)
Soil pH with depth (cm)	0-10	5.68 (± 0.10)	5.90 (± 0.19)	5.53 (± 0.13)	5.86 (± 0.16)	5.52 (± 0.17)
	10-20	5.41 (± 0.10)	5.83 (± 0.19)	5.30 (± 0.18)	5.69 (± 0.19)	5.37 (± 0.12)
	20-30	5.19 (± 0.08)	5.65 (± 0.19)	5.09 (± 0.17)	5.48 (± 0.17)	5.36 (± 0.07)

2.5 Discussion

This study is the first to investigate the relationship between soil C and aboveground functional community change during tropical forest secondary succession, utilising an age-gradient of naturally regenerating tropical forest stands in Panama. My study demonstrated that, whereas other stand and soil characteristics had limited explanatory power for variation in soil C content and stocks, soil C at 0-10 cm depth increased with the relative influence of light-demanding tree species across stands of all age classes and decreased with stand-level basal area. My results suggest that high-quality plant inputs may play a key role in soil C accumulation during secondary succession.

The relationship between soil carbon and aboveground successional processes during tropical forest regrowth

I expected basal area to be a better predictor for soil C than forest age as stand BA is highest in the mid-successional stage stands used in this study (intermediate peak hypothesis; Denslow and Guzman 2000) and therefore, the largest input of organic matter to soil C was assumed to be in the mid-aged, not the oldest forest stands. Stand BA was a stronger predictor for variation in soil C than forest age, but in contrast to my first hypothesis, mean soil C content and stocks decreased with increasing stand BA (Figure 2.3). Although surprising given the assumptions regarding organic matter input, a similar unexpected negative relationship between soil organic C and stand BA was reported for recovering tropical forest in Australia (Paz *et al.*, 2016). Interestingly, the relationship was only detected in soil derived from Andesite, which also underlies much of BCNM and may possibly help explain the unexpected pattern observed in this study. Tree BA was also used to calculate the RI of ACC, DEC and NEG species in stands, therefore it's effect was also assessed as a component of tree functional groups, which my study demonstrated was the strongest predictor of soil C stocks and concentrations in these forest stands.

Soil C with the increasing relative influence of contrasting tree functional groups

My hypothesis rested on the assumption that changes in tree functional composition during secondary succession reflect changes in plant functional attributes (traits) and these influence litter decomposition rates and input of C to the soil. Changes in tree functional composition during succession is generally characterised by a shift from acquisitive (light-demanding) species to more conservative (shade-tolerant) species, as plants adapt to increasing light and nutrient limitation (Wright *et al.*, 2004). My results partially support this general assumption as the proportion of ACC species was higher at young successional stages whereas the proportion of DEC species was highest in the old-growth forest, however the proportions of ACC and DEC species in mid-successional stands did not conform to the expected patterns of shifts in functional groups (Appendix A:Table S2.1).

These functionally distinct tree groups are associated with contrasting plant traits which can have a strong influence on soil C and nutrient cycling. Plant traits most relevant to soil C cycling are those which control the input and stabilisation of soil C and/or those that influence soil C loss, but there is often a 'trade-off' between the two. Light-demanding species are associated with 'competitor' traits (Grime, 1974) which can influence the input of C to soils through the generation of large amounts of nutrient rich organic matter due to their rapid growth rate and photosynthetic capacity, but C can be quickly released back to the atmosphere during more rapid litter decomposition (De Deyn, Cornelissen and Bardgett, 2008). Conversely, shade-tolerant species have traits which allow them to be more stress-resistant and produce longer-lived, but poor-quality (e.g. high C:N, high lignin:N) organic matter (Grime *et al.*, 1996) which inhibits litter decomposition (Kutsch, Bahn and Heinemeyer, 2009).

The functional tree groups used in this study, based on species-specific light response, are assumed to correspond well with the popular definitions of 'light-demanding'/pioneer and 'shade-tolerant'/climax species (Dent, DeWalt and Denslow, 2012) and be a proxy for acquisitive and conservative life-history strategies. Based on these assumptions, and as the quantity of litterfall was not seen to vary significantly between SF and OG stands (Denslow and Guzman, 2000), I expected that soil C input from litter might be more highly concentrated from DEC species than from ACC species (De Deyn, Cornelissen and Bardgett, 2008); and along with associated traits such as greater leaf toughness and the presence of defence compounds

(Bardgett, 2017) would decompose more slowly, resulting in a build-up of soil C in stands with a greater proportion of DEC species. However, the opposite was observed in these forest stands, with higher soil C found in stands with a greater proportion of light-demanding species.

The assumption that litter decomposition rates differ between the two contrasting tree functional groups is maintained (Cornwell *et al.*, 2008); but the relationship between litter decomposition rate and the incorporation of plant derived C into soil is often disconnected (Prescott, 2010; Cotrufo, Wallenstein and Boot, 2013). Plant litter decomposition drives SOM formation but does not directly control the accumulation and stabilisation of soil organic carbon (SOC), the largest component of SOM. Instead, a strong and growing body of research postulates that soil microbial products are the major contributors of C to SOM and that the products of successive microbial processes increase C stability in SOM through aggregation and chemical bonding in the soil matrix via what has been termed the Microbial Efficiency-Matrix Stabilisation framework (MEMS) (Cotrufo, Wallenstein and Boot, 2013). The MEMS framework hypothesises that the input of C from labile plant substrates (e.g. sugars) drive microbial processes and thus, the production of increasingly stable C compounds as they are more efficiently used by soil microbes than C from more recalcitrant plant material (Cotrufo, Wallenstein and Boot, 2013). My results support this hypothesis as soil C content and stocks were highest in stands with a higher relative influence of light-demanding ACC species (Figures 2.4a, 2.5a); the litter of which is expected to contain more labile carbon compounds than that of the more shade-tolerant DEC species.

Effect of former land-use and soil properties on soil carbon

The influence of former land-use is one possible explanation why studies of secondary tropical forest succession have not found a consistent pattern of increasing soil C with stand age (Martin, Bullock and Newton, 2013; Orihuela-Belmonte *et al.*, 2013). Previous soil disturbance, fertilizer application, or nutrient depletion by agricultural use have distinct and often contrasting effects on plant growth and soil C turnover, which can persist for decades (Detwiler, 1986) and obscure trends in soil C accumulation over time, but contrary to my third hypothesis, the influence of past land-use on soil C content or stocks was not apparent at any depth increment. The effect of disturbance (e.g. from ploughing) on initial soil C loss is well documented (Guo and Gifford, 2002; Don, Schumacher and Freibauer, 2011), and I therefore

expected to detect differences between the stands used for crops, those used for pasture and the OG stands, however, the characterisation of former land-use relied on brief descriptions compiled from largely anecdotal accounts (Denslow and Guzman 2000), and therefore soil disturbance is only inferred and thus, may not accurately reflect current soil conditions . The sites included in my study spanned a range of soil types and key soil properties, including differences in soil N and pH, which can also influence soil C storage. Previous work in the secondary forest stands of the chronosequence showed no relationship between soil C storage and land-use or the underlying geology, but a strong relationship between soil C and soil N stocks (Jones *et al.*, 2019). I observed a strong relationship between soil C, N and pH in the present study including the old-growth forest stands. These relationships are perhaps unsurprising, given that C and N are major components of soil organic matter (SOM) and soil pH is a key control of microbial community composition and decomposition processes (Fierer and Jackson, 2006; Tripathi *et al.*, 2016). In my study, the similar patterns of decline in soil C, soil N and soil pH with depth largely explain the relationships among these variables. Hence, neither land-use nor soil properties fully explain the patterns in soil C accumulation during secondary succession. A further possible explanation for the lack of clear differences in soil C among stands with distinct former land use and soil type is that subsequent forest regrowth over time has weakened the effects of past disturbance (Grimm *et al.*, 2008; Jones et al. 2019) and that input from present day vegetation has a greater influence on soil C storage.

Further research

The soil C content and stocks measured in this study provide only a snapshot observation and do not consider the rate of soil C turnover; particularly CO_2 release through microbial respiration, an important component of soil C dynamics in tropical forests. The rate of CO_2 efflux in tropical forests is largely determined by the mineralisation of labile organic C compounds by soil microorganisms during the decomposition of organic material. The differences in plant litter quality between tree functional groups may also result in differences in microbial community composition, which in turn can strongly influence soil C cycling. For example, the poor quality litter produced from conservative strategy (DEC) species should in theory, promote the preferential growth of fungi (rather that bacteria) which inhibits the cycling of C and nutrients

and thus leads to greater soil C storage, whereas high quality litter from acquisitive (ACC) strategists should encourage a greater proportion of bacteria in microbial communities during decomposition which is linked with more rapid C and nutrient cycling but also higher soil C loss (Bardgett, 2017). However, this theory has largely been developed from studies in grassland ecosystems and therefore these broad assumptions may not hold true in tropical forests, nevertheless, investigating changes in soil microbial community composition and function during tropical forest secondary succession may provide valuable insight into soil C cycling during secondary succession in recovering tropical forests. Another possible explanation for the positive relationship between soil C and light-demanding tree species is the effect of roots. Plant root traits influence soil C cycling through the release of labile C in to soil from root exudates, which stimulates microbial activity and the release of CO_2 back to the atmosphere and may also influence soil C cycling through traits related to nutrient foraging and associations with mycorrhizal fungi (De Deyn, Cornelissen and Bardgett, 2008). Root traits may help explain variation in soil C, and although sometimes shown to be only weakly coupled with leaf traits (De Deyn, Cornelissen and Bardgett, 2008), would most likely also be determined by tree functional group. Therefore, tree functional groups, characterised as light-demanding and shade-tolerant species, remain the best predictor of soil C content and stocks in these stands of naturally regenerating tropical forest.

2.6 Conclusions

This study identifies a strong link between the dominant functional group of trees and soil C content and stocks during tropical forest secondary succession and provides an interesting starting point for further research. Improving our ability to predict, and crucially, increase soil C accumulation during tropical forest regrowth will not only help improve global C models but help mitigate atmospheric CO_2 concentrations through more efficient forest management practices and reforestation initiatives. My results indicate that successional changes in tree functional groups during forest regrowth influence soil C content and stocks and specifically, that the relative influence of light-demanding tree species in a stand is positively correlated with soil C content and stocks at 0-10 cm depth. I propose that the broad distinction between ACC and DEC species and their relative importance in stands provides the basis for a useful, quantitative method to investigate how successional dynamics in tree communities and

associated plant functional traits influence ecosystem scale C dynamics in recovering secondary tropical forests and could be used to promote natural regrowth and influence species selection in forest restoration strategies.

3 Soil carbon dynamics are linked to tree species shade-tolerance along an age gradient of naturally regenerating tropical forest.

3.1 Abstract

Secondary regrowth forests are the dominant type of forest cover in the tropics and are thus increasingly important for their role in the global carbon (C) balance. During recovery, tropical secondary forests rapidly accumulate aboveground biomass and whilst substantial amounts of plant-derived carbon are assimilated belowground through decomposition processes, soil C dynamics during forest regrowth have received much less attention. During secondary succession, shifts in tree community growth strategies from light-demanding to shade-tolerant species are accompanied by changes in litter quality, which may influence rates of C turnover both directly, via litter decay rates, and indirectly via the influence of tree functional groups on the decomposition environment. To explore the links between tree functional traits and soil carbon dynamics, I conducted an *in situ* litter decomposition experiment across an age gradient of naturally regenerating tropical forest. I used litter mixtures created from tree species differing in their shade tolerance as a key functional characteristic, including a single-species and bare-soil control. I observed litter mass loss and soil respiration as measures of C turnover over a five-month period. As expected, litter from light-demanding species decomposed more rapidly than that of shade-tolerant species and there was a corresponding temporal response in soil respiration reflecting differences in litter decay rates. Surprisingly, there was no unidirectional effect of forest successional age on litter decay rates or soil respiration. However, soil respiration from the litter treatment containing both light-demanding and shade-tolerant species was significantly higher in the younger than older forest stands. This study highlights the potential importance of functionally diverse plant inputs for soil carbon dynamics in tropical forests. Links between litter traits and soil microbial communities could further clarify the role of functional diversity in soil C dynamics and storage during secondary tropical forest succession.

3.2 Introduction

Increasing importance of secondary forests

Tropical forests are the largest terrestrial C sink as they are responsible for around *c*. 40% of net primary production (NPP; Cleveland *et al.*, 2011) and store *c*. 45% of terrestrial C (Anderson-Teixeira *et al.*, 2016), sequestering around half of global anthropogenic CO_2 emissions (Yin, Wu and Li, 2018). Land-use change from human activity such as logging and agricultural expansion continues to remove vast areas of intact tropical forest annually (Chazdon and Guariguata, 2016), and has altered the functioning of remnant tropical forests, including above-ground C dynamics (e.g. Qie *et al.*, 2017). Agricultural abandonment, as part the common practice of swidden agriculture along with recovery from selective logging, has created a mosaic of naturally regenerating 'secondary' or 'regrowth' forest throughout the tropics. These recovering forests now make up over half of all tropical forests and due to current land-use practices, their increase is expected to continue (Chazdon, 2014). As such, regrowth forests have received much attention and are increasingly looked upon as crucial providers of tropical forest ecosystem services for both biodiversity conservation (Chazdon *et al.*, 2009) and climate regulation (Poorter *et al.*, 2016). Tropical forest regrowth can be an effective C sink due to rapid C accumulation in aboveground biomass (Pan *et al.*, 2011) which is estimated to reach 90% of intact forests after *c.* 66 years (Poorter *et al.*, 2016) . Hence, secondary tropical forests play an important role in mitigating the effects of rising atmospheric CO_2 concentrations.

Despite the importance of secondary forest regrowth for C sequestration, we know little about the mechanisms of C accumulation belowground (Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013). Soils make up the largest C pool in the terrestrial biosphere (Yang, Luo and Finzi, 2011) and account for over half of the C stock in tropical forests (Don, Schumacher and Freibauer, 2011). Tropical forest soils are an important part of the global C balance but our understanding of soil C dynamics during secondary forest regrowth is hampered by the complexity of biogeochemical processes and interactions, the lack of studies in tropical regions, and inconsistent patterns of soil C losses and gains during forest disturbance and recovery (Yang, Luo and Finzi, 2011; Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013;

Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017). Although the cycling and storage of C in tropical forest soils is partly determined by climate, soil characteristics and land-use history (Marín-Spiotta and Sharma, 2013) changes in the quality and quantity of plant-derived organic matter during forest regrowth are expected to have a significant impact on soil C dynamics.

Changes in species composition during succession and impact on soil C dynamics

Predictable and measurable changes in tree species composition and functional groups during secondary succession are largely driven by changes in the availability of resources (light, water, and nutrients) as forests mature. During early succession, an abundance of light reaches the forest floor and fast-growing 'pioneer' species out-compete slower-growing species to dominate the canopy. However, once the canopy closes (after *c.* 20 years; Denslow and Guzman, 2000), light becomes a limiting factor and conditions favour the slower-growing, shade-tolerant species that thrive in the understory. Over time, the shorter-lived pioneer species are gradually replaced by more long-lived species associated with old-growth forests, resulting in a progression towards a dominance of shade-tolerant species in later succession (Dent, DeWalt and Denslow, 2012; Chazdon, 2014; Whitfeld *et al.*, 2014). The shift from light-demanding to shade-tolerant species can also be characterised as a shift in the resource use strategy of the dominant tree species, from 'acquisitive' to 'conservative' along a fast-slow plant economic spectrum (Reich, 2014). This is reflected in a set of coordinated leaf functional traits (Conti and Díaz, 2013). Light-demanding species that invest in fast growth are characterised as having 'high quality' palatable leaves, which have high nutrient concentrations (e.g. N, P), high specific leaf area (SLA) and low content of structural compounds such as lignin (Wright *et al.*, 2004; Chazdon, 2014). Conversely, shade-tolerant species preferentially invest in structure, defence and longevity, resulting in leaves characterised as 'low quality', typically with low nutrient concentrations, high fibre and lignin content and greater concentrations of foliar defence compounds such as tannins and phenols (Wright *et al.*, 2004; Ostertag *et al.*, 2008). Hence, the shift from light-demanding to shade-tolerant tree functional groups during secondary succession is accompanied by marked changes in the quality of litter inputs, which will influence the rates of C and nutrient cycling both directly, via litter decomposition rates,

and indirectly via the influence of tree functional groups on the decomposition environment, for example, via nutrient exchanges with microbial communities Cornelissen *et al.*, 1999; Jewell *et al.*, 2017).

Influence of changing plant functional characteristics on litter decomposition and soil C turnover

Plant litter decomposition plays a fundamental role in terrestrial ecosystem nutrient cycles and C dynamics (Sayer, 2006; Prescott, 2010; Sayer and Tanner, 2010). During decomposition, C and nutrients are sequentially broken down and made available to plants, beginning with the more labile and soluble compounds (Kutsch, Bahn and Heinemeyer, 2009). This preferential break down of litter by decomposer organisms results in an early release of soluble nutrients and labile C forms and an accumulation of structural compounds and recalcitrant C in remaining litter over time (Krishna and Mohan, 2017). Plant-derived C compounds are either mineralised and returned to the atmosphere as CO_2 or immobilised in the soil matrix (Kutsch, Bahn and Heinemeyer, 2009) and this balance can eventually determine whether a forest functions as a C source or sink. Hence, even slight changes affecting the decomposition of plant material can have a significant effect on C dynamics (Sayer *et al.*, 2011). The rate of litter decomposition is driven by both abiotic (climate and soil), and biotic (litter quality and the decomposer community) factors. Although moisture and temperature are strong predictors of decomposition rates globally (Powers *et al.*, 2009), neither tend to be limiting in tropical rainforest environments, so plant litter quality is the dominant control on litter decomposition rates (Cornwell *et al.*, 2008; Hattenschwiler *et al.*, 2011). Consequently, changes in the quality of leaf litter inputs as a result of shifts in tree functional groups during secondary succession are likely to have a significant impact on litter decomposition rates and C turnover.

Despite a growing body of research assessing the relationship between the fast-slow continuum of the plant economic spectrum and C turnover, much of our knowledge is derived from temperate studies focussing on a limited number of species, which may not apply to highly diverse tropical forests. Although the influence of specific leaf traits, such as foliar N concentrations, on litter decomposition rates has been well studied (Santiago, 2007; Cornwell

et al., 2008; Bakker, Carreño-Rocabado and Poorter, 2011; Szefer *et al.*, 2017), there is a growing body of evidence to suggest that decomposition of species mixtures cannot necessarily be predicted from the decay rates of individual species (Gartner and Cardon, 2004; Gessner *et al.*, 2010; Jewell *et al.*, 2017). Decomposition rates of mixed species litter are often "non-additive", whereby the litter in mixtures decomposes more rapidly (synergistic) or more slowly (antagonistic) than would be expected based on the decay rates of individual component species. Non-additive effects likely arise as a result of nutrient transfer between different litter types, which facilitates the decomposition of more recalcitrant litter compounds (Hättenschwiler, Tiunov and Scheu, 2005), via the release of secondary metabolites which can inhibit decomposition (Chomel *et al.*, 2016), or due to increased variation in physical microhabitat and decomposer interactions (Hättenschwiler, Tiunov and Scheu, 2005). Hence, litter mixtures might capture more of the complex interactions between plant litter traits and decomposer communities and better represent the influence of tree functional characteristics on soil C dynamics at the stand or community level.

The functional characteristics and diversity of the living tree community also affect soil C dynamics by influencing the community composition and activity of decomposer organisms, particularly soil microbial communities that are the powerhouse behind litter decomposition processes (Prescott and Grayston, 2013). The presence and activity of soil microbes is strongly related to C turnover and storage, not only during the initial decomposition of plant material, but also because there is mounting evidence that soil microbial products are the major contributors of soil C (Cotrufo, Wallenstein and Boot, 2013; Liang, Schimel and Jastrow, 2017). The microbial C use efficiency (MEMS) framework (Cotrufo, Wallenstein and Boot, 2013) proposes that the products of successive microbial turnover increase C stability in soil organic matter (SOM) through aggregation and chemical bonding in the soil matrix. According to the MEMS framework, plant inputs of labile C compounds may play a greater role in soil C accumulation than previously assumed, as labile compounds are used more efficiently, which fuels microbial turnover and results in the production of increasingly stable C compounds (Cotrufo, Wallenstein and Boot, 2013) .

The results of Chapter 2 suggest that microbial turnover of labile plant material contributes to SOM formation during secondary succession, as soil C content and stocks were highest in secondary forest stands with a greater relative influence of light-demanding species, which are

expected to have easily-degradable litter containing more labile carbon compounds than shade-tolerant species. And, due to the expected corresponding shift in the abundance and activity of decomposer groups during succession; from labile C specialists to those more adapted to the breakdown of recalcitrant C compounds, we might expect higher rates of soil C turnover in the younger than older forest stands and a general decrease in soil C turnover and accumulation with increasing tree community shade tolerance.

Hence, changes in the functional characteristics of tree species during secondary forest succession might have a substantial impact on soil C dynamics and storage. A mechanistic understanding of the links between soil C dynamics and plant functional traits during secondary tropical forest succession could therefore provide crucial information for forest management to maximise soil C storage and help parameterise ecosystem models (e.g. Pugh *et al.*, 2019).

In the present study, I conducted an *in-situ* litter decomposition experiment across an age gradient of naturally regenerating tropical forest to explore the links between tree functional traits and soil carbon dynamics. I used litter mixtures from tree species differing in their shade tolerance as a key functional characteristic, and measured litter mass loss and soil respiration to represent C turnover to test the following hypotheses:

1. Litter mixtures from light-demanding tree species will represent a high-quality resource to microbial decomposers, with high nutrient but low structural fibre content, and will therefore decompose faster than litter mixtures from slow-growing shade-tolerant trees.

2. Respiration rates will reflect differences in patterns of litter mass loss, with higher respiration rates from litter mixtures representing light-demanding species, especially during the early stages of decomposition, and lower respiration rates from litter representing shade-tolerant species.

3. Based on the shift from light-demanding to shade-tolerant tree species during secondary succession, young forest stands will have higher rates of litter decomposition and soil respiration than old forest stands.

3.3 Methods

Study site

The experiment took place across four stands of naturally regenerating regrowth forest in an established chronosequence within the Barro Colorado National Monument (BCNM) Panama, Central America. The climate is classified as moist tropical with a distinct dry season from January to April with a mean annual temperature of around 27°C and an average annual rainfall of 2600 mm, of which 90% falls in the rainy season (Windsor, 1990). Soils are described as clay-rich oxisols and silty-clay alfisols on sedimentary and volcanic parent materials (Yavitt, 2000) but do not differ significantly in soil C and nutrients (Chapter 2; Yavitt, 2000; Grimm *et al.*, 2008). The BCNM chronosequence consists of permanent plots in 10 forest stands: two stands for each of four forest age classes of secondary forest (SF), and two old growth forest (OG) stands for comparison, each at least 5 ha in size. The OG stands are aged at >500 years old (Dent, DeWalt and Denslow, 2012) and secondary forest (SF) stands are currently 40, 60, 90 and 120 years old (Denslow and Guzman, 2000). I selected a subset of four stands (subsequently 40Y, 60Y, 90Y and OG; Table 3.1) to represent a gradient of secondary regrowth forest succession. Within each stand, I established five replicate blocks, spaced at least 20 m apart on level terrain, avoiding obvious disturbances (e.g. footpaths, canopy gaps, animal activity) as far as possible.

Table 3.1 Stand and soil characteristics for subset of four secondary forest stands (40, 60, 90 years old and OG) in Panama, Central America used in a litter decomposition experiment; where RI ACC = relative abundance of accelerating growth tree species, RI DEC = relative abundance of decelerating growth tree species, BA = total tree basal area, C = total soil carbon, N = total soil nitrogen, C:N = carbon:nitrogen ratio and pH = soil pH. Soil characteristics are given as means and standard error for n = 8 at 0-10 cm sampling depth. Superscript numbers indicate the data source, where 1 = The geological composition of the Panama Canal Watershed (STRI GIS Laboratory), 2 = Denslow and Guzman (2000), and 3 = Chapter 3 of this thesis. Different super-script letters indicate significant differences among forest age classes at $p < 0.05$ determined by ANOVAS with Turkey post-hoc comparisons and correction for multiple comparisons.

Forest age (years)	Geology[1]	Land-use[2]	RI ACC (%)[3]	RI DEC (%)[3]	BA (m^2/ha)[3]	C Stock (Mg/ha^2)[3]	C (%)[3]	N (%)[3]	C:N[3]	pH[3]
40	Tb	Swidden	43.37	42.70	21.62	29.75 (± 9.20)	5.22 (± 0.81)	0.42^{ab} (± 0.09)	12.83^{ab} (± 0.98)	5.69^{b} (± 0.16)

60	Tb	Plantation	26.15	56.15	22.06	24.53 (± 3.56)	4.81 (± 0.35)	0.44^{a} (± 0.04)	11.10^{ab} (± 0.38)	6.39^{a} (± 0.09)
90	Tcv/Tcm	Swidden	19.77	68.75	40.29	18.05 (± 3.35)	3.76 (± 0.35)	0.26^{b} (± 0.03)	14.35^{a} (± 0.44)	5.68^{b} (± 0.18)
OG	Tb	Old growth	10.10	75.22	25.92	19.95 (± 0.91)	3.99 (± 0.09)	0.40^{ab} (± 0.01)	10.06^{b} (± 0.41)	5.78^{ab} (± 0.14)

Litter treatments representing tree functional groups

To create functionally distinct litter mixtures, I used species-specific data on tree growth response to increasing light (Rüger *et al.* 2009) to represent tree community shade tolerance. Briefly, species were assigned to one of two growth-response categories; 'accelerating' or 'decelerating' growth with increasing light based on a light effect parameter ranging from -0.6 to 3.3 (Rüger *et al.* 2009; Chapter 2). Accelerating species (light effect >1) represent light-demanding, resource acquisitive 'pioneer' species, whereas decelerating species (light effect <1) represent shade-tolerant, resource conservative species (Rüger *et al.* 2009). To test the hypothesis that changes in tree functional groups during secondary succession explain differences in decomposition rates and soil respiration I created five distinct litter treatments: 1) litter from species with an accelerating growth response to increasing light levels (ACC); 2) litter from species with a decelerating growth response to increasing light levels (DEC); 3) a mixture of ACC and DEC species (MIX); 4) natural mixed litter unique to each forest stand (NAT); and 5) a non-forest standard litter, *Saccharum spontaneum* L. (STD). I also included bare soil controls in each experimental block. Henceforth, the ACC, DEC and MIX litters are referred to collectively as 'functional litter treatments', whereas the NAT and STD litters are referred to as 'standard litter treatments'.

To ensure that the litter treatments represented changes in tree functional groups during succession, while reflecting species composition at the study site, I used the following criteria to choose species within each growth response category, based on tree census data for the study sites: (a) the species showed a clear trend (positive or negative) in relative abundance with forest age; (b) the species were representative of earlier or later stages of succession based on other studies, and (c) the species' leaf traits are considered broadly representative of successional stage. Based on the above criteria, the ACC treatment included litter from *Luehea*

seemannii Triana & Planch and *Miconia argentea* (Sw.) DC. to represent a young, light-demanding, secondary forest community and the DEC treatment included litter from *Protium panamense* (Rose) I.M.Johnst. and *Tetragastris panamensis* (Engl.) Kuntze to represent a shade-tolerant, old secondary or old-growth forest community (Figure 3.1; Table 3.2). The MIX treatment included all four species to represent intermediate successional stages.

The tree species *Luehea seemannii* (Figure 3.1a) is described as one of the dominant species of secondary forests in all areas on the Pacific half of the Panama Canal Area. It also occurs sparsely in old-growth forest as a large tree but rare as a sapling, only appearing in natural tree-fall clearings (Condit, Pérez and Daguerre, 2011). *Miconia argentea* (Figure 3.1b) is described as being one of the most abundant species of secondary forests in the Panama Canal Area, occurring only where there is light in natural clearings within the forest (Condit, Pérez and Daguerre, 2011). *Protium panamense* (Figure 3.1c) is described as a widespread species on the Caribbean half of the isthmus of Panama and abundant at Barro Colorado, occurring only in the forest interior (Condit, Pérez and Daguerre, 2011). Finally, *Tetragastris panamenis* (Figure 3.1d) is described as a very widespread species in Panama and is one of the dominant trees in the old-growth forest canopy on BCI, with abundant saplings in understorey (Condit, Pérez and Daguerre, 2011). In addition, as a standard litter treatment (STD), I included *Saccharum spontaneum* L., locally known as 'Paja Blanca' (white straw), a C_4 grass that was introduced to the area to prevent soil erosion along the Panama Canal but does not occur within the forests.

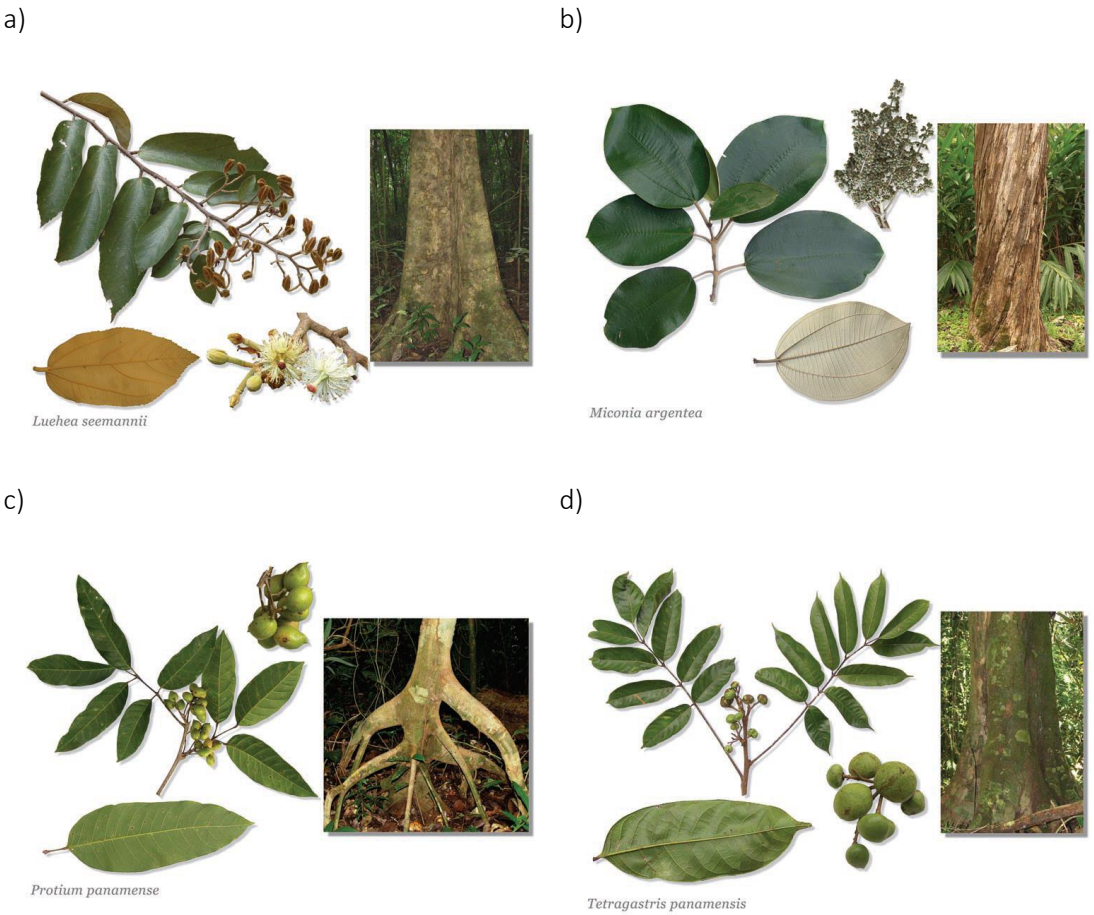


Figure 3.1 Leaves, flowers, fruit and trunk of the four tree species a) *Luehea seemannii*, b) *Miconia argentea*, c) *Protium panamense* and d) *Tetragastris panamensis*, which were selected to represent a,b) light-demanding trees common in young secondary tropical forest stands and c,d) shade-tolerant trees older secondary forest and old growth tropical forest stands, in Panama, Central America. Source: (Condit, Pérez and Daguerre, 2011).

The selected species representative of younger secondary forest functional communities generally decreased in IV % with increasing forest age across the chronosequence and have relatively high foliar nitrogen content high specific leaf area (SLA), and relatively low leaf mass area (LMA) and leaf density (Table 3.2), which is regarded as characteristic of more light-demanding species (Chazdon, 2014). Conversely, the selected species representative of older secondary/old growth forest tree communities generally increased in IV with increasing forest

age and have comparatively low foliar nitrogen, low SLA, and relatively high LMA and leaf toughness (Table 3.2).

Table 3.2 Selected functional characteristics of four tree species used to create three functionally distinct litter treatments (ACC, DEC and MIX) for a litter decomposition experiment in the BCNM Panama, Central America, where; mean (b) = species specific light effect parameter indicating either accelerating or decelerating growth (>1= accelerating growth (ACC), <1= decelerating growth (DEC), SLA = specific leaf area, LDMC = leaf dry matter content. Data source indicated with superscript 1 = Ruger *et al*, (2009), and superscript 2 = S.J. Wright *et al*, (2012) accessed from the TRY Database (http://www.try-db.org).

Species name	Species code	Litter treatment	Mean (b)[1]	Leaf density[2] (g cm^{-3})	Leaf N[2] (mg/g)	SLA[2] (mm^2/mg^-)	LDMC[2] (g/g)
Luehea seemanni	LUEHSE	ACC, MIX	2.00	0.30	22.38	17.02	0.42
Miconia argentea	MICOAR	ACC, MIX	1.87	0.31	20.63	14.44	0.32
Protium panamense	PROTPA	DEC, MIX	0.67	0.50	16.39	11.04	0.41
Tetragastris panaensis	TET2PA	DEC, MIX	0.43	0.61	15.69	10.00	0.49

Initial litter collection, processing, and analyses

I collected freshly fallen litter for the four tree species (LUEHSE, MICOAR, PROTPA, TET2PA) during the dry season from February to April 2017, which coincided with the period of highest litterfall and minimised decomposition prior to collection. Litter was collected from the forest floor at least once a week to ensure fresh samples, avoiding specimens with visual signs of decay or disease, and species identity was double-checked in the laboratory. To obtain representative samples, I collected litter from beneath *c.* 10 individuals of each species within the age class of forest stand they represented. I also collected a mixture of freshly fallen leaves in each of the five blocks per stand which were then homogenised across the stand to create a stand-specific natural mixed litter (NAT) treatment, and the non-forest standard litter (*Saccharum spontaneum)* from three locations spaced *c.* 20 m apart within the BCNM. To characterise the litter from the four species and evaluate possible intra- and interspecific variation, I also collected replicate samples of freshly fallen litter for each species from a total of five different

individuals (or groups of individuals, where it was not possible to distinguish the litter among closely grouped individuals) from age-representative stand(s) for litter nutrient analyses. All litter samples were oven-dried separately for 48 hours at 65°C to constant weight directly after collection. To achieve a standard size of litter fragments for the decomposition experiment, I removed petioles and cut larger leaves into pieces of <10 cm length. I then created the three functional litter treatments (ACC, DEC, MIX) using equal mass of the constituent species (Table 3.3).

Table 3.3 Treatments used in a decomposition experiment in Panama, Central America, showing the proportions and total dry weight (g) of each litter type in three functional litter treatments, and the total dry weight of litter for natural litter and standard litter treatments.

Treatment	*Description*	*Species*			
		LUEHSE	MICOAR	PROTPA	TET2PA
1. ACC	Accelerating species	50% (6 g)	50% (6 g)		
2. DEC	Decelerating species			50% (6 g)	50% (6 g)
3. MIX	Mix of ACC and DEC	25% (3 g)	25% (3 g)	25% (3 g)	25% (3 g)
		Natural litter	STD		
4. NAT	Natural litter	100% (12 g)			
5. STD	Standard litter		100% (12 g)		
6. CTL	Bare soil control				

To determine the initial chemical and fibre content of litter treatments, I ground a subset of air-dried litter from each species to < 1 mm. One composite sample from each species was analysed by HNO_3 digestion and ICP-OES detection for total mineral elements: Phosphorus(P) Potassium(K), Calcium(Ca), Magnesium(Mg), Sodium(Na), Manganese(Mn), Zinc(Zn), Aluminium (Al), Boron(B), Chromium (Cr), Copper(Cu), Iron(Fe) and Nickel(Ni) at the soil laboratory of the Smithsonian Tropical Research Institute in Panama, and a further two samples were analysed for P, K, Ca and Mg plus selenium (S) at a commercial laboratory (Central Analytical Laboratory, SRUC Veterinary Services, Midlothian UK). I analysed total litter C and N from five replicates per species using high temperature combustion gas chromatography on a Vario El III C/N analyser (Elementar, Stockport, UK) at Lancaster University. I determined total fibre and lignin content from three replicate samples per species using the acid detergent extraction described by Van Soest, Robertson and Lewis (1992); I analysed one set of samples at Lancaster University by a two-step extraction method to determine acid detergent fibre

(ADF) and acid detergent lignin (ADL). Briefly, 1 g of ground sample was placed in a crucible with 1 g of acetanilide and boiled for 1 hour with 100 ml of acid detergent solution (ADS) and four drops of n-Octanol using a FOSS fibertec™ 8000 fibre analysis system (FOSS, Hilleroed Denmark). Samples were rinsed with distilled water until acid-free and soaked with reagent-grade acetone then dried overnight at 105°C before weighing. Weighed samples were then soaked in in 25 ml H2SO4 (72%) and stirred every hour for 3 hours, rinsed with hot distilled water and dried overnight at 105°C. The samples were then placed in a furnace at 525°C for 3 hours then left to cool in desiccators at room temperature before weighing. To calculate total extracted fibre content (ADF), the weight of the processed sample was subtracted from the original sample weight. Lignin content was determined by subtracting the weight of the sample from the final stage from the weight of total extracted fibre (ADF). Both stages were corrected using blanks. To provide sufficient replicates, another two sets of samples were analysed using the acid detergent extraction method (ADF and ADL) and the neutral detergent extraction method (NDF and NDL) at a commercial laboratory, (Central Analytical Laboratory, SRUC Veterinary Services, Midlothian UK).

Mesocosm installation

To link measurements of litter decomposition and soil respiration, I used *in situ* mesocosms to delimit the experimental area from surrounding soil and litter. Mesocosms are an effective method to measure both litter decomposition and soil respiration in a single system; they provide comparable litter mass loss measurements to litter-bags while minimising disturbance and maintaining natural environmental conditions (Laird-Hopkins *et al.*, 2017). The mesocosms were 20 cm diameter PVC tubes cut to *c.* 15-cm lengths and sunk into the soil to a depth of 2 cm (Figure 3.2). The mesocosms were installed and natural litter removed in April 2017, at least two weeks before the first measurements to allow the soil to recover from initial disturbance. I installed 12 mesocosms in each of the five replicate blocks per stand, with two mesocosms for each of the five litter treatments and two mesocosms without litter (bare soil controls; CTL). One of the two mesocosms per treatment was used for repeated monthly measurements, and the second (duplicate) was used for destructive sampling during the experiment. Hence, I

installed a total of 240 mesocosms (four stands, five replicate blocks, six treatments, two sets), giving *n* = 5 replicate values for each measurement and treatment. To contain the litter treatments within the mesocosms I used mesh 'baskets' (10-mm plastic mesh), which provided maximum contact between the litter and the soil during decomposition but also allowed for the removal of the litter to measure mass loss during the experiment (Laird-Hopkins *et al.*, 2017) and to take belowground respiration (SR_B) measurements from the underlying mineral soil (Figure 3.3). To calculate the appropriate dry weight of litter to use in the decomposition experiment, I collected standing litter from the forest floor in August 2016. I sampled four points in two stands of each forest age class by placing a 20-cm diameter section of PVC tube on the forest floor and cutting carefully cut around the outer edge to separate the sample from the surrounding litter. The samples were dried for 48 hours at 65˚C and weighed to determine the average litter standing crop dry weight per unit area. In May 2017, each basket received 12 g dry weight of litter based on the average standing litter dry weight measured across the chronosequence and the tops of the mesocosms were covered with mesh (10-mm) to minimise additional inputs of natural litter.

Figure 3.2 Soil mesocosms in of one of five replicate blocks, used for a litter decomposition experiment across an age gradient in four forest stands of naturally regenerating tropical forest in the Barro Colorado Nature Monument, Panama, Central America.

Figure 3.3 Experimental mesocosm showing the mesh basket which allowed the removal of the contained litter treatment for mass loss and soil respiration measurements during a litter decomposition experiment in the Barro Colorado Nature Monument, Panama, Central America.

Field measurements

To investigate the effects of functionally distinct litter treatments on soil and litter-derived CO_2 efflux from microbial respiration during litter decomposition I took monthly measurements of soil respiration (CO_2 efflux) from May to October 2017 (6 months) using an infra-red gas analyser attached to a 20-cm diameter soil survey chamber (Li-8100; LI-COR Biosciences, Lincoln, NE, USA). I partitioned total soil respiration (SR) into litter-derived respiration (SR_L) and belowground respiration (SR_B), by first measuring SR over the decomposing litter, then removing the baskets with litter from each mesocosm to measure SR_B, and estimating SR_L from the difference between SR and SR_B. To limit the effects of disturbance and CO_2 trapped below the litter, I waited *c*. 20 minutes (E. Sayer, pers. comm.) after the removal of litter baskets

before measuring SR_B. Measurements of SR_B and SR_L were not made in August due to time constraints. To account for the influence of soil moisture and temperature on respiration rates, I measured soil water content at 0-6 cm depth using a Thetaprobe (Delta-T Devices, Cambridge, UK) at three points within 1 m of each mesocosm, and soil temperature at a depth of 0-10 cm in the same area using a soil temperature probe (Fisher Scientific, Leicestershire, UK).

Sample collection

To assess the decomposition of the litter treatments and their influence on soil properties, I collected soil and litter samples from the duplicate mesocosms after four months of decomposition in September 2018 and from the remaining mesocosms at the end of the study in October 2018. The second sampling occurred earlier than planned because of substantial mass loss from two of the litter treatments (ACC and STD). All samples were sealed in individual plastic bags, transported to the laboratory within two hours, stored at 4°C and processed within two days.

Litter sampling and decay rate calculation

I removed the baskets with the remaining litter from each mesocosm and sealed them in individual plastic bags. To measure litter mass loss and decay rate, I first weighed the samples in 'field condition' then carefully picked through the litter on a clean sheet of aluminium foil to remove any large clumps of soil and extraneous material before weighing them again. I then weighed a subsample of this litter in a pre-weighed tin and washed it carefully for 2 minutes to remove any remaining soil particles before drying at 60°C for 48 hours and weighing again to calculate litter dry mass (g).

The litter decay rate *k* was calculated from the dry mass of litter remaining in each mesocosm at the end of the experiment using the following equation (Olson 1963):

$$\ln\left(\frac{X}{X_0}\right) = -k\mathrm{t} \qquad \text{Eq.1}$$

Where: *t* is time in years, *X* is litter dry mass at collection, X_0 is the initial litter dry mass (12 g).

Litter mass loss data was used to show comparisons in figures but as it was more normally distributed, I used decay rate data in models.

Soil sampling and analysis

I collected three soil cores (0-5 cm depth) from each mesocosm using a 4.8-cm diameter punch soil corer (after first carefully removing the litter layer), to make one composite sample per mesocosm plus a representative 'block' soil sample from outside of the mesocosms at the end of the experiment to allow comparison of soil from experiments with undisturbed 'natural' soil (4 stands x 5 blocks x 7 treatments = 140 samples in each duplicate set). Fresh subsamples were taken from each composite sample to determine soil moisture content and pH. To determine soil total C and N content, I ground subsamples of homogenized, air-dried soil using a ball mill (Mixer Mill 400, Retsch®, Haan, Germany). Total C and N was analysed on 30 mg soil samples by high temperature combustion gas chromatography on a Vario El III C/N analyser (Elementar, Stockport, UK) at Lancaster University. Extractable P and K were measured using the modified Morgan's method at a commercial laboratory (Central Analytical Laboratory, SRUC Veterinary Services, Midlothian UK) and soil pH was measured on a 1:3 mixture of fresh soil and deionised water using a STARTER 2100 Bench pH meter (OHAUS, New Jersey, USA) or Mettler Toledo® Seven Compact® pH meter (Leicester, UK).

Data analysis

All statistical analyses were performed in R version 3.5.2 (R Core Team, 2018). Data were log-transformed where necessary to meet model assumptions. First, to isolate the overall influence of stand on litter decay rates, I compared means from the final time-point decay rate data for the standard litter treatments (NAT and STD) using linear models (*lm* function). I then assessed the influence of treatment and stand (and their interaction) on the decay rates of functional litter treatments (ACC, DEC and MIX) in linear models, dropping non-significant (p-value > 0.05) terms sequentially to achieve a minimum adequate model (Crawley, 2007).

To assess which of the litter mixtures best represented natural forest litter in each stand, I used linear models to compare the decay rate of natural (NAT) litter with those of the three functional litter treatments for each stand separately. To assess the influence of stand on SR, I compared five months of active decomposition data (thus excluding May) for the bare soil controls (CTL) and the STD litter treatments using linear mixed effects models (*lmer* function; lme4 package (Bates *et al.*, 2015)) with month and replicate block modelled as a random effects and stand as a fixed effect. Then, to assess broad temporal patterns in SR_L between functional litter treatments in reference to litter decay rate and mass loss, I calculated mean SR values for two decomposition stages (early = June and July, late = September and October) and assessed the effects of stand, functional litter treatment and decomposition stage on SR_L using linear mixed effects models with stand, litter treatment and decomposition stage and their interactions as fixed effects, and month and replicate block as random effects. Significance was determined by sequentially dropping terms until a minimum adequate model was reached, using AIC and p-values to check for model improvement. I used Tukey's honest significant difference (HSD) for *post hoc* comparisons among factors in linear models (*lm*). Statistics for all linear mixed effects models (*lmer*) are given for the comparison of the final model to the corresponding null model using likelihood ratio tests and p-values for individual factors were derived by Satterthwaite's approximation using the package lmerTest (Kuznetsova, Brockhoff and Christensen, 2017) and the model fit was assessed using diagnostic plots (Crawley, 2007).

3.4 Results

Mean nutrient content variation among standard and functional litter treatments

Litter chemical properties varied significantly among functional treatments. In support of my first hypothesis, the ACC species litter treatment had significantly higher P, K, Mg, Na, S, Ca, Cu and Zn than DEC species, but lower content of acid detergent fibre (ADF) and neutral detergent fibre (NDF; Table 3.4). Total litter C content differed significantly among all treatments and surprisingly, was highest in the ACC and lowest in the DEC treatment ($F_{3,16}$ = 156.3, p < 0.001; Table 3.3). Total N was significantly higher and the C:N ratio was significantly lower in the STD treatment than all other litters but did not vary significantly among the functional litter

treatments. As expected, the chemical concentrations in the MIX litter samples are intermediate between the ACC and DEC species.

Table 3.4 Litter properties from three functional litter treatment mixtures and a standard litter treatment used in a decomposition experiment in the BCNM, Panama, Central America; where ACC = light-demanding (accelerating growth) species, DEC = shade-tolerant (decelerating growth) species, MIX = mixture of light-demanding and shade-tolerant species, and STD = non-forest standard litter treatment. ADF = acid detergent fibre, NDF = neutral detergent fibre, TC = total carbon, TN = total nitrogen, CN ratio = carbon:nitrogen, L:N ratio = lignin:nitrogen. Litter properties given as means and standard errors for *n* = 5 (TC, TN, C:N ratio), *n* = 3 (P, K, Mg, Na, Ca, Fe, Zn), *n* = 2 (ADF, NDF, S) and *n* = 1 (lignin, L:N ratio). Different super-script letters indicate significant differences among litter treatments at $p < 0.05$, determined by ANOVAs with Tukey post-hoc comparisons and correction for multiple comparisons. STD treatment consists of single species '*Saccharum spontaneum'*. See table 3.3 for species and treatment descriptions.

	Litter treatment			
	ACC	DEC	MIX	STD
ADF (%)	46.58 [b]	57.30 [a]	51.94 [b]	51.13 [b]
	(±3.08)	(±0.23)	(±1.42)	(±4.23)
NDF (%)	39.23 [d]	46.65 [b]	42.94 [c]	75.10 [a]
	(±0.18)	(±0.55)	(±0.36)	(±0.20)
Lignin (%)	20.50	22.43	21.46	4.00
TC (%)	43.19 [a]	39.63 [d]	41.41 [c]	42.16 [b]
	(±0.15)	(±0.14)	(±0.06)	(±0.11)
TN (%)	1.15 [b]	1.01 [b]	1.08 [b]	1.68 [a]
	(±0.10)	(±0.05)	(±0.07)	(±0.05)
C:N Ratio	38.76 [a]	39.68 [a]	39.22 [a]	25.20 [b]
	(±2.92)	(±1.87)	(±2.39)	(±0.82)
L:N Ratio	17.53	22.43	21.46	2.38
P (mg/g)	0.58 [b]	0.33 [d]	0.45 [c]	0.94 [a]
	(±0.02)	(±0.03)	(±0.02)	(±0.03)
K (mg/g)	5.69 [b]	1.37 [d]	3.53 [c]	15.37 [a]
	(±0.25)	(±0.21)	(±0.23)	(±0.47)
Mg mg/g	3.44 [a]	2.60 [c]	3.02 [b]	0.99 [d]
	(±0.12)	(±0.15)	(±0.13)	(±0.10)
Na (mg/g)	1.55 [a]	0.92 [b]	1.24 [ab]	0.59 [c]
	(±0.07)	(±0.24)	(±0.16)	(±0.07)
S (mg/g)	3.22 [a]	1.03 [c]	2.13 [b]	2.32 [b]
	(±0.39)	(±0.08)	(±0.23)	(±0.05)

Ca (mg/g)	16.52 [a]	14.35 [c]	15.44 [b]	2.08 [d]
	(±0.63)	(±0.37)	(±0.50)	(±0.20)
Cu (mg/g)	8.04 [a]	4.59 [c]	6.31 [b]	8.91 [a]
	(±0.22)	(±0.49)	(±0.34)	(±0.22)
Fe (mg/Kg)	63.84 [b]	75.69 [b]	69.76 [b]	120.72 [a]
	(±6.63)	(±13.47)	(±9.95)	(±14.50)
Zn (mg/Kg)	41.93 [a]	8.23 [d]	25.08 [b]	17.15 [c]
	(±2.81)	(±0.92)	(±0.96)	(±0.93)

The influence of stand on decomposition of standard litter treatments

There was a strong influence of forest stand on the decomposition of the two standard litters STD and NAT (Figure 3.4; Table 3.5) but there was no clear directional relationship between decay rate and stand age. Overall, litter decayed at higher rates in the 60Y stand, and lower rates in the 90Y stands. STD litter decomposed faster than NAT litter across all forest stands and hence, the decay rates of standard litters among stands was best represented by the model including treatment and stand (without interaction; $F_{7,26}$ = 10.96, $p < 0.01$), which explained *c.* 71 % of variation. The decay rate in the 60Y stand was significantly higher than in the 40Y and 90Y stands for STD ($F_{3,10}$ = 6.56, p = 0.010) and the 90Y stand for NAT ($F_{3,16}$ = 5.54, p = 0.008) when analysed separately, but decay rates did not differ significantly among the other stands.

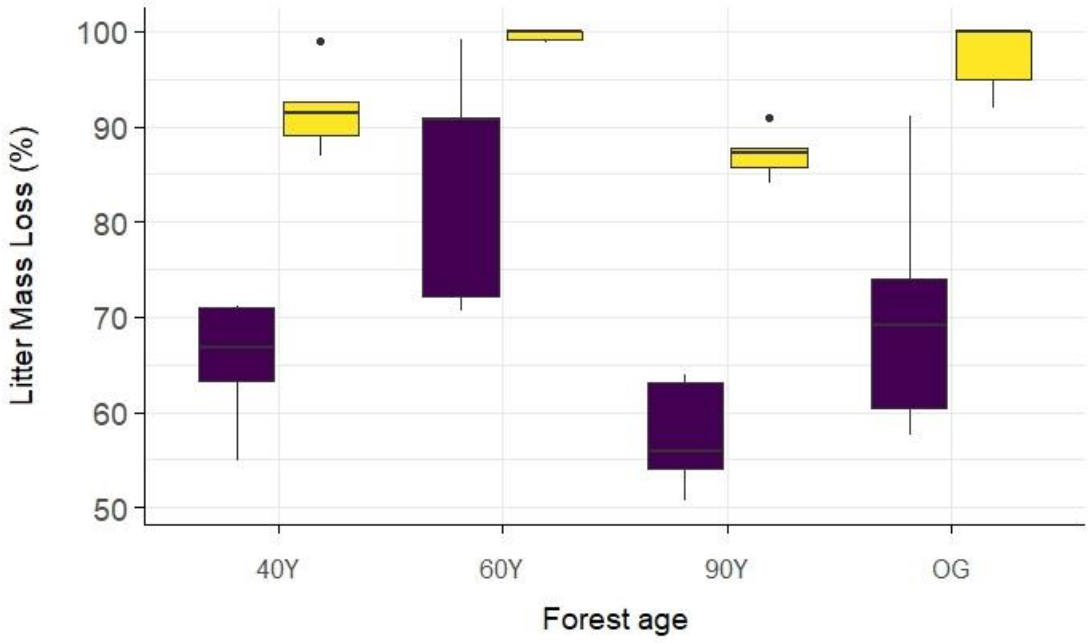


Figure 3.4 Litter mass loss (%) of two litter treatments after five months of a litter decomposition experiment across an age gradient of four naturally recovering tropical forest stands in the BCNM Panama, Central America. Non-forest standard litter (STD) = yellow, stand specific natural litter (NAT) = dark blue. Boxes denote the 25th and 75th percentiles and median lines are given for *n* = 5, whiskers indicate values up to 1.5 x the interquartile range, and dots indicate outliers.

Table 3.5 Litter decay rates (*k*) for three functional litter treatments and two standard litter treatments across an age gradient of four tropical forest stands (40, 60, 90 year old SF and OG) after five months of a litter decomposition experiment in the BCNM Panama, Central America; where DEC = shade-tolerant (decelerating growth) species litter, ACC = light-demanding (accelerating growth) species litter, MIX = mixture of decelerating and accelerating species litter, NAT = stand specific natural litter and STD = non-forest standard litter. Litter decay rates (*k*) given as means and standard error for n= 5. Significant differences between stands indicated with uppercase superscript and between litter treatments (excluding STD) with lowercase superscript determined by Tukey *post-hoc* analyses at $p < 0.05$.

	Functional litter treatments			*Standard litter treatments*	
Forest age	DEC	ACC	MIX	NAT	STD
OG	$1.02^{c\ AB}$ (± 0.15)	$5.14^{a\ AB}$ (± 1.29)	$2.27^{b\ AB}$ (± 0.29)	$3.24^{ab\ AB}$ (± 0.68)	6.63^{AB} (± 0.57)
90Y	$0.98^{c\ AB}$ (± 0.10)	$2.47^{a\ B}$ (± 0.26)	$1.73^{b\ B}$ (± 0.10)	$2.08^{ab\ B}$ (± 0.15)	4.97^{B} (± 0.23)
60Y	$1.41^{c\ A}$ (± 0.07)	$6.35^{a\ A}$ (± 1.59)	$3.73^{b\ A}$ (± 0.46)	$5.81^{ab\ A}$ (± 1.55)	10.90^{A} (± 0.38)
40Y	$0.90^{c\ B}$ (± 0.06)	$2.85^{a\ AB}$ (± 0.19)	$1.70^{b\ B}$ (± 0.14)	$2.59^{ab\ AB}$ (± 0.20)	6.66^{B} (± 1.59)

Stand and treatment effect on the decay rate of functional litter treatments

There was a clear separation in the decay rates of functional litter treatments in all stands and although there was no directional pattern with stand successional stage, there was a significant effect of forest stand on litter decay rates. Therefore, the model containing treatment and stand (without interaction) was found to best explain variation in the decay rates of functional litter treatments ($F_{5,53} = 43.36$, $p < 0.001$; Figure 3.5). Decay rates differed significantly among functional litter treatments: across all stands, ACC decomposed fastest and DEC slowest, with intermediate rates of mass loss for MIX litter (treatment effect: $p = < 0.01$; Figure 3.5; Table 3.5). Overall, the decay rates for all functional litter treatments were highest in the 60Y forest and lowest in the 90Y forest (stand effect: $p = 0.02$) but differences among stands varied with

litter treatment: decay rates in the 60Y stand were significantly higher than the 90Y stand for the ACC (p = 0.021) and MIX (p < 0.001) treatments and significantly higher than in the 40Y forest for the DEC (p = 0.029) treatment. The decay rate of MIX litter was also significantly higher in the 60Y than 40Y (p < 0.001) forest stand.

The comparison of decay rates between natural (NAT) litter and functional litter treatments demonstrated that the decay rate of NAT litter lay between the decay rates of MIX and ACC litter, but were consistently greater than the decay rates of DEC litter across all forest stands (Table 3.5).

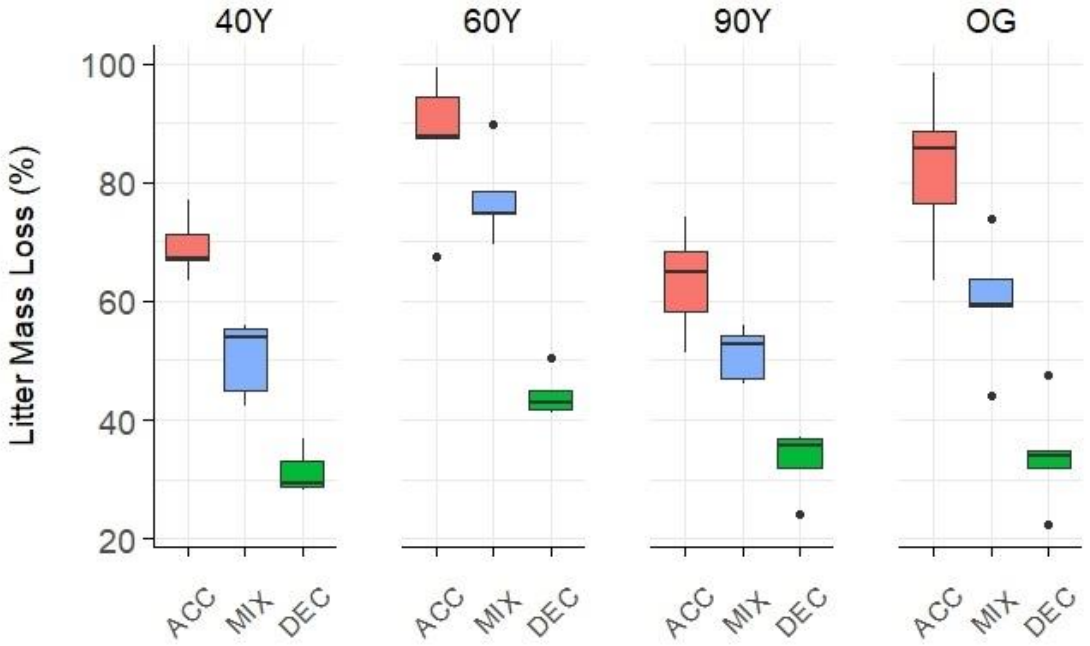
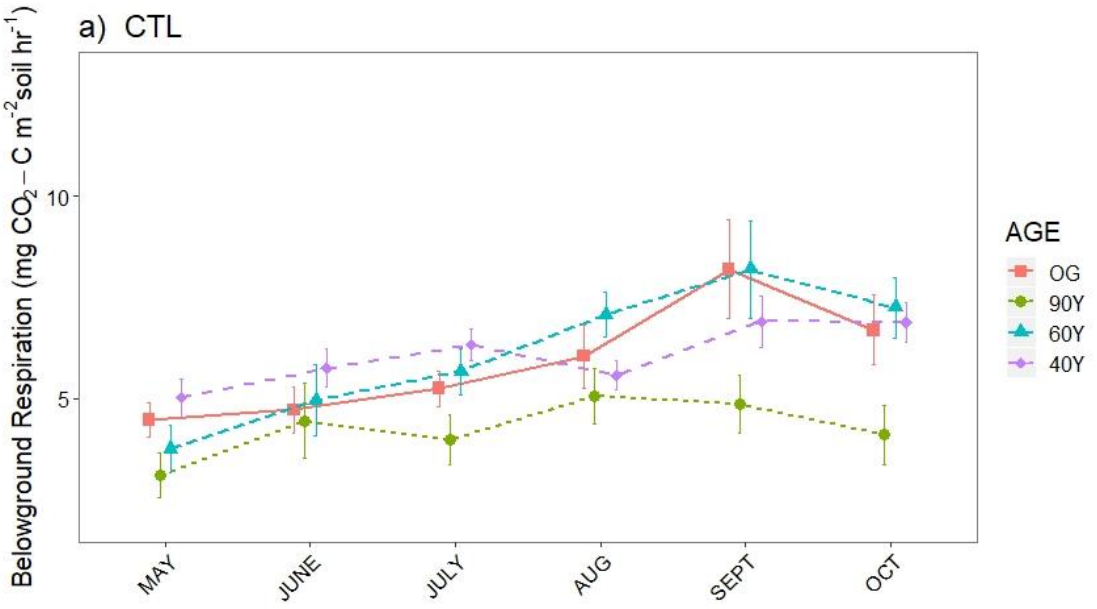


Figure 3.5 Litter mass loss of three functionally distinct litter mixtures after five months of decomposition across an age gradient of tropical forest in Panama, Central America; ACC = light-demanding, accelerating growth species (red), DEC = shade-tolerant, decelerating growth species (green), and MIX = a mixture of light-demanding and shade-tolerant species (blue). Boxes denote the 25th and 75th percentiles and median lines are given for n = 5, whiskers indicate values up to 1.5 x the interquartile range, and dots indicate outliers. See table 4.2 for treatment descriptions. 40Y, 60Y and 90Y refer to stand ages (years since last disturbance), OG refers to old growth, undisturbed forest.

Influence of forest stand on total soil respiration

The comparison of respiration rates across stands demonstrated that SR from the STD and NAT litter treatments, and SR_B from the CTL treatments differed significantly among stands but there was no pattern with stand age across the whole time series, with month modelled as a random effect. SR_B generally increased throughout the experiment and was significantly lower in the 90Y forest than the other three stands (χ^2 = 32.91, p < 0.001; Figure 3.6a), which did not differ. SR from the STD litter treatment was significantly lower in the 90Y stand and significantly higher in the 60Y stand compared to the 40Y and OG stands, and significantly higher in the 40Y stand compared to the OG stand (χ^2 = 47.19, p < 0.001; Figure 3.6b). SR from the NAT treatment was significantly lower in the 90Y compared to the 60Y and 40Y stands and significantly higher in the 60Y stand than the two older stands (χ^2 = 12.79, p = 0.005; Figure 3.6c).



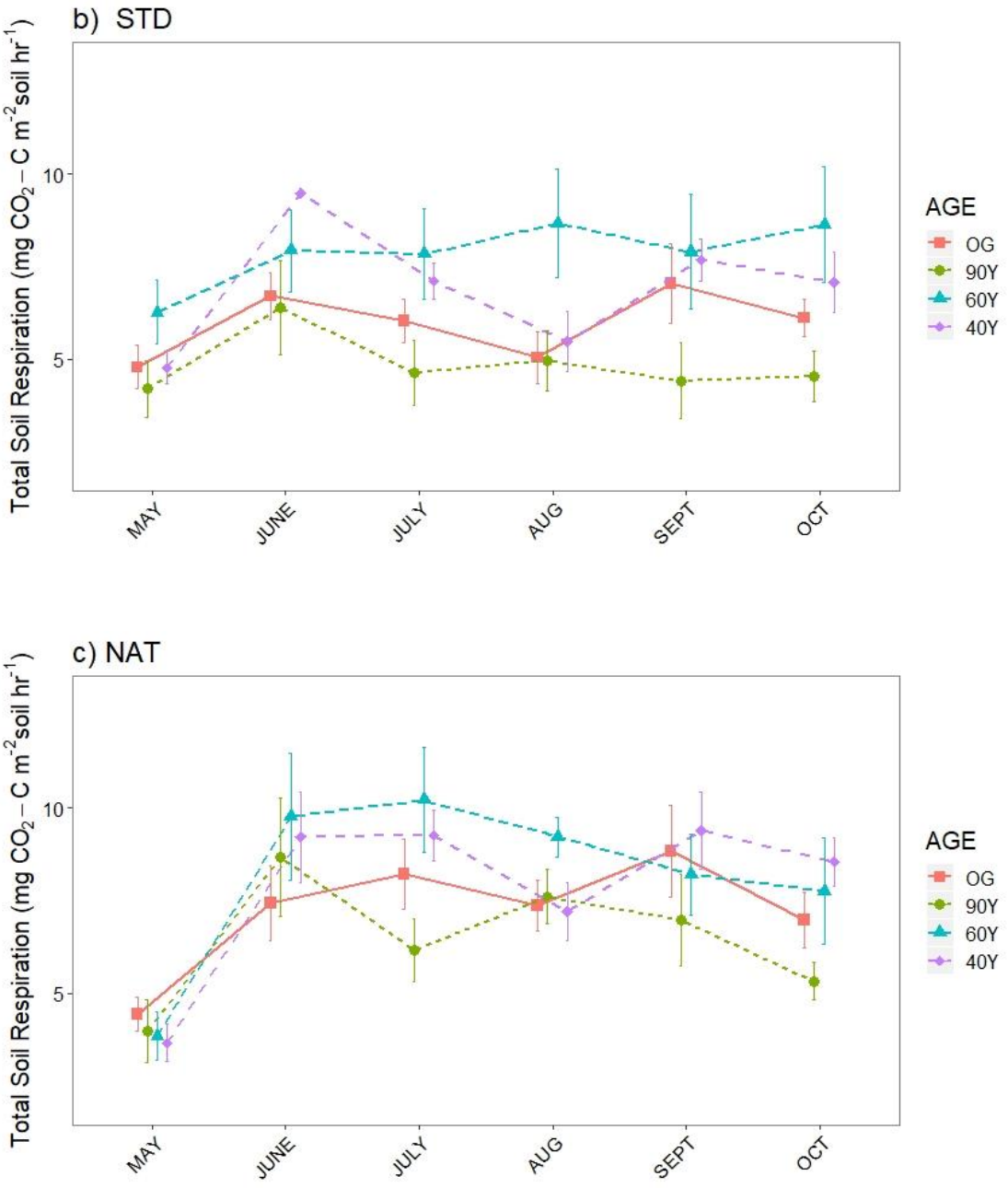


Figure 3.6 Soil respiration during six months of a litter decomposition experiment across an age gradient of four naturally regenerating tropical forest stands in Panama, Central America; OG = red squares, 90Y = olive green circles, 60Y = turquoise blue triangles and, 40Y = purple diamonds. Separate figures show a) belowground respiration (SR_B) from the bare soil control (CTL) treatment, b) total soil respiration (SR) from the decomposition of non-forest standard litter (STD) treatment and c) total soil respiration from the decomposition of natural litter (NAT). Means and standard errors are shown for *n* = 5 per time point. Full six months respiration data shown but active litter decomposition commenced in June, hence respiration data from May was excluded from analyses for the STD and NAT treatments

Influence of functionally different litter treatments on total soil respiration during decomposition

Across all stands, the temporal patterns of SR differed among functional litter treatments: SR from the DEC treatment increased from the first month of decomposition (June) until the end of the experiment (October) whereas the ACC treatment generally increased until July but declined towards the end of the experiment (Figure 3.7). SR from the MIX treatment initially tracked the SR of the ACC treatment but was more similar to SR from the DEC treatment in the final month of the study (Figure 3.7).

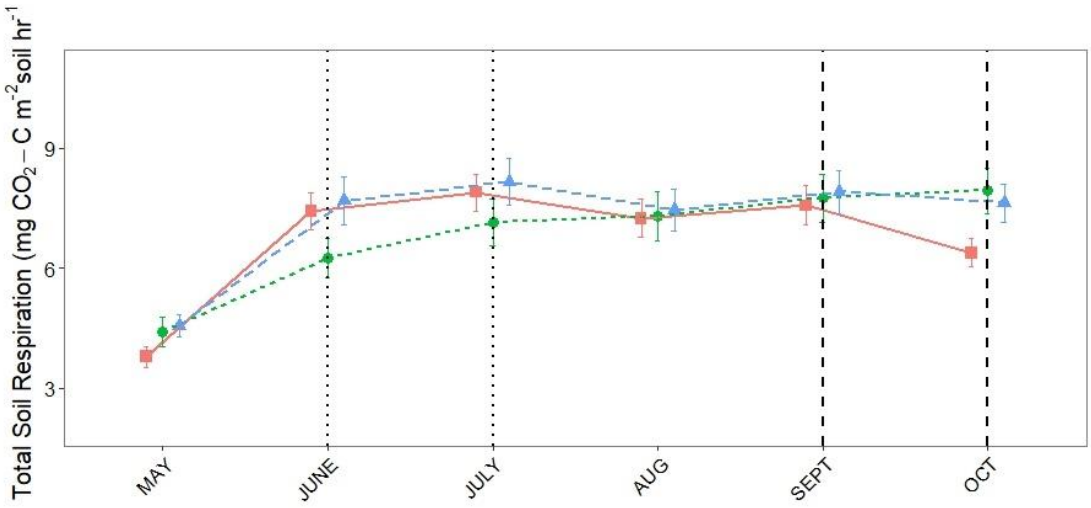


Figure 3.7 Mean soil respiration (SR) of three functional litter mixtures: light-demanding, accelerating growth species (ACC) = red squares, shade-tolerant, decelerating growth species (DEC) = green circles, and a mixture of light-demanding and shade-tolerant species (MIX) = blue triangles (see table 4.2 for description), from four forest stands measured over six months of a litter decomposition experiment in the BCNM Panama, Central America. Dotted lines indicate 'early' and dashed lines indicate 'late' decomposition stages. Full six months respiration data shown but active litter decomposition commenced in June, hence respiration data from May was excluded from analyses.

Functional litter treatment and forest stand influenced SR during decomposition and the effect differed among stands (Figure 3.8) therefore, the model that best explained variation in SR included litter treatment, stand and their interaction (χ^2 = 56.94, p < 0.001). This interaction was largely explained by the significantly higher SR in the MIX treatment in the two younger (40Y and 60Y) stands compared with the two older (90Y and OG) stands (χ^2 = 40.24, p < 0.001; Figure 3.9c). SR in the ACC treatment was significantly lower in the 90Y stand than other stands and significantly higher in the 40Y than the OG stand (χ^2 = 19.1, p < 0.001; Figure 3.9a). SR in the DEC treatment was significantly higher in the 60Y than other stands and significantly lower in the 90Y than OG stand (χ^2 = 19.46, p < 0.001; Figure 3.9b).

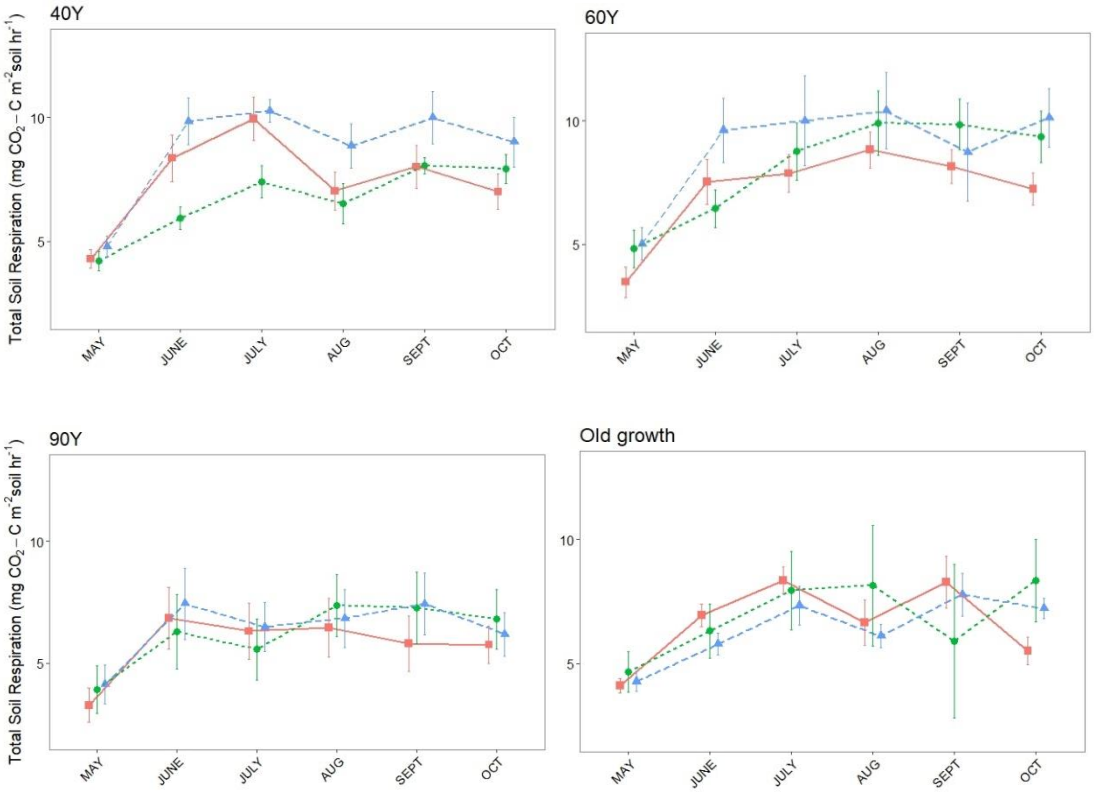


Figure 3.8 Comparison of total soil respiration (SR) during the decomposition for three functionally different tree litter mixtures: light-demanding species (ACC) = red squares, shade-tolerant species (DEC) = green circles, and a mixture of ACC and DEC (MIX) = blue triangles, among three stands of naturally recovering tropical forest (aged, 40, 60 and 90 years) and an old growth stand, measured over six months of a litter decomposition experiment in the BCNM Panama, Central America. Full six months respiration data shown but active litter decomposition commenced in June, hence respiration data from May was excluded from analyses.

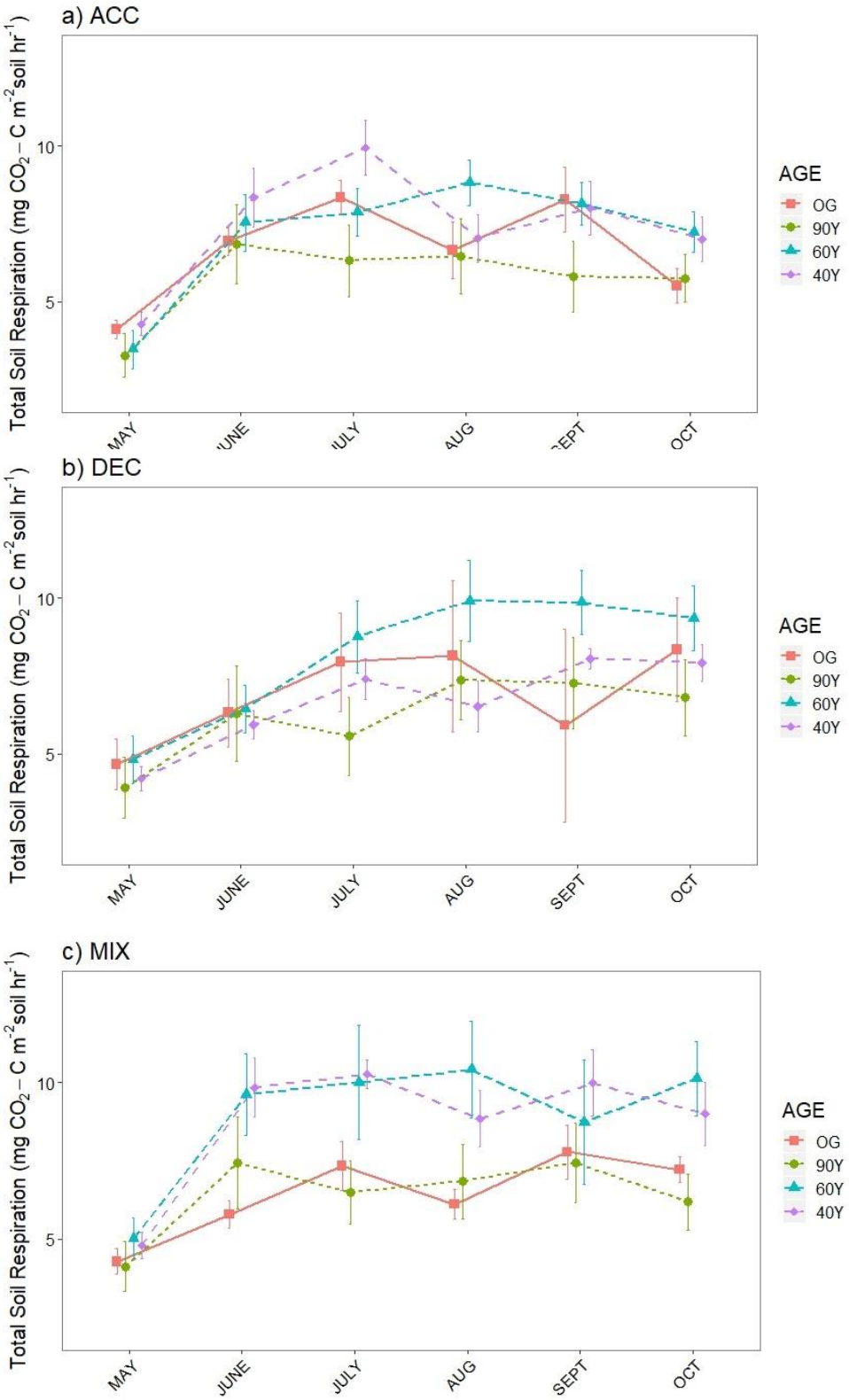

a) ACC
b) DEC
c) MIX
Total Soil Respiration (mg CO_2 – C m^{-2}soil hr^{-1})
10
5
MAY
JUNE
JULY
AUG
SEPT
OCT
AGE
OG
90Y
60Y
40Y

Figure 3.9 Soil respiration from three functional litter treatments during six months of a litter decomposition experiment across an age gradient of four naturally regenerating tropical forest stands in Panama, Central America; OG = red squares, 90Y = olive green circles, 60Y = turquoise blue triangles and, 40Y = purple diamonds. Separate figures show a) litter from light-demanding, accelerating growth species (ACC), b) litter from shade-tolerant, decelerating growth species (DEC) and c) mixed litter from light-demanding and shade-tolerant species (MIX). Means and standard errors are shown for n = 5 per time point. Full six months respiration data shown but active litter decomposition commenced in June, hence respiration data from May was excluded from analyses

Analysis of the litter-derived portion of respiration (SR_L) using mean values per decomposition stage confirmed the change in highest respiration rates between early and late stage decomposition for ACC and DEC treatments. In early decomposition (June and July) SR_L from ACC was significantly higher than DEC (but not MIX), however, in late stage decomposition (September and October) SR_L differed significantly between all functional treatments: DEC > MIX > ACC. The effect of stand was also an important predictor for variation in SR_L due to significantly higher SR_L in the 40Y stand than the other stands and marginally significantly higher SR_L in the 60Y stand than two older stands (p = 0.057). Therefore, the model that best explained variation in SR_L included treatment, decomposition stage (and their interaction) and forest stand (χ^2 = 44.68, p < 0.001; Figure 3.10).

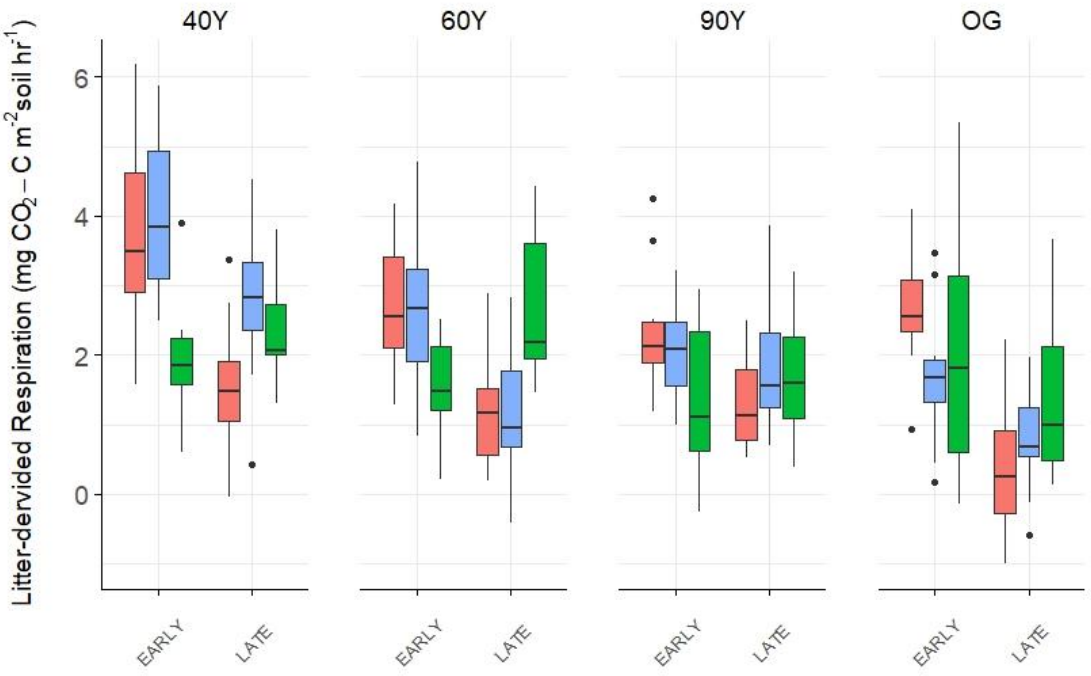


Figure 3.10 Mean litter-derived respiration (SR_L) for three functional litter mixtures: light-demanding, accelerating growth species (ACC) = red, a mixture of light-demanding and shade-tolerant species (MIX) = blue. and decelerating growth species and shade-tolerant (DEC) = green, across an age gradient of four naturally regenerating tropical forest stands measured at two decomposition stages: 'Early' (June and July) and 'Late' (September and October) during a litter decomposition experiment in the BCNM Panama, Central America. 40Y, 60Y, 90Y and OG refer to stand ages. Boxes denote the 25th and 75th percentiles and median lines are given for *n* = 10, whiskers indicate values up to 1.5 x the interquartile range, and dots indicate outliers.

3.5 Discussion

Litter from light-demanding 'ACC' species decomposed more rapidly than that of shade-tolerant 'DEC' species and there was a corresponding temporal response in SR and SR_L reflecting differences in litter decay rates. However, despite evidence to support the first two hypotheses, there was no clear successional trajectory for litter decay rates or SR along the age gradient of tropical forest stands.

Differences in litter decay rates are related to distinct traits of tree functional groups

Litter quality and decay rates conformed to expectations based on the differences in growth strategies between tree functional groups. As hypothesised, the ACC litter treatment representing early-successional light-demanding species had higher nutrient concentrations and lower content of structural fibre than the DEC treatment, which represented late-successional shade-tolerant trees. The differences in litter quality clearly reflect the shift in resource investment of trees during secondary succession, from fast-growing acquisitive to slow-growing conservative strategies (Chazdon, 2014; Reich, 2014). The differences in litter quality of the ACC and DEC treatments were reflected in the faster decay rates of ACC litter and slower decomposition of DEC litter. The decay rates of MIX litter were largely additive, i.e. appeared to be intermediate as compared with the other two treatments. Differences between the decay rates of accelerating and decelerating species litter in this study are comparable with the decay rate of 'pioneer' and 'old growth' species litter mixtures at a nearby study site (Laird-Hopkins *et al.*, 2017), thus supporting the assumption that species in this study are representative of early and late succession.

Surprisingly, some litter properties differed between the two single species in each functional litter mixture more than expected, resulting in lower variability between the composite litter treatments of the two opposing functional mixes than anticipated. For example, the ACC species *Luehea seemannii* had higher ADF and NDF values than the DEC species TET2PA, and the N content of TET2PA was closer to the ACC species MICOAR than the DEC species PROTPA (Appendix B Table S3.1). Since the experiment dealt with a relatively small species pool, and because traits tend to be highly coordinated (e.g. Wright et al., 2004) it was not possible to formally test the influence of specific traits on decomposition rates. In BCNM, litter P and K concentrations and lignin:N may be particularly important; these traits varied significantly between ACC and DEC species litter and in a fertilization experiment conducted in the BCNM, litter decomposition increased with P addition whereas cellulose (the primary constituent of leaf litter) decomposition increased with both P and K (Kaspari *et al.*, 2008). Similarly, litter P and K content have been shown to be more important than N in explaining litter decomposition in a nearby forest stand (Kerdraon *et al.*, 2020). This effect may be partly explained by the low

concentrations of P and K found in BCNM soils (Barthold, Stallard and Elsenbeer, 2008; Kaspari *et al.*, 2008), thus increasing the importance of litter-derived nutrients as a source for decomposer organisms. Hence, the litter properties and decay rates in the present study suggest that leaf traits related to the life-history strategy of tree functional groups have a considerable influence on litter decomposition, providing evidence to support my first hypothesis.

Similar patterns of litter decomposition and soil respiration across stands

Soil respiration rates largely mirrored litter decomposition rates across stands and among litter treatments. High respiration rates in bare-soil controls indicate that SR is strongly influenced by belowground processes (e.g. root respiration from dominant trees), which will contribute to the sizeable differences in SR among stands. Nonetheless, the rates of SR and litter decomposition followed much the same order among stands: 60Y > 40Y ≥ OG > 90Y. In further support of my second hypothesis, the differences in SR_L among the functional litter treatments during early- and late-stage decomposition demonstrated the strong influence of different litter types on soil microbial activity via the accessibility and availability of labile compounds from decaying plant material (Berg and McClaugherty, 2007). Higher SR and SR_L from ACC species litter during the early stages of decomposition correspond to rapid loss of highly soluble compounds during the first month of decomposition (Kutsch, Bahn and Heinemeyer, 2009) whereas higher SR and SR_L from DEC litter during the later stages of decay could reflect the greater amount of substrate remaining, coupled with greater activity of slow-growing microbes capable of degrading tougher C compounds (Waldrop MP , Balser TC, 2000; Kutsch, Bahn and Heinemeyer, 2009). Hence, the differences in SR from ACC and DEC litter during early- and late-stage decomposition likely reflect the sequential breakdown and processing of labile C during decomposition (Kutsch, Bahn and Heinemeyer, 2009; Powers *et al.*, 2009).

Limited evidence for declining soil C turnover with stand age

Soil C turnover is strongly linked to plant growth rates and the quality of plant inputs (Schlessinger and Andrews, 2000; De Deyn, Cornelissen and Bardgett, 2008). Based on the increasing relative influence of shade-tolerant tree species with forest age among the four stands, and the observed relationship between the relative influence of light-demanding (ACC) species and soil C (content and stocks) at 0-10 cm (Table 3.1), I therefore expected that C turnover would be fastest in younger stands, and this would be reflected in higher rates of litter decay and SR in younger compared to the older forest stands.

In contrast to my initial hypothesis, variation in decay rates and SR of standard treatments among stands was not explained by shifts in tree functional groups among forest age classes, as the highest decay rates were recorded in the 60Y stand, and the lowest in the 90Y stand. Surprisingly, despite wide variation in soil C content and the relative importance of tree functional groups (Table 3.1), there was no difference in litter decay rates between the youngest (40Y) and oldest (OG) forest stands. Similarly, there was no clear successional trajectory of SR, although variation among stands generally mirrored those for litter decay rates with the highest values recorded in the 60Y stand and lowest in the 90Y stand.

Rather than forest age, local soil conditions may have been especially important in determining decay and SR rates. Higher rates of decay and soil respiration in the 60Y stand can be at least partly attributed to higher soil water content, which likely boosted decomposition at the start of the experiment and during a period of low rainfall in September and October (STRI Physical Monitoring Program) and may partially explain the significantly lower decay rates and SR in the 40Y compared to the 60Y stand. Similarly, slightly lower soil temperatures in old-growth forests are often a result of reduced light levels in the understorey (Denslow and Guzman, 2000; Lebrija-Trejos *et al.*, 2010), which may also help maintain litter moisture levels optimum for decomposition. Hence, although my third hypothesis of declining decomposition rates and soil respiration with forest age was rejected, it is possible that the influence of stand-level functional development was confounded by differences in microclimate among stands.

Differences in key soil properties among stands also contribute to the observed patterns of litter decomposition and soil respiration. Soil C and N content were highest in the 40Y and 60Y stands (Chapter 3; Jones *et al.*, 2019), which will contribute to the higher rates of SR by

sustaining greater microbial biomass (Fierer *et al.*, 2009). Correspondingly, the lower rates of decay and SR in the 90Y stand coincide with lower soil N content compared to all other stands (Table 3.1). Nitrogen availability can constrain decomposition processes and C turnover as cellulose degradation often increases with the increasing availability of N whereas lignin degradation can be confounded by N availability (Kutsch, Bahn and Heinemeyer, 2009), which may particularly influence early stage litter decomposition. Finally, variation in C turnover rates among stands could also be explained by differences in other soil chemical and nutrient properties and their relationship with soil microbial community composition and function. For example, higher soil pH in the 60Y stand (Table 3.1) could also contribute to the higher rates of litter decay and SR in this stand compared to the others, as soil pH exerts a strong influence on soil microbial community composition (Fierer and Jackson, 2006; Rousk, Brookes and Bååth, 2009), which in turn controls rates of C turnover (Schimel and Schaeffer, 2012). Given the strong links between soil properties and tree functional composition at my study sites (Chapter 2), it is not possible to disentangle the influence of tree functional composition on soil C turnover from the influence of soil properties, however, it is clear that both act in concert to shape decomposition processes during secondary forest succession.

Using functional litter treatments to represent the influence of tree functional groups during succession

Although the functional litter treatments represented theoretical shifts in litter traits during secondary succession, the comparison between litter treatments and natural litter across stand revealed the importance of functional diversity in stands of all ages. As the proportion of light-demanding (ACC) and shade-tolerant (DEC) species shifts towards a dominance in DEC species with increasing forest age (Table 3.1), I expected the ACC treatment to be more representative of the younger forest and the DEC treatment more representative of older forest litter. As such I anticipated the decay rate and SR from natural (NAT) litter would be closer to that of the ACC species litter in the younger (40Y and 60Y) stands but closer to DEC species litter in the older (90Y and OG) stands. Although the decay rate of NAT litter was most similar to ACC in the youngest (40Y) forest stand, NAT litter in the two oldest stands (90Y and OG) and the 60Y stand

decomposed twice as fast as DEC litter, and slightly faster than MIX litter (Table 3.5). It was surprising that the decay rate of NAT litter was not significantly different from ACC litter in any of the stands, particularly the two oldest stands, which had a higher proportion of DEC species. A possible explanation for this is that NAT litter from each stand was collected during the dry season, which is when the majority of litterfall occurs in this region (Wieder and Wright, 1995); and although research remains somewhat unclear, particularly in the tropics, it is feasible that litter collected during the dry season could contain a predominance of deciduous species leaves compared with the rest of the year. Leaves from deciduous species are generally characterised as being short lived with low structural investment and fast decomposition rates (Cornelissen, 1996) and thus could explain why NAT litter decomposed more rapidly than expected.

Interestingly, although there was no clear successional trajectory for litter decay rate or SR from standard litter treatments (CTL, STD and NAT), SR from the MIX treatments was significantly higher in the younger than older stands (Figure 3.9c). Functionally diverse litter mixtures such as the MIX treatment might be better able to capture differences in C turnover among functionally distinct tree communities because the treatment targets the broadest range of microbial species and processes. For example, a study in Canada using a functionally diverse litter treatment with four common species, revealed significant differences in decomposition rates among communities differing in species diversity (Jewell *et al.*, 2017). In the present study, all the forest stands contained a mixture of ACC and DEC species, and there was also wide variation in litter traits between species within the same functional mixture (Appendix B: Table S3.1). Hence, mixed litter from both old growth (shade-tolerant) species and 'pioneer' (light-demanding) species may provide a more realistic and directly comparable litter treatment to assess C turnover across highly diverse forest stands.

3.6 Conclusions

Based on the shifts in tree species composition from light-demanding to shade-tolerant species during secondary succession, and the assumption that chemical and physical traits of leaf litter reflect differences in species life-history strategies, I expected that rates of litter decomposition and soil respiration would decline with forest stand age. My study demonstrated that the decay rate of litter mixtures representing light-demanding and shade-tolerant species followed the

expected patterns of mass loss and influenced soil respiration rates. Although there was no clear pattern of declining C turnover with forest age, I identified interactions between litter mixtures and stand properties that indicate changes in soil C dynamics with shifts in plant traits during succession, but also that local soil environmental factors have a considerable role to play. My research highlights the potential importance of functionally diverse plant inputs for soil microbial activity in tropical forests, and future work on the links between litter traits and soil microbial communities could further clarify the role of functional diversity in soil C dynamics and storage during secondary tropical forest succession.

4 Soil microbial community composition is related to tree community shade-tolerance and soil chemical properties

4.1 Abstract

Secondary regrowth forests are increasingly important for their role in the global carbon (C) balance. Despite containing over half of all tropical forest C, our understanding of C storage and cycling in forest soils during secondary succession remains limited but is likely to be impacted by the shift in tree community resource-use strategy from light-demanding to shade-tolerant species and the reduction in litter quality. I hypothesise that this change in litter quality influences rates of soil C turnover via changes to soil microbial abundance and structure, and specifically a shift towards K-strategist dominated decomposer microbial communities with decreasing organic matter quality. This study assessed relationships between soil microbial community composition, tree community shade-tolerance and soil C turnover along a successional gradient of recovering tropical forest stands in Panama. Overall, stands dominated by light-demanding tree species had higher microbial biomass but the prediction that stands dominated by shade-tolerant species would have higher relative abundances of K-strategist decomposers was not supported. Although there was no clear trajectory of shifts in microbial community structure with forest age across the chronosequence, results revealed there were significant differences in microbial biomarker groups between the OG and SF stands, and that litter decomposition and soil respiration rates decreased with an increasing ratio of gram-positive to gram-negative bacteria.

These results demonstrate a clear link between tree community shade-tolerance and soil microbial community structure across stands of different ages and suggest that more localised relationships exist between soil microbial communities and tree functional composition, which influence decomposition and soil respiration. As soil microbes play a fundamental role in soil C cycling and storage, understanding the relationship between aboveground tree community functional groups and the composition and activity of belowground microbial communities will allow us to better predict chances in soil C dynamics during tropical forest regrowth.

4.2 Introduction

Tree functional changes during tropical forest succession

Secondary forests are the dominant forest cover in the tropics (Chazdon, 2014) and they play an important for role in global carbon (C) dynamics as they can rapidly accumulate C in aboveground biomass during regrowth (Pan *et al.*, 2011). Although a clear pattern of increasing C during forest regrowth is rarely observed belowground (Yang, Luo and Finzi, 2011; Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017), it is estimated that a larger proportion of C is stored belowground in tropical forest soils than in aboveground vegetation (Don, Schumacher and Freibauer, 2011), demonstrating that soil C is another critical component of global C dynamics. One potential explanation for why soil C does not increase predictably with forest age is that the influence of tree community composition on biogeochemical processes is more important for soil C accumulation than forest age *per se* as tropical forests regenerate (Chapter 3). Hence, differences in soil C storage among forest sites might be expected due to differences in tree communities during secondary succession. The trajectory of tree community composition can be influenced by a multitude of factors including the degree and cause of original disturbance, soil physicochemical properties and dominance of invasive plant species (Chazdon, 2014) and as such, the functional characteristics of dominant tree species and average community level traits may differ within and among secondary forest stands of the same age (Norden *et al.*, 2015; Boukili and Chazdon, 2017).

Tropical forest secondary succession is typically characterised by a shift in resource-use strategy from fast-growing light-demanding species in early succession, towards a dominance of slow-growing shade-tolerance species in later succession (Dent, DeWalt and Denslow, 2012; Chazdon, 2014; Whitfeld *et al.*, 2014). The shift in species dominance along this fast-slow resource-use continuum during secondary succession is reflected in corresponding shifts in plant functional traits (Conti and Díaz, 2013), which are likely to have a significant influence on the cycling of C and nutrients in forest soils via decomposition processes. Changes in tree functional traits, as the community shifts from light-demanding to shade-tolerant species

(Cornelissen *et al.*, 1999; Jewell *et al.*, 2017) from 'high quality litter', with high nutrient concentrations and relatively high content of more labile C forms (Wright *et al.*, 2004; Chazdon, 2014) to 'low quality' litter, typically with low nutrient concentrations, high fibre and lignin content and greater concentrations of foliar defence compounds such as tannins and phenols which can inhibit decomposition (Wright *et al.*, 2004; Ostertag *et al.*, 2008). Differences in litter quality alter resource availability to soil faunal communities. Hence, differences in functional characteristics between light-demanding and shade-tolerant species, are expected to influence the abundance, composition and activity of soil microbial communities via changes to the quality of organic matter entering the soil food web (Bardgett *et al.*, 2005; Kutsch, Bahn and Heinemeyer, 2009).

Soil microbial community responses to changing resource quality

Soil microbial communities have a significant influence on C dynamics as they are the powerhouse behind organic matter decomposition in soils (Prescott and Grayston, 2013) and ultimately determine the rate of plant-derived C that is either mineralised and returned to the atmosphere as CO_2 or immobilised in the soil (Kutsch, Bahn and Heinemeyer, 2009). Microbial biomass is a key driver of biogeochemical processes and is strongly linked to substrate availability (Kutsch, Bahn and Heinemeyer, 2009), whereby total microbial biomass tends to increase with increasing soil organic matter content (Yao *et al.*, 2000) and litter-derived soluble C content (Fanin, Hättenschwiler and Fromin, 2014) thus suggesting higher rates of microbial C turnover in nutrient-rich substrates. Changes in resource quality may thus have important implications for soil C sequestration, as evidence suggests that labile C compounds are used more efficiently and thus stimulate microbial turnover which results in the production of increasingly stable C compounds (Cotrufo, Wallenstein and Boot, 2013). This process is postulated in the Microbial Efficiency Matrix Stabilisation (MEMS) Framework which states that the products of successive microbial turnover increase C stability in soil organic matter (SOM) through aggregation and chemical bonding in the soil matrix (Cotrufo, Wallenstein and Boot, 2013; Liang, Schimel and Jastrow, 2017). Although decomposition rates can be strongly influenced by moisture and temperature (Powers *et al.*, 2009), in humid tropical forests, the strongest factor determining decomposition rates is the quality of plant-derived organic matter (Marín-Spiotta and Sharma, 2013).

In addition to microbial biomass, microbial community composition and relative abundances of different microbial functional groups can also reveal important information about belowground functioning and changes in resource quality. The metabolization of easily degradable compounds, from nutrient rich organic matter, is generally associated with small-bodied and fast-growing microorganisms termed zymogenous or r-strategists, whereas degradation of more complex structural compounds found in nutrient poor organic matter, requires the activity of larger and slower-growing microorganisms termed autochtonous or K-strategists (Kutsch, Bahn and Heinemeyer, 2009; Zhou *et al.*, 2017). Fungi are typically regarded as K-strategists as they are capable of degrading all biologically formed compounds including recalcitrant polymers such as lignin via the production of enzymes (Kutsch, Bahn and Heinemeyer, 2009). By contrast, bacteria (excluding actinomycetes) often lack enzymes capable of degrading complex biopolymers (Kutsch, Bahn and Heinemeyer, 2009) and may instead metabolise the products of fungi-biopolymer decomposition (Urbanová, Šnajdr and Baldrian, 2015) and are therefore generally considered as r-stategists (Zhou *et al.*, 2017). Nonetheless, different bacterial groups such as Copiotrophic or Gram-negative bacteria (Gneg: e.g. Proteobacteria and Bacteroidetes) and Oligotrophic or Gram-positive bacteria (Gpos; e.g. Acidobacteria and Actinobacteria) are also considered to correspond to r- and K-strategists, respectively (Fierer, Bradford and Jackson, 2007). Given the distinct capacities of microbial functional groups to utilise different resources, the abundance, activity and structure of the microbial decomposer community is expected to change as the quality of organic matter inputs shifts from a predominately nutrient-rich substate containing labile organic compounds (e.g. sugars) to an increasingly complex, nutrient-poor substrate containing a higher proportion of recalcitrant compounds (e.g. lignin). Hence, we would expect a shift from a Gneg-dominated microbial community in forest stands with a high proportion of fast-growing light-demanding tree species, towards a more fungal-dominated microbial community and an increasing relative abundance of Gpos bacteria in stands dominated by shade-tolerant trees.

It is important to consider scale when comparing tree and soil communities. This is complicated by differences in the size and spatial arrangement of the organisms. Tree community composition is usually measured in plots of at least 0.25 ha, whereas microbial communities can change within a few centimetres. However, Barberán *et al.* (2015) report that the 'large neighbourhood' special scale (tree within a 20 m radii of soil sampling points) revealed the

strongest correlation between tree and microbial community composition, suggesting that whereas stand-level analyses might allow the detection of broad patterns, tree communities at the local scale may have the strongest influence on soil microbial communities.

The strong links between resource quality, microbial community composition, and decomposition rates might provide a mechanism for the relationship between tree community shade-tolerance and soil C accumulation in secondary tropical forests (Chapter 3). To explore this, I examined how changes in tree functional characteristics influence soil microbial communities during secondary succession at two spatial scales. First, I evaluated broad patterns by assessing relationships between soil microbial community composition and tree functional characteristics at the stand level (0.32 ha) along a successional gradient of ten naturally recovering tropical forest stands. Second, to investigate the relationships between microbial communities, tree functional groups and soil properties in more detail I conducted a small-scale study in a subset of four stands representing different successional stages (Chapter 4) to test the following hypotheses:

1. Differences in tree functional groups among forest stands will be reflected in a corresponding shift in soil microbial community composition, whereby the ratios of fungi:bacteria and Gpos:Gneg bacteria will increase with increasing tree community shade-tolerance
2. Differences in soil chemical properties among forest stands will influence soil microbial community composition, particularly bacteria.
3. Differences in soil microbial biomass and community composition will be related to soil C turnover, represented by rates of litter decomposition and soil CO_2 efflux, whereby higher soil microbial biomass will have higher rates of soil C turnover.

4.3 Methods

Study site

The study was conducted within a chronosequence of naturally regenerating tropical forest in the Barro Colorado Nature Monument (BCNM), in Panama, Central America, which comprises the 1500-ha Barro Colorado Island (BCI) and five surrounding mainland peninsulas. The climate is classified as moist tropical with a distinct dry season from January to April, a mean annual temperature of *c.* 27°C and an average annual rainfall of 2600 mm, of which 90% falls in the rainy season (Windsor, 1990). Soils are described as clay-rich oxisols and silty-clay alfisols on sedimentary and volcanic parent materials (Yavitt, 2000) but are not considered to differ significantly in soil C and nutrients (Yavitt, 2000; Grimm *et al.*, 2008). To assess the relationship between functional groups of trees and soil microbes at the stand level, I selected permanent plots in 10 forest stands: two stands for each of four forest age classes of secondary forest (SF), and two old growth forest (OG) stands for comparison, each at least 5 ha in size. The OG stands are >500 years old (Dent, DeWalt and Denslow, 2012) and the secondary forest (SF) stands are currently 40, 60, 90 and 120 years old (Denslow and Guzman, 2000). To assess the links between microbial communities and decomposition rates, I selected a subset of four stands (subsequently 40Y, 60Y, 90Y and OG; Chapter 2) representing a gradient of secondary regrowth forest succession. Within each stand, I established five replicate blocks, spaced at least 20 m apart on level terrain, and avoiding obvious disturbances (e.g. trails, canopy gaps, animal activity) as far as possible (further details in Chapter 3).

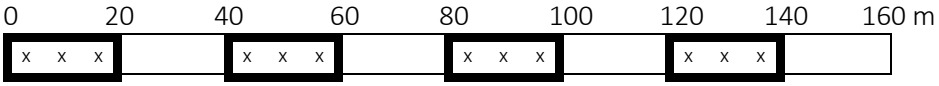
The two spatial scales were used as it was not feasible to do the detailed study across all stands. Therefore, the stand level study captured all points on the age gradient, whereas the block level study better captured the within stand variability and the local tree community effects, but stand were still selected to capture the broadest successional gradient of tree communities.

Tree functional groups

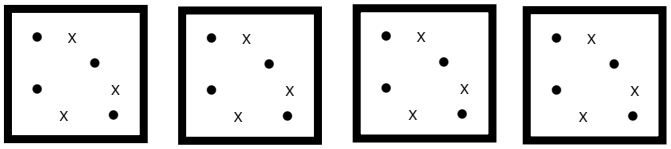
To assess the relationship between functional changes in the tree community and soil microbial community composition during succession, I characterised tree community shade-tolerance

using tree census data and species-specific growth responses to increasing light. Briefly, tree species were assigned to one of two growth-response categories: 'accelerating' or 'decelerating' growth with increasing light, based on a light effect parameter (mean *b*) ranging from -0.6 to 3.3 (Rüger *et al.* 2009; as per Chapters 2 and 3).Accelerating species (ACC; mean *b* >1) represent light-demanding, resource-acquisitive 'pioneer' species, whereas decelerating species (DEC; mean *b* <1) represent shade-tolerant, resource-conservative species (Rüger *et al.* 2009). I used species frequencies (count) and dominance (basal area) to calculate the relative influence of light-demanding (RI ACC) and shade-tolerant (RI DEC) species at the stand-level (0.32 ha; all 10 stands) from 2011 census data (D. Dent, unpublished data). To assess the relationship between tree functional groups and soil microbial communities at a finer scale within the subset of four forest stands, all trees > 200 mm stem diameter at breast height (1.3 m; DBH) within a 20 m^2 radius of the centre of each replicate block were assigned to functional groups as above. RI values for block-level tree functional groups were calculated for each species per block and growth response values were given as the mean light effect value of all species per block (block-level mean *b*).

a) Large-scale soil sampling from four blocks in each of 10 chronosequence stands



b) Fine-scale soil sampling from four blocks in subset of four stands



☐ = sample block
x = soil cores combined to make one composite sample per block
● = one individual sample

Figure 4.1 Soil sampling design for microbial analysis on two spatial scales in a chronosequence of secondary tropical forest in Panama, Central America: a) samples collected along one 160 m transect (10 stands x 4 blocks = 40 samples in total) and b) samples collected from four (20 x 20 m) sample blocks spaced at least 20 m apart in a subset of four stands (4 stands x 4 blocks x 4 samples = 64 samples in total).

Soil sampling

To characterise soil microbial communities and account for the influence of soil physicochemical properties, I sampled soils at the stand level and block level. At the stand level, I established four (20-m x 10-m) sampling blocks, spaced at 40-m intervals along a 160-m transect in each of the ten chronosequence stands (Figure 4.1a). I collected three soil cores (0-10 cm depth) in each of the sampling blocks between May and June 2016 and mixed them to make one composite sample per block (10 stands × 4 blocks = 40 samples in total). To characterise block-level soil microbial community composition, I collected four individual soil cores (0-5 cm depth) from each replicate block within the subset of four stands (4 stand x 4 blocks x 4 cores = 64 sample in total) in September 2017 (Figure 4.1b). To characterise block-level soil properties, I collected an additional three soil cores (0-5 cm depth) in each replicate block within four of the stands and mixed them to make one composite sample per block (4 stands × 4 blocks = 16 samples in total; Figure 4.1b). All samples were collected using a 4.8-cm diameter punch soil corer, sealed individually in plastic bags, and stored at 4˚C within four hours of collection. Subsamples were taken and frozen at -80 °C within 12 hours of collection before being freeze-dried.

Soil physicochemical properties

I measured soil pH on a 1:3 mixture of fresh soil and deionised water using bench pH meter (STARTER 2100, OHAUS, New Jersey, USA) or Mettler Toledo® Seven Compact® pH meter (Leicester, UK). To determine percentage soil C and N content, I ground a *c*. 50 g subsample of homogenized, air-dried soil using a ball mill (Mixer Mill 400, Retsch®, Haan, Germany), Total C and N was analysed on 30 mg soil samples by high temperature combustion gas chromatography on a Vario El III C/N analyser (Elementar, Stockport, UK) at Lancaster University. For the block-level soil samples, extractable P and K were measured using the modified Morgan's method at a commercial laboratory (Central Analytical Laboratory, SRUC Veterinary Services, Midlothian UK)

Soil microbial analyses

Soil microbial community composition was determined by phospho-lipid fatty acid (PLFA) analysis using *c*. 1 g freeze-dried soil. For stand-level analyses in each of the 10 chronosequence stands, PLFAs were extracted form 40 soil samples at 0-10 cm depth using the Bligh and Dyer (1959) method at Lancaster University, and from 64 samples at the block level at 0-5 cm in the subset of four stands (four replicates each of the ACC, DEC, MIX and NAT treatments per stand), following the high-throughput method of Buyer and Sasser, (2012) at a commercial laboratory (Microbial ID Inc., Newark, USA). The two methods were used for time purposes, but data generated are considered comparable. Extracts were analysed and peaks identified by gas chromatography (Perkin-Elmer GC-FID, UK, and Agilent Series II 6890, Palo Alto, USA, respectively) using an internal C19:0 standard at Lancaster University, UK or the Sherlock 6.2™ Microbial Identification System (MIDI, Newark, DE, USA). Total microbial and total fungal biomass were determined from the sum of raw fatty acid peaks (nmol g^{-1} dry weight soil) and relative abundance was calculated for groups of biomarkers representing broad microbial functional types (henceforth: biomarker functional groups; Appendix C: arbuscular mycorrhizal fungi (AM fungi), saprophytic fungi, Gram-positive bacteria, and Gram-negative bacteria. To assess differences in microbial community composition, the ratio of Gram-positive to Gram-negative bacteria (Gpos:Gneg) and the ratio of fungi to bacteria (F:B ratio) were calculated as indicators of variation in microbial community structure (Bardgett, Hobbs and Frostegård, 1996; Zhu *et al.*, 2017).

Litter decomposition and soil respiration rates

To reflect the diversity of decomposing plant litter in tropical forests and thus capture relationships between block-level soil microbial communities, and soil C turnover, I used block-mean litter decay rates (*k*), and total soil respiration (SR_{MEAN}) data, calculated as the mean for five litter treatments per replicate block in each of the four forest stands (4 block x 4 stands = 16 total) from a four-month decomposition experiment (Chapter 3).

Data analyses

All statistical analyses were performed in R version 3.5.2 (R Core Team, 2018), using the vegan package for multivariate analyses (Oksanen *et al.*, 2018) and the lme4 package for linear mixed effects models (Bates *et al.*, 2015). Data were log-transformed or standardised where necessary to meet model assumptions. To assess relationships between soil microbial community composition, tree community shade-tolerance and soil C turnover in the subset of four stands, I first tested the influence of litter treatment on soil microbial community metrics using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities and linear mixed effects models (Appendix C; Table S5.2; Figures S5.1 and 2). The results of these analyses revealed there was no significant difference in soil microbial community composition among litter treatments (Appendix C; Table S5.2; Figures S5.1 and 2). Therefore, I combined PLFA data from four soil samples in each replicate block and used block-mean data in subsequent models.

To examine the effects of changes in tree functional groups on soil microbial community composition during secondary succession at both the stand- and block level, I performed non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities (*metaMDS* function) using PLFA data expressed as relative abundances. Stable solutions with stress scores < 0.2 and r^2 > .95 were used for subsequent analyses, resulting in a two-dimensional solution. I assessed the influence of forest age class (stand-level) and stand (block-level) on soil microbial community composition using a permutational analysis of variance (PERMANOVA; *adonis* function), with 9,999 permutations stratified within blocks. To assess relationships between microbial community composition and litter decay rates, tree functional groups, and soil properties at the block-level, I used the *envfit* function to fit standardised variables as vectors to the NMDS ordinations, and significance values were generated with 9,999 random permutations. I tested the influence of forest age class (stand-level) and stand (block-level) on microbial biomass, biomarker functional groups and soil properties using linear mixed effects models (*lmer* function) and one-way ANOVAs (*lm* function), followed by Tukey's honest significant difference (HSD) *post hoc* test for comparisons among forest age classes and stands at the respective spatial scale. I then assessed relationships between biomarker functional groups, tree functional parameters and soil characteristics at the block-level using stepwise linear regression, with the biomarker functional group as the response variable and soil or tree

functional characteristics as explanatory variables. The initial model included all explanatory variables (soil P, soil K, soil pH, RI.ACC, RI.DEC, and mean *b*) and I checked for variance inflation using the *vif* function in the HH package (Heiberger, 2020). Due to issues of co-linearity between RI.ACC and RI.DEC (variance inflation factor >5), I dropped RI.ACC as an explanatory variable. Subsequent models were then compared with forward and backward selection of variables using AIC values to assess each model fit until a minimum adequate model was reached (*stepAIC* function). Finally, I assessed the relationships between the response variables; mean decay rate and soil respiration rates and soil properties and/or microbial community parameters (soil P, soil K, soil pH, total microbial biomass, fungal biomass, F:B ratio and Gpos:Gneg ratio) using stepwise linear regression and linear mixed effects models following the same steps as described above. For all linear mixed effects models, significance was determined by comparison to appropriate null models (without explanatory variables) using likelihood ratio tests. Results are reported as significant at $p < 0.05$ for linear mixed effects models χ^2 and p values are given for the comparison between the final model and the corresponding null model.

4.4 Results

Stand-level soil microbial community composition along a tropical forest chronosequence

The NMDS ordination showed no clear separation of microbial communities by forest age, whereby microbial communities in secondary forest stands could largely be considered a subset of the old-growth forest community (Figure 4.2). Nonetheless, PERMANOVA revealed significant differences in soil microbial communities among forest age classes ($p = 0.037$), indicating shifts in microbial community structure along the chronosequence.

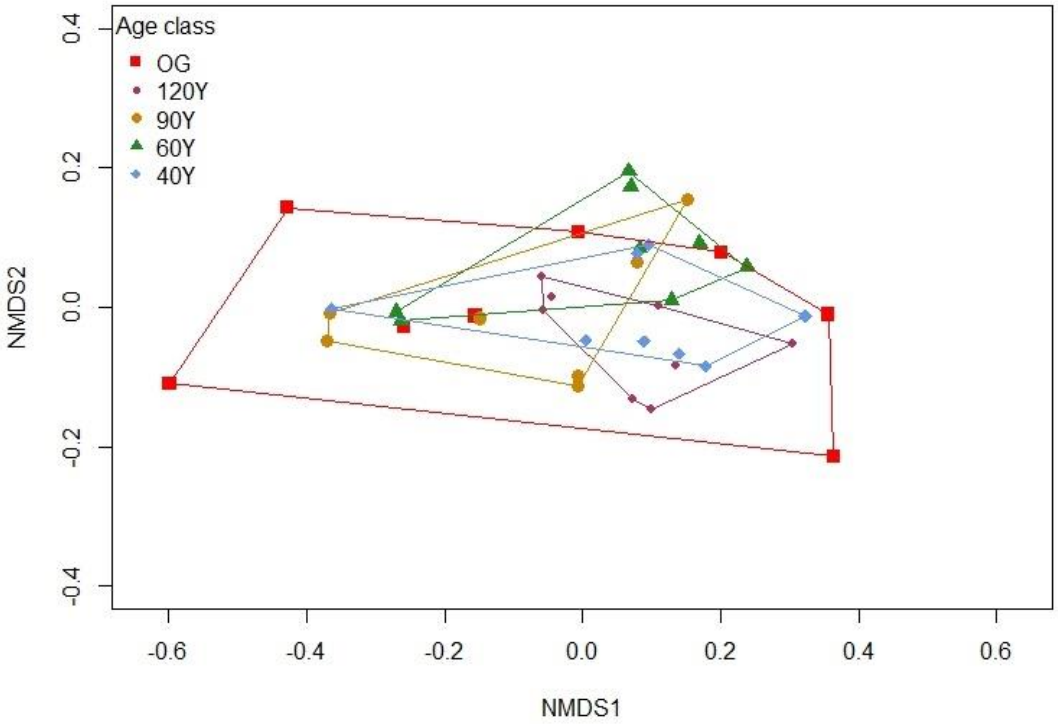


Figure 4.2 NMDS representation of soil microbial community composition in 10 forest stands along an age gradient of naturally regenerating tropical forest; ordinations were based on Bray-Curtis dissimilarities of phospholipid fatty acid (PLFA) biomarkers in soil samples collected at 0-10 cm from four blocks in two stands in each of five age classes: 40 year old (40Y; blue diamonds), 60 year old (60Y; green triangles), 90 year old (90; orange dots), 120 year old (120Y; small maroon dots) and old-growth (OG; > 500 year old; red squares) forest stands in Panama, Central America. Ordinations were based on Bray–Curtis dissimilarities and hulls group samples within age classes.

Relationship between stand-level soil microbial biomass and tree functional groups

At the stand level, soil microbial biomass was related to the relative influence (RI) of tree functional groups. Mean total PLFA biomass (μg g^{-1}) tended to increase with the relative influence of light-demanding (ACC) species and decrease with the relative influence of shade-tolerant (DEC) species resulting in a moderate negative relationship between total PLFA biomass and the ratio of DEC:ACC species ($R^2 = 0.325$, $p = 0.05$; Figure 4.3).

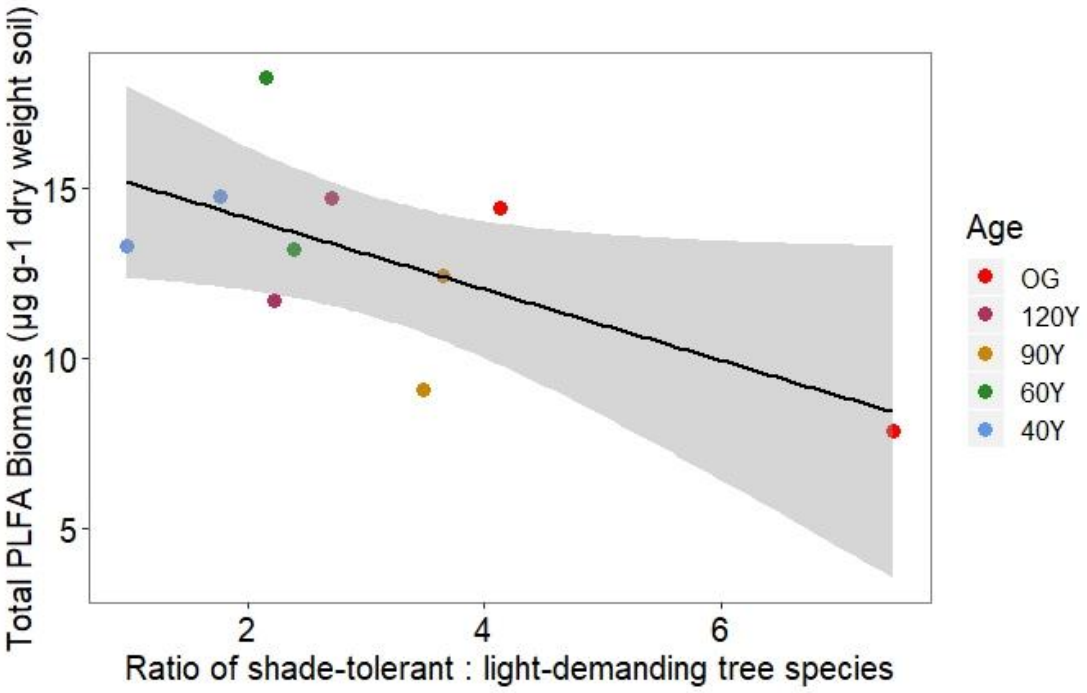


Figure 4.3 Relationship between soil microbial biomass and tree community shade tolerance in 10 forest stands of five age classes along a gradient of naturally regenerating tropical forest in Panama, Central America; where microbial biomass is represented by total phospholipid fatty acids (PLFA) in soils sampled at 0-10 cm, and shade-tolerance is represented by the ratio of shade-tolerant to light-demanding tree species at the stand level (0.32 ha); the forest age classes comprise: 40 year old (40Y; blue), 60 year old (60Y; green), 90 year old (90; orange), 120 year old (120Y; maroon) and old-growth (OG; > 500 year old; red).

Block-level soil microbial community composition in four tropical forest stands

Tree community functional characteristics, C turnover and soil properties among four forest stands

Of the 47 tree species censused, 40 species were assigned to tree functional groups, accounting for *c.* 93 % of individuals. Although block-level mean *b* (species growth response to light) was twice as high in the 40Y than 90Y stand, and mean RI ACC in the two younger stands was more than double that in the two older stands, variability among blocks within each stand was high. Only the RI of shade-tolerant species (RI DEC) differed significantly among stands, whereby RI DEC was significantly higher in the OG stand than the 40Y and 60Y stands ($F_{3,12}$ = 5.34, p = 0.014;

Table 5.1). Due to high values for RI ACC and DEC (e.g. 100 %) in some blocks, the ratio of DEC:ACC species was not used to characterise tree functional groups at the block-level. Soil properties differed among the four forest stands (Table 4.1), whereby soil C was significantly higher in the 40Y and 60Y compared to 90Y stand ($F_{3,12}$ = 6.14, p = 0.009) and N ($F_{3,12}$ = 7.74, p = 0.004) was significantly higher in the two younger (40Y and 60Y) stands than older stands, but the C:N ratio did not differ among stands. Soil pH in the 60Y stand was significantly higher than in the OG stand ($F_{3,12}$ = 5.43, p = 0.014; Table 5.1) but soil P and K did not differ significantly among stands. Block-mean litter decay rate (k) differed among stands ($F_{3,12}$ = 8.12, p = 0.003; Table 4.1) which was explained by significantly higher mean k in the 60Y than the 40Y and 90Y stands but there was no significant difference in block-mean total soil respiration (SR) among stands (Table 4.1).

Table 4.1 Tree community metrics, metrics representing soil C turnover, soil chemical properties and microbial community metrics for four tropical forest stands along a successional gradient in Panama, Central America; where 40Y, 60Y and 90Y denote the estimated stand age in years, and OG Is old-growth forest, where Mean *b* = mean growth response value to increasing light; RI DEC = relative influence of shade-tolerant species and RI ACC = relative influence of light-demanding species, SR_{MEAN} = mean total soil respiration. Total soil respiration and decay rate measurements are from a separate study (Chapter 4). Means and standard errors are given for *n* = 4 replicate blocks; significant differences among stands based on post-hoc tests are indicated by different superscript letters.

	Forest stand age			
Stand mean values	40Y	60Y	90Y	OG
Tree community metrics				
Mean *b*	1.07 ±0.24	0.81 ±0.15	0.52 ±0.17	0.59 ±0.07
RI DEC (%)	24.13 ±14.56^{b}	33.73 ±22.36^{b}	77.69 ±15.76^{ab}	99.6 ±0.25^{a}
RI ACC (%)	59.69 ±16.19	64.01 ±21.73	22.31 ±15.76	0.18 ±0.18
Metrics of soil C turnover				
Litter decay rate (k)	2.64 ±0.24^{b}	4.16 ±0.38^{a}	2.10 ±0.24^{b}	3.25 ±0.36^{ab}
Total soil respiration (SR_{MEAN})	7.60 ±0.29	8.73 ±1.05	5.70±1.17	6.82 ±0.86
Soil chemical properties				
Total carbon (C; %)	8.18 ±0.74^{a}	7.96 ±1.03^{a}	4.65 ±0.68^{b}	5.15 ±0.37^{ab}
Total nitrogen (N; %)	0.58 ±0.06^{a}	0.57 ±0.07^{a}	0.30 ±0.05^{b}	0.33 ±0.03^{b}
C:N ratio	14.22 ±0.20	14.50 ±0.51	15.90 ±0.99	16.06 ±0.36
pH	5.88 ±0.08^{ab}	6.30 ±0.08^{a}	5.82 ±0.16^{ab}	5.41 ±0.25^{b}
Phosphorus (mg/kg)	4.53 ±0.95	9.86 ±2.34	5.59 ±0.07	4.26 ±1.27
Potassium (mg/kg)	725.96 ±86.56	1095.81 ±205.51	866.71 ±93.20	918.19 ±26.15
Microbial community metrics				
Total microbial biomass (Nmol g^{-1})	393.20 ±23.79^{a}	344.05 ±10.05^{a}	256.53 ±18.93^{b}	278.09 ±25.08^{b}
Fungal biomass (Nmol g^{-1})	29.26 ± 3.13^{a}	27.55 ±1.66^{ab}	19.11 ±1.15^{b}	22.50 ±2.41^{ab}
AM fungi (%)	3.22 ±0.10^{ab}	3.26 ±0.09^{ab}	3.05 ±0.10^{b}	3.63 ±0.19^{a}
Fungi: Bacteria ratio	0.13 ±0.01	0.14 ±0.00	0.13 ±0.00	0.14 ±0.01
Gram positive bacteria (%)	32.75 ±0.88^{a}	32.13 ±0.41^{ab}	33.23 ±0.32^{a}	29.56 ±0.75^{b}
Gram negative bacteria (%)	35.52 ±0.82^{ab}	33.98 ±0.43^{b}	35.07 ±1.29^{b}	41.43 ±2.47^{a}
Gpos: Gneg ratio	0.93 ±0.05^{a}	0.95 ±0.01^{a}	0.95 ±0.04^{a}	0.72 ±0.05^{b}

Influence of soil properties and tree functional groups on soil microbial community composition

At the block level, NMDS ordination revealed a clear separation of soil microbial communities among stands, particularly between the older (OG and 90Y) and younger (60Y and 40Y) forest stands (Figure 4.4; PERMANOVA: $p < 0.001$). Vector fitting to the NMDS ordination revealed the RI of DEC species ($p = 0.032$), soil pH ($p = 0.039$) and mean *b* ($p = 0.048$) explained separation along the first axis whereas soil C and N content best explained separation along the second axis (NMDS2 = 0.047, $p = 0.003$; Figure 4.4). Hence, the combination of local tree community shade-tolerance and soil properties explained differences in soil microbial community composition among stands.

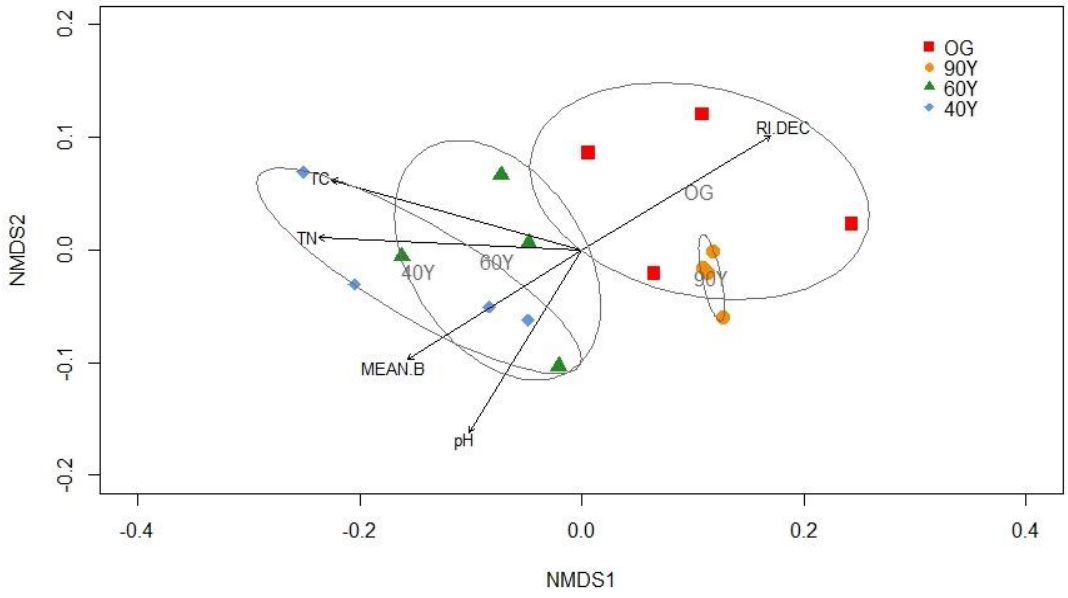


Figure 4.4 NMDS representation of soil microbial community composition in four forest stands along an age gradient of naturally regenerating tropical forest in Panama, Central America; ordinations were based on Bray-Curtis dissimilarities of phospholipid fatty acid (PLFA) biomarkers in soil samples collected from 0-5cm in four blocks per stand, where 40 year old (40Y; blue diamonds), 60 year old (60Y; green triangles), 90 year old (90Y; orange circles) and old growth (OG; > 500 years old; red squares) forest stands. Significant ($p < 0.05$) relationships between ordination axes and soil properties or tree functional characteristics (at 20 m^2 radius) are fitted as vectors (black arrows), where RI.DEC is the relative influence of shade-tolerant (decelerating growth) tree species (%) per block, MEAN.B is the block mean species

growth response to increasing light, pH is soil pH, TC is total carbon (%) and TN is total soil nitrogen (%); ellipses group blocks within stands based on 99% confidence intervals.

Microbial communities at the block-level differed among stands in both biomass and structure. Fitting the relative abundances of microbial functional groups as vectors to the NMDS ordination revealed that differences in the ratio of Gpos:Gneg bacteria (NMDS1 = 0.981, p = 0.004) along with a non-significant trend for the relative abundance of AM fungi (p = 0.052) explained separation along the first axis and differences in total microbial biomass (NMDS2 = 0.996, $p < 0.001$) and total fungal biomass (NMDS2 = 0.920, $p = 0.001$) best explained separation along the second axis. The separation of stands was partly explained by higher total microbial biomass in the 40Y and 60Y stands, compared to the 90Y and OG stands ($F_{3,12}$ = 9.44, p = 0.002; Figure 4.5a; Table 4.1), and higher fungal biomass in the 60Y compared to the 90Y stand ($F_{3,12}$ = 4.40, p = 0.026; Figure 4.4b). There was also a lower relative abundance of Gram-positive biomarkers ($F_{3,12}$ = 6.65, p = 0.007; Figure 5.4f) and Gpos:Gneg ratio ($F_{3,12}$ = 7.03, p = 0.006; Figure 4.4g) in the OG stand compared to the three secondary forest stands, but a higher relative abundance of Gram-negative bacteria ($F_{3,12}$ = 5.20, p = 0.016) in the OG stand compared to the three secondary forest stands. AM fungal biomarkers were higher in the OG than 90Y stand ($F_{3,12}$ = 3.51, p = 0.049) the but there was no difference in the fungi:bacteria ratio among stands (Figure 4.5d; Table 4.1).

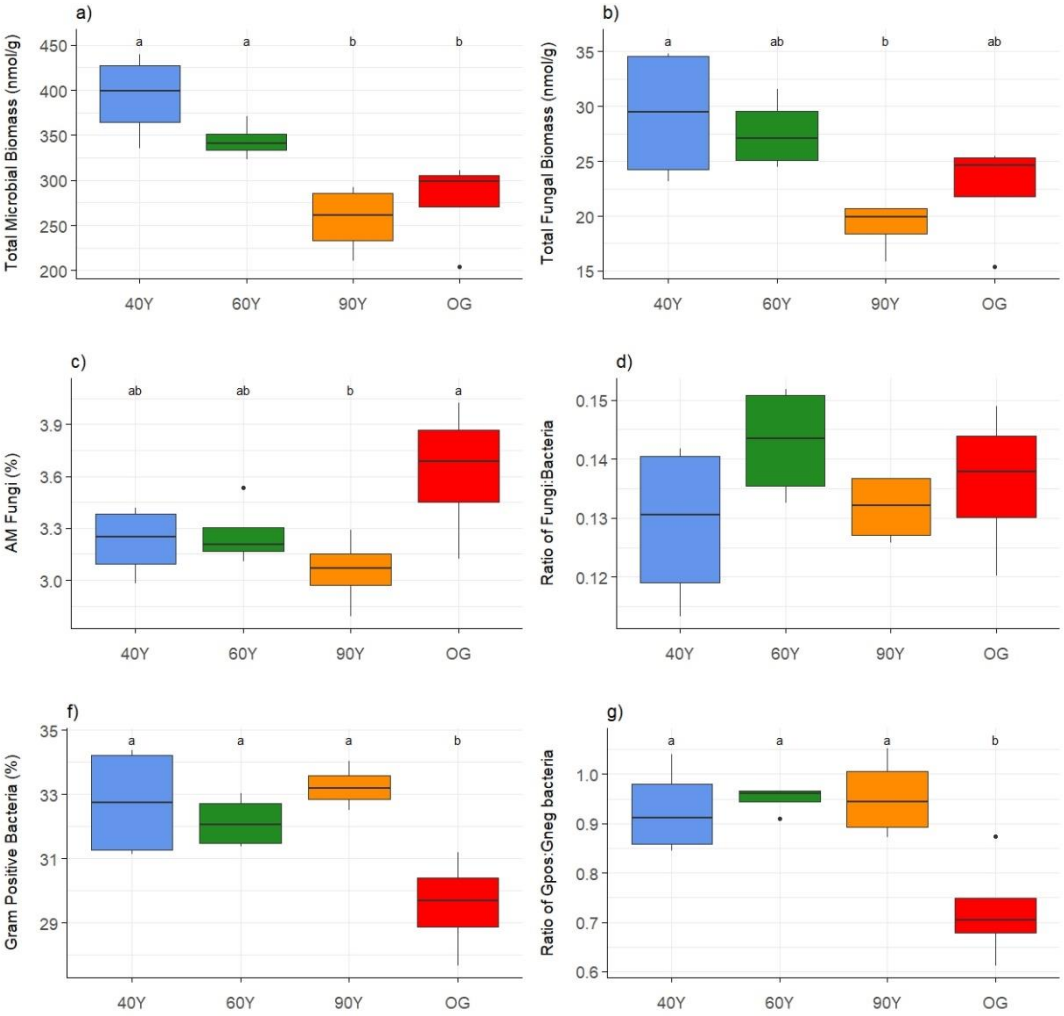
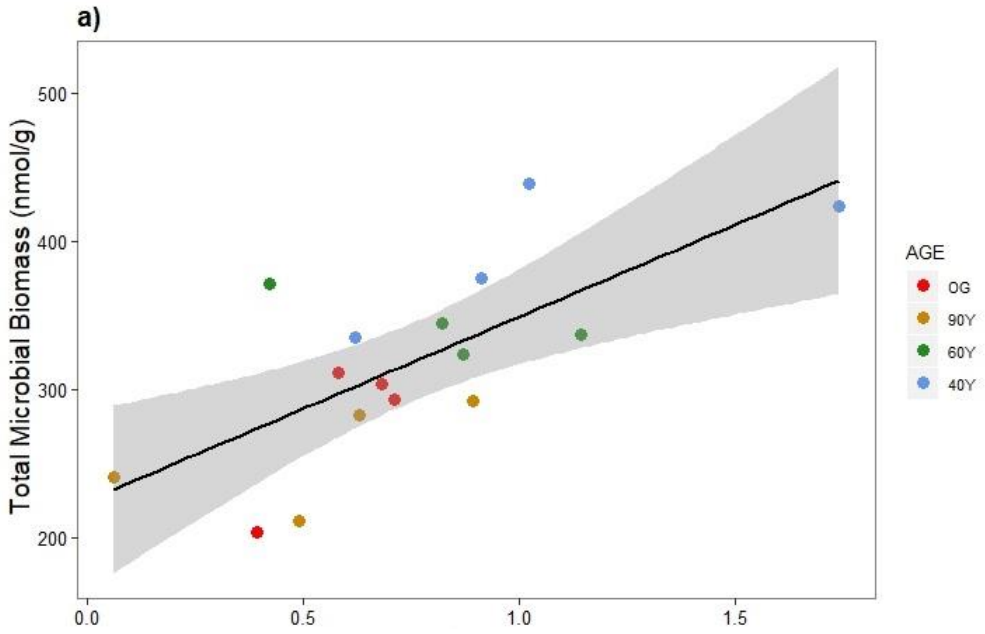


Figure 4.5 Soil microbial biomarker groups from PLFA analysis along a successional gradient of four forest stands (40 year old, 60 year old, 90 year old and OG = old-growth (>500 year old) forest) for a) total microbial biomass, b) total fungal biomass, c) the relative abundance of arbuscular mycorrhizal fungi (AM fungi), d) the ratio between fungi and bacteria, e) the relative abundance of gram-negative bacteria, f) the relative abundance of gram-positive bacteria, and g) the ratio between gram-positive and gram-negative bacteria. Soil was sampled from 0-5 cm depth, PLFA data from each replicate block were combined resulting in n = 4 per stand. Significant differences among stands were determined by Tukey *post-hoc* analyses and indicated by different letters at $p < 0.05$. Boxes denote the 25th and 75th percentiles and median lines, whiskers indicate values up to 1.5 x the interquartile range, and dots indicate outliers.

Combined influence of tree functional groups and soil properties on soil microbial biomass and biomarker groups

Stepwise multiple linear regressions revealed relationships between microbial community parameters and soil K, soil pH and mean *b* (full model results in Appendix C). Total microbial biomass and fungal biomass declined with increasing soil K content and increased with soil pH and mean *b* (R^2 = 0.60, p = 0.003 and R^2 = 0.40, p = 0.030 for total and fungal biomass, respectively; relationship between microbial biomass and mean *b* shown in Figure 4.6) but there were no significant relationships between the fungi:bacteria ratio and any stand or soil properties. The relative abundance of AM fungi increased with increasing soil K concentrations and declined with increasing soil pH (R^2 = 0.33, p = 0.03). There was no relationship between the abundance of Gram-positive bacteria and any of the measured stand or soil characteristics. However, the abundance of Gram-negative bacteria declined strongly with increasing soil pH (R^2 = 0.62, p < 0.001) and the Gpos:Gneg ratio increased with increasing pH (R^2 = 0.40, p = 0.005). Hence, although soil properties exerted control on the relative abundances of microbial functional groups, stands dominated by light-demanding trees determined higher levels of total microbial biomass and fungal biomass.



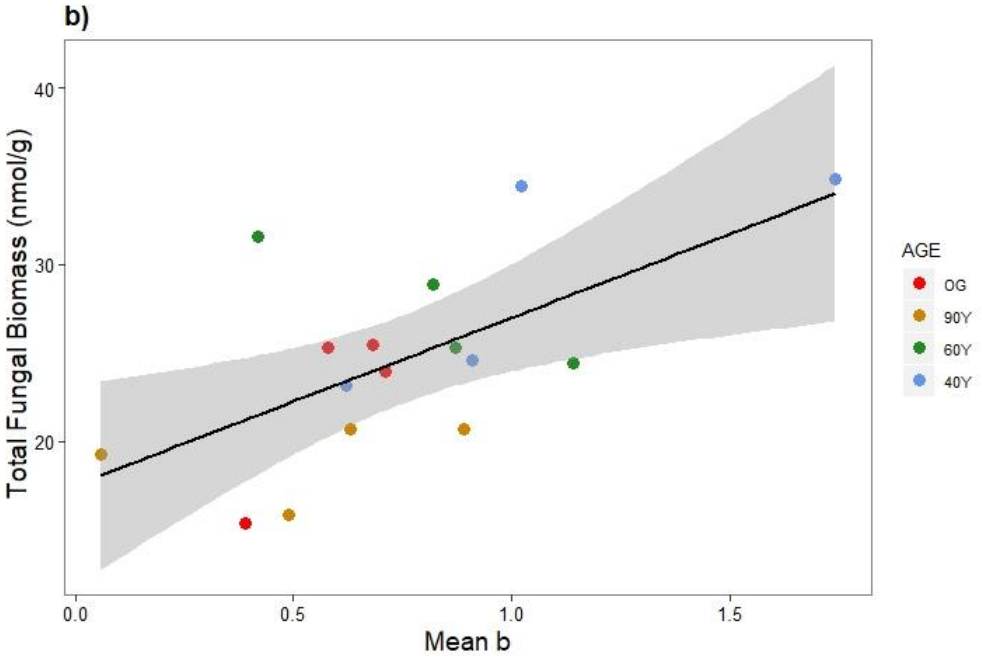


Figure 4.6 Relationship between a) total soil microbial biomass and b) total fungal biomass and block-mean tree community growth response to light (mean *b*; Rüger *et al.,* 2009) in four forest stands in a tropical forest in Panama, Central America; where microbial biomass is represented by total phospholipid fatty acids (PLFA) in soils sampled at 0-5 cm and stands are represented by age (years since last disturbance event and OG = old-growth forest).

Linking soil microbial communities with soil C turnover

Relationships between representative measures of soil C turnover (response terms) and microbial community parameters were indicated with stepwise multiple linear regressions (full models in Appendix C). The best model for litter decay rates included fungal (or total) biomass, the Gpos:Gneg ratio, soil K and soil pH (R^2 = 0.72; p < 0.001), whereby decay rates declined strongly with increasing Gpos:Gneg ratio, and increased with fungal biomass, soil K and soil pH. Total soil respiration (SR_{mean}) was best explained by total microbial biomass, the F:B and Gpos:Gneg ratios, and soil P and K, whereby SR_{mean} increased with increasing microbial biomass, P and K but declined with increasing ratios of F:B and Gpos:Gneg (R^2 = 0.71, p = 0.002).

4.5 Discussion

To my knowledge, this is the first study to assess the relationship between tree functional groups and soil microbial communities along a chronosequence of tropical forest stands, using tree community shade-tolerance as the key functional characteristic. My study tested the assumption that the top-down influence of dominant tree functional groups on the soil food web would in turn, influence the abundance, structure and activity of soil microbial communities. I demonstrated clear links between tree community shade-tolerance and soil microbial community structure across stands of different ages. Although the shifts in microbial functional groups did not conform to expectations, my findings nonetheless demonstrated strong relationships between microbial community structure and local dominance of shade-tolerant trees, and the local abundance of specific microbial functional groups explained differences in litter decomposition and soil respiration rates.

Stand-level relationships between tree and microbial functional community composition

Across the chronosequence, I expected that increasing shade-tolerance of the tree community, and the corresponding decline in organic matter quality with forest age, would be reflected by changes in soil microbial communities. However, although there were stand-level differences in microbial community structure, there were no clear differences in individual microbial functional groups along the successional gradient, and I did not observe the expected increase in the relative abundances of K-strategist decomposer organisms (i.e. increasing ratios of F:B and Gpos:Gneg bacteria). Nonetheless, despite the broad scale of the study (0.32 ha per stand), I revealed that total and fungal microbial biomass tended to decline with increasing dominance of shade-tolerant tree species, represented by the DEC:ACC ratio. As the soil microbial biomass is considered to be directly related to the quantity and quality of organic matter in the soil food web (Yao *et al.*, 2000), the moderately significant stand-level relationships between tree community shade-tolerance and microbial biomass suggest that tree functional groups influence microbial communities via organic matter quality, with greater microbial biomass in stands of light-demanding species with higher litter quality (Fanin, Hättenschwiler and Fromin 2014). It is noteworthy that soil microbial communities in the secondary forest stands largely

represented subsets of the old-growth forest microbial community (Figure 5.1) indicating that microbial communities may develop in complexity during forest recovery.

The lack of strong relationships between tree species composition and microbial community structure at the stand level could be explained by differences in soil chemical properties among stands, particularly differences in soil pH (Chapter 2). Soil pH is widely considered one of the most influential factors affecting the microbial community (Rousk, Brookes and Bååth, 2009) and although it is often reported to have a stronger effect on bacteria (e.g. Barberán *et al.*, 2015), fungal communities are also influenced by soil pH (Bachelot *et al.*, 2016).

The increase in both total and fungal biomass with increasing soil pH is largely consistent with other studies covering a similar pH range (e.g. Rousk, Brookes and Bååth, 2009). As differences in soil pH can occur due to many factors (e.g. vegetation type, soil type, land-use practices) and can influence other soil properties such as C and nutrients (Rousk, Brookes and Bååth, 2009) it is not possible to disentangle the relative influence of soil pH and tree community composition on soil microbial communities. Differences in former land-use could also obscure relationships between above- and belowground communities, because the legacy effect of former agricultural land-use practices can influence soil physicochemical properties for decades (Foster *et al.*, 2003). Although there is insufficient information to formally analyse the impact of land-use history on soil microbial communities for the forest stands in this study, the legacy of former land-use practices could also explain some of the unexpected trends in microbial community structure.

Block-level relationships between tree and microbial functional community composition

There were clear differences in microbial communities among stands when measurements were made at a finer scale. The differentiation of microbial communities between young secondary forests (40Y and 60Y) and old-growth forest was particularly striking (Figure 4.3). Stronger relationships between tree functional groups and soil microbial communities were expected at the block level because the soils were taken within the radius of influence of the individual trees. For example, Barberán *et al,* (2015) revealed the tree community within a 20

m radius of soil sampling points had the strongest correlation between tree and microbial community composition. Although the results from the stand and block-level studies may not be directly comparable because of the different sampling times, block-level analyses revealed a stronger influence of tree communities on soil microbial communities than tree communities at the stand-level.

Although there was some variability among replicate blocks in the subset of four stands, differences in the proportions of tree functional groups at the block-level generally conformed to the pattern of increasing relative influence of shade-tolerant tree species with stand age (Table 4.1). Hence, the subset of stands used in this study are likely to be representative of a natural successional gradient, demonstrating concurrent shifts in above- and belowground community composition.

Interestingly, the within-stand variation in soil microbial communities was smallest in the 90Y stand (Figure 4.4), which may overlie a different geological formation from the other stands (40Y, 60Y and OG; Appendix B). Although measured soil properties (N, P, K and pH) did not differ between the 90Y and other stands, it is plausible that the 90Y stand had distinct soil physical characteristics such as clay content and water retention (Baillie *et al.*, 2007) which in turn can have a significant influence on soil microbial communities (Lauber *et al.*, 2008).

Although organic matter quality was not explicitly measured as part of this study, I previously demonstrated that litter representing ACC species decomposes more rapidly than litter representing DEC species, and hence the greater microbial biomass in blocks dominated by light-demanding tree species is likely explained by greater inputs of high-quality litter (Yao *et al.*, 2000; Bray, Kitajima and Mack, 2012). Nonetheless, in contrast to my initial hypothesis, there was no clear relationship between tree community shade-tolerance and structural metrics of the microbial community. I expected that the F:B and Gpos:Gneg ratios would increase with the relative influence of shade-tolerant trees, reflecting increasing abundance of K-strategist decomposer organisms in stands with low-quality litter inputs (Fierer, Bradford and Jackson, 2007; Zhou *et al.*, 2017). However, there was no relationship between tree community composition and F:B ratios and, surprisingly, the lowest Gpos:Gneg ratios were measured in the old-growth forest stand (Figure 4.5d and g; Table 4.1).

The difference between old-growth and secondary forests was particularly striking for Gneg bacteria, and it is possible that the lower abundance of Gneg bacteria in secondary forest stands

is related to abiotic stress, rather than resource quality. Younger forest stands have a more open canopy, which can result in greater variation in soil temperature and water content (Craven, 2015). Soil drying introduces matric and osmotic stress which will affect microbial community composition (Burns et al., 2013), and Gneg bacteria are thought to be more susceptible to dry conditions (Fierer, Bradford and Jackson, 2007) whereas Gpos bacteria are considered more stress tolerant (Waldrop MP , Balser TC, 2000). By contrast the F:B ratio is controlled largely by resource stoichiometry (Waring, Averill and Hawkes, 2013) because fungi have an advantage over bacteria at higher C:N ratios, whereas bacterial inhibition of fungal growth is stronger on low C:N substrates (Rousk and Bååth, 2007; Rousk *et al.*, 2008). These expected patterns in F:B ratios are based largely on evidence from temperate systems, where nitrogen is more likely to be limiting to microbial activity – this may not apply in many tropical systems; indeed, N is not considered limiting in the study forest, and the soil C:N ratios did not differ among stands.

Although soil nutrients had limited explanatory power for microbial community composition, both total microbial biomass and fungal biomass decreased with increasing soil K content. Soil K is considered limiting in these soils and may preferentially bind to humic substances (Barthold, Stallard and Elsenbeer, 2008; Kaspari *et al.*, 2008) and it is also an important nutrient required by fungi for organic matter decomposition (Kaspari *et al.*, 2008) so could be considered a limiting nutrient for soil microbial communities and activity. Interestingly, a fertilization study conducted nearby within the BCNM reported no significant effect of soil K on microbial biomass but instead a significant positive effect of soil P (Turner and Wright, 2014), thus the negative relationship between microbial biomass and soil K in this study is surprising. However, soil P and K appeared to influence respiration rates, whereby SR increased with increasing P and K. Litter decay rates also increased with increasing soil K, suggesting links between soil nutrient availability and microbial activity, which warrant further investigation

Links between soil microbial community composition and soil carbon turnover

As microbial biomass is strongly related to soil biogeochemical cycling and forms a large proportion of soil organic C (Simpson *et al.*, 2007; Kutsch, Bahn and Heinemeyer, 2009), I expected that soil C turnover (litter decomposition and soil respiration) would be higher in soils with greater microbial biomass. Surprisingly, microbial biomass was a weaker predictor for decay rates and soil respiration than microbial structure. Changes in microbial community composition alone can account for a substantial proportion of the variation in respiration rates during litter decomposition (Strickland *et al.*, 2009) but microbial communities in the soil at 0-5 cm depth are likely to differ from the microbial communities on the litter surface (McGuire *et al.*, 2012). Nonetheless, the decline in decay rates and soil respiration with increasing Gpos:Gneg ratio suggests that soils with high a relative abundance of Gneg bacteria are characterised by high rates of microbial activity and soil C turnover, which likely indicates that they preferentially use labile substrate (Bardgett, Mommer and Vries, 2014). According to the MEMS framework, rapid turnover of labile organic matter by copiotrophic microbes such as Gneg bacteria would accelerate soil organic matter formation (Cotrufo, Wallenstein and Boot, 2013), and hence distinct bacterial communities among stands might contribute to the observed patterns of soil carbon accumulation with increasing relative influence of light-demanding trees (Chapter 2).

Soil respiration, but not decay rates, also declined with increasing F:B ratios, which might also reflect differences in metabolic rates of fungi and bacteria, in particular because fungi tend to store more C than they metabolize (Singh *et al.*, 2010). It is noteworthy that soil P and K content also explained some of the variation in total soil respiration, as both nutrients are thought to be limiting to plant growth in the study area (Sayer and Tanner, 2010; Yavitt *et al.*, 2011).

It is conceivable that overlap in soil microbial communities from old-growth and secondary forest stands is a result of the high plant diversity in tropical forests. Functionally diverse microbial communities comprising both r- and K-strategists are likely required to decompose mixed litter originating from a wide range of tree functional groups (Kerdraon *et al.*, 2019, 2020). Although my results provide initial evidence for links between tree functional groups, microbial community structure and soil C turnover in secondary tropical forests, more in-depth

analyses of microbial community composition (e.g. using sequencing techniques) and metabolic capacities is required to fully determine the mechanistic basis for these relationships.

4.6 Conclusions

This study reveals clear links between tree functional groups and soil microbial communities in secondary tropical forest stands. Although there was no clear trajectory of shifts in microbial community structure with forest age across the chronosequence, my findings suggest that more localised relationships exist between soil microbial communities and tree functional composition, which influence decomposition and soil respiration. Overall, stands dominated by light-demanding tree species had higher microbial biomass but the assumption that stands with a higher relative influence of shade-tolerant species would have higher relative abundances of K-strategist decomposers was unsubstantiated. My study highlights the important top-down influence of tree functional groups on soil microbial biomass and community composition, despite the important role of soil physicochemical properties which may obscure relationships between plant-derived organic matter and soil microbial communities in these forest stands. The unexpected shifts in microbial functional groups could underpin the relationship between tree community shade-tolerance and soil carbon storage during secondary forest succession and help explain the disconnect between above- and belowground C pools during forest recovery.

Given the importance of secondary tropical forests for the global carbon cycle, further work on the links between soil microbial community composition and tree functional traits could elucidate important mechanisms of soil organic matter formation during secondary forest succession.

5 General discussion

Secondary tropical forest regrowth is becoming increasingly important for C sequestration and storage (Poorter *et al.*, 2016; Powers and Marín-Spiotta, 2017). Aboveground biomass C accumulates rapidly during regrowth after disturbance (Poorter *et al.*, 2016), but there is no clear pattern of soil C accumulation over time during forest recovery (Yang, Luo and Finzi, 2011; Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017). As tropical forests store more C belowground than in aboveground vegetation (Don, Schumacher and Freibauer, 2011), there is a pressing need to improve our understanding of soil C dynamics during tropical forest secondary succession. Whereas aboveground biomass C accumulation over time is a direct result of rapid tree growth during forest recovery (Poorter *et al.*, 2016), soil C storage depends on both the quantity and quality of organic matter inputs from plants (Metcalfe, Fisher and Wardle, 2011; Castellano *et al.*, 2015). The quality and quantity of plant inputs is determined by tree species composition, which changes during secondary succession and can differ markedly between forest stands of similar ages (Norden *et al.*, 2015; Boukili and Chazdon, 2017). Hence, I hypothesised that functionally distinct tree communities during secondary succession would have a significant influence on soil C dynamics and storage via the quality of litter inputs to the soil. The body of work presented in this thesis provides compelling evidence in support of my initial hypothesis, demonstrating that soil C accumulation is more closely related to tree functional composition than forest age, and explores some of the potential mechanisms through which changes in the functional composition of trees can influence soil C dynamics.

5.1 Characterisation of tree communities during secondary succession

The characterisation of tree communities was an important component of my work as it underpinned my overarching hypothesis, as well as the hypotheses of the individual studies presented in each chapter. I characterised tree communities using growth responses to increasing light values (Rüger *et al.*, 2009) to assign species to functional groups. I estimated the relative influence (RI) of tree functional groups based on their frequency (number of individuals) and dominance (basal area). Hence, my approach uses species growth responses

as a proxy for resource-use strategy, and accounts for the relative proportion of tree functional groups to estimate their influence on biogeochemical cycling. My approach and results differ from those of Dent, DeWalt and Denslow (2012), who previously characterised tree community shade-tolerance along the chronosequence using the same species-specific growth response to increasing light values (Rüger *et al.*, 2009). However, in their study, Dent, DeWalt and Denslow (2012) used community-weighted mean values based on tree basal area to define the functional composition of forest stands and reported that tree community shade-tolerance increased with increasing stand age. By contrast, my approach accounted for the potential influence of many small individuals within a functional group, as well as the influence of large trees which I viewed as important in determining plant-soil interactions. Hence, my results suggest that the relative influence of tree community functional groups does not necessarily shift predictably during secondary succession in these forests. Importantly, the relative influence of light-demanding and shade-tolerant species was a significant predictor for C in the surface soil, which suggests that forest management to increase belowground C storage could be achieved via the functional composition of tree communities. It is now important to investigate whether similar relationships between the tree functional groups (as characterised in this study) and soil C content exist across other forest sites and at larger spatial scales.

It is encouraging that the broad functional classifications I used in my thesis revealed such a strong relationship between tree community shade-tolerance and soil C content and demonstrates the potential for this approach to be extended. Notably, the functional classification I used was based on a single trait axis (the growth-survival trade-off), whereas a recent study suggests that a more detailed picture of forest dynamics during secondary succession can be achieved using two trait axes, resulting in five plant functional types (Rüger *et al* 2020). Importantly, the inclusion of a second trait axis (the stature-recruitment trade-off), allowed the authors to differentiate short-stature pioneers from the long-lived pioneers, which form a significant proportion of the canopy in secondary forest stands in the BCNM and in other tropical forests (Rüger *et al.*, 2020), and explains the high RI values for light-demanding species in the late secondary and old-growth forest stands in my study. Therefore, future tests of the relationships between the functional structure of tree communities and soil C accumulation could benefit from the expanded categories of plant functional types proposed by Rüger *et al* (2020).

5.2 Linking tree functional groups to decomposition processes and soil respiration

My initial results revealed that soil C content and stocks in the surface soil (0-10 cm depth) increased with the relative influence of light-demanding tree species across the 10 chronosequence stands. Although these results supported my initial hypothesis, the direction of the relationship was unexpected, as I hypothesised that C accumulation would be promoted by the slow turnover of nutrient-poor litter derived from slow-growing shade-tolerant species. However, this unexpected result led me to consider an alternative hypothesis, whereby the input of labile organic material to soils promotes microbial turnover, and the products of successive microbial processing results in increasingly stable C compounds being incorporated into the soil matrix (the MEMS framework; Cotrufo, Wallenstein and Boot, 2013). Hence, based on my results in Chapter 2, I hypothesised that that the surface input of high-quality litter would explain soil C accumulation via increased rates of soil C turnover. I tested this hypothesis in Chapter 3 using an *in situ* litter decomposition experiment with functionally distinct litter treatments. My study demonstrated that litter representing light-demanding tree species (ACC) decomposed significantly faster than litter representing shade-tolerant species (DEC), and that changes in soil respiration reflected decomposition dynamics, whereby total soil (SR) and litter-derived (SR_L) respiration rates were significantly higher for the ACC than DEC treatment in the first two months of decomposition, whereas in the last two months the pattern was reversed with higher SR and SR_L for DEC compared to ACC treatments. However, the effects of litter type were consistent across stands, and overall differences in decay rates and soil respiration among stands were not related to tree community shade-tolerance. My results suggest that although the quality of leaf litter reaching the forest floor may indeed influence soil C turnover via litter decay rates and soil respiration, these pathways do not fully explain the relationship between tree functional groups and soil C in these stands.

Although the results of Chapter 3 revealed that litter decay rates and the temporal response of soil respiration differed with both litter quality and forest stand, the weak relationship between these measures of soil C turnover and the RI of tree functional groups, suggests that other processes connect tree community functional groups with soil C accumulation. As my study only considered the influence of aboveground litter inputs, the lack of a clear relationship

between tree functional groups and soil C turnover in this study could be explained by the influence of roots. Previously, aboveground inputs of plant organic matter were considered the primary contributor to soil C (e.g. Clark *et al.*, 2001), however there is increasing recognition that belowground inputs may be of more importance, in particular the release of labile dissolved organic carbon (DOC) from root exudates (Gross and Harrison, 2019; Sokol and Bradford, 2019). Fine root turnover and the input of labile C via root exudates make a major contribution to the formation of soil organic matter (I Brunner *et al.*, 2013; Bardgett, Mommer and De Vries, 2014; Sokol and Bradford, 2019), and are expected to form mineral-stabilized soil C more efficiently than aboveground inputs, due in part to their direct entry into the mineral soil and proximity to the microbial community within the rhizosphere (Sokol and Bradford, 2019). At my study site, fine root biomass is largely concentrated in the surface soil horizons (*c*. 50 % at 0-5 cm and 75% at 0-10 cm depth; Yavitt *et al.*, 2011) and there is evidence to suggest that roots influence C cycling in these forests, for example via increased litter decomposition in the presence of roots (Nottingham *et al.*, 2013) and increased soil C stocks with increasing fine root biomass (Cusack *et al.*, 2018).

The quality and quantity of root inputs are likely to differ strongly among tree functional groups and could contribute to the observed relationship between tree community shade tolerance and soil C accumulation. However, whereas there are clear global patterns in foliar traits (the leaf economics spectrum; Wright *et al.*, 2004; Reich, 2014), root traits do not necessarily follow the same principles (Mommer and Weemstra, 2012; Kramer-Walter *et al.*, 2016). Indeed, we know very little about differences in root traits among plant functional groups, especially in the tropics, in part due to the difficulty of studying rhizosphere processes without disturbing them (Vitousek and Sanford, 1986). Hence, although the potential effect of root inputs on soil C dynamics was beyond the scope of my study, further research using root ingrowth cores (Brunner *et al.*, 2013; Nottingham *et al.*, 2013), root decomposition studies, and trenching methods (Hanson *et al.*, 2000; Sayer and Tanner, 2010) to partition root-rhizosphere respiration, and isotope labelling techniques to trace C pathways though the food soil web (Pausch and Kuzyakov, 2018) may reveal important mechanisms underlying the relationship between tree community functional groups and soil C.

5.3 Soil microbial communities link tree functional groups and soil carbon dynamics

The study presented in Chapter 4 revealed relationships between tree functional groups and soil microbial communities both within and between stands. Assessed at a comparatively broad spatial scale (0.32 ha) in each of the 10 chronosequence stands, my results indicated there was a relationship between soil microbial communities and tree functional groups, which differed among stands. Although the relationship was only moderately significant, soil microbial biomass increased with decreasing shade-tolerance of the tree community, indicating that organic inputs from light-demanding trees could enhance soil C accumulation by promoting microbial growth. The same pattern emerged within stands, whereby soil microbial biomass was significantly lower in blocks dominated by shade-tolerant trees. Interestingly, the broad-scale analyses revealed that microbial communities in the SF stands appeared to be a subset of the OG forest communities. This suggests that previously disturbed SF may have a narrower microbial community than undisturbed OG stands and that the microbial community develops over long timescales (e.g. many decades to hundreds of years). The analyses of microbial communities within the subset of four forest stands also demonstrated a clear separation in microbial community composition between the two older (90Y and OG) and two younger (40Y and 60Y) stands, which was largely explained by tree community shade-tolerance.

The combined results of the studies in Chapters 3 and 4 provide some evidence to support to my hypothesis that microbial processing of labile C input from nutrient rich plant material would enhance soil C accumulation (Cotrufo, Wallenstein and Boot, 2013). The decomposition experiments in Chapter 3 demonstrated strong links between litter quality and decay rates, whereas the negative relationship between the soil microbial biomass and the relative influence of shade-tolerant tree species in Chapter 4 suggest that the overall microbial contribution to soil C, such as microbial necromass and the products of microbial C processing (Cotrufo, Wallenstein and Boot, 2013), might be more important than rates of soil C turnover represented by litter decomposition and respiration (Liang, Schimel and Jastrow, 2017).

Surprisingly, I found no clear relationships between tree functional groups and microbial community structure or specific microbial biomarker groups. I had expected an increase in the relative abundance of K-strategists with increasing tree community shade-tolerance, but the results of the study in Chapter 4 instead revealed that there were no clear relationships

between specific microbial biomarker groups and tree functional groups. Nonetheless, differences in microbial functional groups revealed indirect links between soil microbial communities and tree functional groups, as litter decay rates and soil respiration declined with increasing Gpos:Gneg ratio, but increased with an increasing proportion of light-demanding species, suggesting that soils with high relative abundance of Gneg bacteria are characterised by high rates of microbial activity and soil C turnover.

5.4 Potential influence of time, depth and soil characteristics on soil carbon accumulation

My results also provide evidence to suggest that there may be a stabilizing effect in soils as forests mature over time, particularly at depth. For example, soil C at 20-30 cm depth was slightly higher in the OG than SF stands and interestingly, there was lower variation between the 10-20 and 20-30 cm depth increments in the OG than in the four SF age classes (Figure 2.2b; Table 2.3). A possible explanation for this is that as well as taking time for organic C to penetrate into deeper soil layers, the decrease in temperature, oxygen and nutrients with increasing soil depth, means microbial access becomes more limited which in turn leads to slower rates of C turnover and enables larger proportions of stable C to be stored at greater soil depths (e.g. Fontaine *et al.*, 2007). Therefore, soil disturbances which alter the microbial environment at depth, such as agricultural land-use practices, may potentially disrupt the ability of deeper soils to accumulate C. In a recent global meta-analysis, Chen *et al.* (2020) highlight the importance of soil depth and time for the accumulation of SOC content and stock in mixed species environments and propose that roots are the main influence on soil C in deeper soil. As half of global soil carbon is stored at depths below 30 cm (Balesdent *et al.*, 2018), and the immense C storage capacity in tropical soils is largely attributed to their depth (Jobbágy and Jackson, 2000), these results emphasise the need to assess relationships between tree functional characteristics and soil C at a range of soil depths.

The physical protection and residence time of soil C at depth may be strongly influenced by soil physical and chemical properties. For example, soil clay content has been shown to have a stronger influence on soil C than vegetation (and climatic factors) at depth (Jobbágy and

Jackson, 2000; Gray, Bishop and Wilson, 2016). Hence, C accumulation at the surface is likely to be linked to tree functional groups because labile C inputs from high quality litter and rhizodeposits kick-start soil microbial biomass growth, and sequential microbial processing then results in increasingly stable soil C compounds (Cotrufo, Wallenstein and Boot, 2013). By contrast, the incorporation of soil C at greater depths is likely to be a function of time and soil physical and chemical properties. Isolating the influence of soil physical and chemical properties with controlled experiments may help unravel relationships between tree community functional groups and soil C dynamics. However, as my study demonstrates, despite considerable spatial heterogeneity in soil properties, soil C turnover is strongly influenced by vegetation. Therefore, a better understanding of the plant traits and pathways that underpin the relationship between tree species and soil C accumulation at the community scale will help us to better predict soil C dynamics during forest regrowth, and identify practical solutions to increase soil C sequestration through tropical forest restoration activities.

5.5 Where my study fits in to wider research

Studies assessing the relationship between tree communities and soil C dynamics in tropical forests are limited and comparisons are challenging because of the different approaches used to characterise the (functional) composition of tree communities and soil C turnover. Nevertheless, several studies have identified relationships between tree species and soil C dynamics, which my results may help to further elucidate. For example, in an 80 year old experimental plantation in the Democratic Republic of the Congo, Bauters *et al.* (2017) found that tree functional composition (explained by leaf C concentration and litter N and Ca) strongly affected pH at 0-5 cm, which in turn had a strong influence on soil C, thus suggesting that trees control soil C and nutrient concentrations primarily via their influence on soil pH. A study by Bréchet *et al.* (2009) using an experimental monospecific tropical plantation of French Guiana, revealed a strong relationship between soil respiration and tree species, which was explained by leaf litterfall, basal area, and litter (and soil) P. Although my study did not indicate direct relationships between the RI of tree functional groups and soil pH and P content, the significant positive relationship between soil pH and both microbial biomass and litter decay rate, and the positive relationship between soil P and soil respiration (SR_{MEAN}) reported in Chapter 4 suggest these soil properties have a strong influence on the soil microbial community both in terms of

abundance and activity which drive soil C dynamics. Studies assessing the influence of specific tree functional groups on soil C dynamics in the tropics are fewer still and have yielded mixed results. For example, in a lowland wet tropical forest in Costa Rica, Keller *et al.*(2013) assessed the influence of tree species on soil biogeochemistry between legume and non-legume tree functional groups, but found no direct relationships between tree functional groups and soil C. Whereas in a study in central Amazonia van Haren *et al.* (2010) found only weak relationships between tree species and CO_2 fluxes, but that soil CO_2 fluxes were higher in close proximity to large trees; they concluded that species-dependent resource acquisition strategies, such as those underlying species-specific growth rates and nutrient demand functions, are likely to be important for predicting ecosystem feedbacks to climate change. Similarly, in monodominant stands on abandoned pasture in Costa Rica Russell *et al.* (2010) reported that differences in species effects on forest C balances were primarily related to differences in growth rates, partitioning of C among biomass components, tissue turnover rates, and tissue chemistry. Thus, the work presented in this thesis complements these studies by linking soil C accumulation to the resource-use strategies of tree functional groups via litter quality and soil microbial communities and activity. Consequently, my studies highlight several promising lines of investigation into the relationships between tree functional groups and C dynamics and provide an important contribution to this emerging field of research.

5.6 Future directions

Natural forests sequester around 40 times more C than plantations (Lewis *et al.*,2019). However, despite being the most widely applicable and cost-effective method of tropical forest restoration, naturally regenerating forests are currently overlooked in favour of more financially productive commercial plantations (Lewis *et al.*, 2019). The ability to predict and increase soil C accumulation during tropical forest regrowth could provide a vital motivating factor to encourage the inclusion of natural regrowth forests in land management and restoration schemes in the fight against climate change.

Precise and accurate determination of current and potential soil C sequestration over large spatial and temporal scales during tropical forest regrowth would be expensive and time-

consuming. Instead, indicator-based methods, which integrate several environmental and soil properties related with SOC storage, have been proposed as a promising alternative (Wiesmeier *et al.*, 2019) as they are generally more time- and cost-effective. Vegetation type is one of the key indicators but the effect of tree species on SOC storage in forests is still unknown (Wiesmeier *et al.*, 2019). Classifying forest by age does not adequately capture patterns in soil C (Yang, Luo and Finzi, 2011; Li, Niu and Luo, 2012; Marín-Spiotta and Sharma, 2013; Martin, Bullock and Newton, 2013; Powers and Marín-Spiotta, 2017) but the strong relationship between tree community shade-tolerance and soil C revealed in this study provides an important step towards identifying a potentially important indicator for soil C storage, which can be tested in other forests and at larger spatial scales. To maximise soil C sequestration and inform land management practices future research should investigate whether the influence of light-demanding species on surface soil C accumulation is maintained despite repeated disturbance events (e.g. management of commercial forestry plantations, agroforestry schemes, selective logging).

5.7 Concluding remarks

As far as I am aware, this study is the first to investigate the relationship between soil C dynamics and tree community functional change during tropical forest secondary succession, accounting for the relative influence of shade-tolerant and light-demanding species. I highlight some of the potential pathways by which tree community composition could influence soil C storage via the quality and quantity of litter inputs representing substrate for the soil microbial community. More information on root traits and rhizosphere inputs, combined with high-resolution analyses of soil microbial communities might greatly improve our mechanistic understanding of soil C dynamics during tropical forest secondary succession and thus enable more accurate estimates of the C sequestration potential in naturally recovering tropical forests, taking into account both above- and belowground components. Overall, the research presented in this thesis demonstrates that the tree functional composition of secondary forests could be one of the main factors determining belowground C storage and therefore, my work represents an important first step towards using tree functional groups to predict soil C accumulation in secondary tropical forests.

6 References

Amazonas, N. T. *et al.* (2011) 'Nitrogen dynamics during ecosystem development in tropical forest restoration', *Forest Ecology and Management*, 262(8), pp. 1551–1557. doi: 10.1016/j.foreco.2011.07.003.

Anderson-Teixeira, K. J. *et al.* (2016) 'Carbon dynamics of mature and regrowth tropical forests derived from a pantropical database (TropForC-db)', *Global Change Biology*, 22(5), pp. 1690–1709. doi: 10.1111/gcb.13226.

Arroyo-Rodríguez, V. *et al.* (2017) 'Multiple successional pathways in human-modified tropical landscapes: new insights from forest succession, forest fragmentation and landscape ecology research', *Biological Reviews*, 92(1), pp. 326–340. doi: 10.1111/brv.12231.

Baccini, A. *et al.* (2017) 'Tropical forests are a net carbon source based on aboveground measurements of gain and loss.', *Science (New York, N.Y.)*, 358(6360), pp. 230–234. doi: 10.1126/science.aam5962.

Bachelot, B. *et al.* (2016) 'Long- - lasting effects of land use history on soil fungal communities in second- - growth tropical rain forests', *Ecological Applications*, 26(6), pp. 1881–1895. doi: 10.1890/15-1397.1.

Baillie, I. *et al.* (2007) *Semi-Detailed Soil Survey of Barro Colorado Island, Panama*, *Smithsonian Tropical Research Institute*. Available at: http://biogeodb.stri.si.edu/bioinformatics/bci_soil_map/documentation/BCI_soil_report_complete.pdf.

Bakker, M. A., Carreño-Rocabado, G. and Poorter, L. (2011) 'Leaf economics traits predict litter decomposition of tropical plants and differ among land use types', *Functional Ecology*, 25(3), pp. 473–483. doi: 10.1111/j.1365-2435.2010.01802.x.

Balesdent, J. *et al.* (2018) 'Atmosphere–soil carbon transfer as a function of soil depth', *Nature*. Springer US, 559(7715), pp. 599–602. doi: 10.1038/s41586-018-0328-3.

Barberán, A. *et al.* (2015) 'Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest', *Ecology Letters*, 18(12), pp. 1397–1405. doi: 10.1111/ele.12536.

Bardgett, R. D. *et al.* (2005) 'A temporal approach to linking aboveground and belowground ecology', *Trends in Ecology & Evolution*, 20(11). doi: 10.1016/j.tree.2005.08.005.

Bardgett, R. D. (2017) 'Plant trait-based approaches for interrogating belowground function', *Biology and Environment*, 117B(1), pp. 1–13. doi: 10.3318/bioe.2017.03.

Bardgett, R. D., Hobbs, P. J. and Frostegård, Å. (1996) 'Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland', *Biology and Fertility of Soils*. doi: 10.1007/BF00382522.

Bardgett, R. D., Mommer, L. and De Vries, F. T. (2014) 'Going underground : root traits as drivers of ecosystem processes', *Trends in Ecology & Evolution*. Elsevier Ltd, 29(12), pp. 692–699. doi: 10.1016/j.tree.2014.10.006.

Barlow, J. *et al.* (2016) 'Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation', *Nature*. Nature Publishing Group, 535(7610), pp. 144–147. doi: 10.1038/nature18326.

Barthold, F. K., Stallard, R. F. and Elsenbeer, H. (2008) 'Soil nutrient-landscape relationships in a lowland tropical rainforest in Panama', *Forest Ecology and Management*, 255(3–4), pp. 1135–1148. doi: 10.1016/j.foreco.2007.09.089.

Bates, D. *et al.* (2015) 'Fitting linear mixed-effects models using lme4', *Journal of Statistical Software*, 67(1). doi: 10.18637/jss.v067.i01.

Bauters, M. *et al.* (2017) 'Functional Composition of Tree Communities Changed Topsoil Properties in an Old Experimental Tropical Plantation', *Ecosystems*. Springer US, 20(5), pp. 861–871. doi: 10.1007/s10021-016-0081-0.

Berg, B. and McClaugherty, C. (2007) *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*. 2nd edn. Springer Berlin Heidelberg.

Bligh, E. G. and Dyer, W. J. (1959) 'A rapid method of total lipid extraction and purification', *Canadian Journal of Biochemistry and Physiology*, 37(1), pp. 911–917. doi: 10.1139/y59-099.

Bond-lamberty, B. *et al.* (2018) 'Globally rising soil heterotrophic respiration over recent decades', *Nature*. Springer US, 560, pp. 81–83. doi: 10.1038/s41586-018-0358-x.

Boukili, V. K. and Chazdon, R. L. (2017) 'Environmental filtering , local site factors and landscape context drive changes in functional trait composition during tropical forest succession', *Perspectives in Plant Ecology, Evolution and Systematics*. Elsevier GmbH., 24, pp. 37–47. doi: 10.1016/j.ppees.2016.11.003.

Bray, S. R., Kitajima, K. and Mack, M. C. (2012) 'Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate', *Soil Biology and Biochemistry*. Elsevier Ltd, 49, pp. 30–37. doi: 10.1016/j.soilbio.2012.02.009.

Bréchet, L. *et al.* (2009) 'Do tree species characteristics influence soil respiration in tropical forests? A test based on 16 tree species planted in monospecific plots', *Plant and Soil*, 319(1–2), pp. 235–246. doi: 10.1007/s11104-008-9866-z.

Brunner, I *et al.* (2013) *Belowground Carbon Turnover in European Forests : Fine Roots , Mycorrhizal Mycelia , Soil Organic Matter and Soil Models. A Technical Report for National C reporters, LULUCF experts and ecosystem modellers. COST Action*

FP0803. Birmensdorf, Switzerland.

Brunner, I. *et al.* (2013) 'Fine-root turnover rates of European forests revisited: An analysis of data from sequential coring and ingrowth cores', *Plant and Soil*, 362(1–2), pp. 357–372. doi: 10.1007/s11104-012-1313-5.

Burns, R. G. *et al.* (2013) 'Soil Biology & Biochemistry Soil enzymes in a changing environment : Current knowledge and future directions', *Soil Biology and Biochemistry*. Elsevier Ltd, 58, pp. 216–234. doi: 10.1016/j.soilbio.2012.11.009.

Buyer, J. S. and Sasser, M. (2012) 'High throughput phospholipid fatty acid analysis of soils', *Applied Soil Ecology*. Elsevier B.V., 61, pp. 127–130. doi: 10.1016/j.apsoil.2012.06.005.

Castellano, M. J. *et al.* (2015) 'Integrating plant litter quality , soil organic matter stabilization , and the carbon saturation concept', *Global Change Biology*, 21, pp. 3200–3209. doi: 10.1111/gcb.12982.

Chazdon, R. (2016) 'Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics', *Science Advances*, 333(6045), pp. 988–93. doi: 10.1126/science.1201609.

Chazdon, R. L. *et al.* (2009) 'The potential for species conservation in tropical secondary forests', *Conservation Biology*, 23(6), pp. 1406–1417. doi: 10.1111/j.1523-1739.2009.01338.x.

Chazdon, R. L. (2014) *Second Growth: The Promise of Tropical Forest Regeneration in an Age of Deforestation*. The University of Chicago Press. doi: 10.1017/CBO9781107415324.004.

Chazdon, R. L. and Guariguata, M. R. (2016) 'Natural regeneration as a tool for large-scale forest restoration in the tropics: prospects and challenges', *Biotropica*, 48(6), pp. 716–730. doi: 10.1111/btp.12381.

Chen, X. *et al.* (2020) 'Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis', *Biological Reviews*, 95(1), pp. 167–183. doi: 10.1111/brv.12554.

Chomel, M. *et al.* (2016) 'Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling', *Journal of Ecology*, 104(6), pp. 1527–1541. doi: 10.1111/1365-2745.12644.

Clark, D. A. *et al.* (2001) 'Net primary production in tropical forests: an evaluation and synthesis of existing field data', *Ecological Applications*, 11(2), pp. 371–384.

Cleveland, C. C. *et al.* (2011) 'Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis', *Ecology Letters*, 14(12), pp. 1313–1317. doi: 10.1111/j.1461-0248.2011.01711.x.

Comita, L. S. *et al.* (2007) 'Patterns of woody plant species abundance and diversity in the seedling layer of a tropical forest', *Journal of Vegetation Science*, 18, pp. 163–174. doi: 10.1658/1100-9233.

Condit, R., Chisholm, R. A. and Hubbell, S. P. (2012) 'Thirty Years of Forest Census at Barro Colorado and the Importance of Immigration in Maintaining Diversity', *PLoS ONE*, 7(11), pp. 1–6. doi: 10.1371/journal.pone.0049826.

Condit, R., Hubbell, S. P. and Foster, R. B. (1996) 'Changes in tree species abundance in a Neotropical forest : impact of climate change', *Journal of Tropical Ecology*, 12(2), pp. 231–256. doi: 10.1017/S0266467400009433.

Condit, R., Pérez, R. and Daguerre, N. (2011) *Trees of Panama and Costa Rica*. Princeton, New Jersey: Princeton University Press. doi: 10.5860/choice.48-5080.

Conti, G. and Díaz, S. (2013) 'Plant functional diversity and carbon storage - an empirical test in semi-arid forest ecosystems', *Journal of Ecology*, 101(1), pp. 18–28. doi: 10.1111/1365-2745.12012.

Cornelissen, J. H. C. (1996) 'An Experimental Comparison of Leaf Decomposition Rates in a Wide Range of Temperate Plant Species and Types', *Journal of Ecology*, 84(4), pp. 573–582.

Cornelissen, J. H. C. *et al.* (1999) 'Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents', *New Phytologist*, 143(1), pp. 191–200. doi: 10.1046/j.1469-8137.1999.00430.x.

Cornwell, W. K. *et al.* (2008) 'Plant species traits are the predominant control on litter decomposition rates within biomes worldwide', *Ecology Letters*, 11(10), pp. 1065–1071. doi: 10.1111/j.1461-0248.2008.01219.x.

Cotrufo, M. F., Wallenstein, M. D. and Boot, C. M. (2013) 'The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization : do labile plant inputs form stable soil organic matter ?', *Global Change Biology*, (19), pp. 988–995. doi: 10.1111/gcb.12113.

Craven, D. (2015) 'Changing gears during succession : shifting functional strategies in young tropical secondary forests in young tropical secondary forests', *Oecologia*, 179, pp. 293–305. doi: 10.1007/s00442-015-3339-x.

Crawley, M. J. (2007) *The R Book.* Chichester, UK: John Wiley & Sons.

Cusack, D. F. *et al.* (2018) 'Soil carbon stocks across tropical forests of Panama regulated by base cation effects on fine roots', *Biogeochemistry*, 137(1–2), pp. 253–266. doi: 10.1007/s10533-017-0416-8.

Dalling, J. W. and Denslow, J. S. (1998) 'Soil seed bank composition along a forest chronosequence in seasonally moist tropical forest , Panama', *Journal of Vegetation Science*, 9, pp. 669–678.

Davidson, E. A. *et al.* (2007) 'Recuperation of nitrogen cycling in Amazonian forests

following agricultural abandonment', *Nature*, 447. doi: 10.1038/nature05900.

Denslow and Guzman (2000) 'Variation in stand structure, light and seedling abundance across a tropical moist forest chronosequence, Panama', *Journal of Vegetation Science*, 11(2), pp. 201–212. doi: 10.2307/3236800.

Dent, D. H., DeWalt, S. J. and Denslow, J. S. (2012) 'Secondary forests of central Panama increase in similarity to old-growth forest over time in shade tolerance but not species composition', *Journal of Vegetation Science*, 24(3), pp. 530–542. doi: 10.1111/j.1654-1103.2012.01482.x.

Detwiler, R. P. (1986) 'Land use change and the global carbon cycle: the role of tropical soils', *Biogeochemistry*, 2(1), pp. 67–93. doi: 10.1007/BF02186966.

DeWalt, S. J., Maliakal, S. K. and Denslow, J. S. (2003) 'Changes in vegetation structure and composition along a tropical forest chronosequence: implications for wildlife', *Forest Ecology and Management*, 182(1–3), pp. 139–151. doi: 10.1016/S0378-1127(03)00029-X.

Dewalt, S. J., Schnitzer, S. A. and Denslow, J. S. (2000) 'Density and diversity of lianas along a chronosequence in a central Panamanian lowland forest Density and diversity of lianas along a chronosequence in a central Panamanian lowland forest', *Journal of Tropical Ecology*, 16(1), pp. 1–19.

De Deyn, G. B., Cornelissen, J. H. C. and Bardgett, R. D. (2008) 'Plant functional traits and soil carbon sequestration in contrasting biomes', *Ecology Letters*, 11(5), pp. 516–531. doi: 10.1111/j.1461-0248.2008.01164.x.

Don, A., Schumacher, J. and Freibauer, A. (2011) 'Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis', *Global Change Biology*, 17, pp. 1658–1670. doi: 10.1111/j.1365-2486.2010.02336.x.

Fanin, N., Hättenschwiler, S. and Fromin, N. (2014) 'Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil', *Plant and Soil*, 379, pp. 79–91. doi: 10.1007/s11104-014-2051-7.

FAO (2015) *Global Forest Resources Assessment 2015*. FAO Forest. UN Food and Agriculture Organization, Rome. Available at: http://www.fao.org/forestry/fra2005/en/.

FAO (2018) *The state of the worlds forests 2018 - Forest pathways to sustainable development.* Rome. Available at: www.fao.org/publications.

Fierer, N. *et al.* (2009) 'Global patterns in belowground communities', *Ecology Letters*, 12(11), pp. 1238–1249. doi: 10.1111/j.1461-0248.2009.01360.x.

Fierer, N., Bradford, M. A. and Jackson, R. B. (2007) 'Toward an Ecological Classification of Soil Bacteria', *Ecology*, 88(6), pp. 1354–1364. doi: 10.1890/05-1839.

Fierer, N. and Jackson, R. B. (2006) 'The diversity and biogeography of soil bacterial communities', *Proceedings of the National Academy of Sciences of the United States of America*, 103(3), pp. 626–631.

Foley, J. A. *et al.* (2005) 'Global Consequences of Land Use', *Science*, 309, pp. 570–575.

Fontaine, S. *et al.* (2007) 'Stability of organic carbon in deep soil layers controlled by fresh carbon supply', *Nature*, 450(7167), pp. 277–280. doi: 10.1038/nature06275.

Foster, D. *et al.* (2003) 'The Importance of Land-Use Legacies to Ecology and Conservation', *BioScience*, 53(1), p. 77. doi: 10.1641/0006-3568(2003)053[0077:tiolul]2.0.co;2.

Friedlingstein, P. *et al.* (2019) 'Global Carbon Budget 2019', *Earth System Science Data*, (11), pp. 1783–1838.

Gartner, T. B. and Cardon, Z. G. (2004) 'Decomposition dynamics in mixed-species leaf litter', *Oikos*, 2(104), pp. 230–246.

Gessner, M. O. *et al.* (2010) 'Diversity meets decomposition', *Trends in Ecology & Evolution*. Elsevier Ltd, 25(6), pp. 372–380. doi: 10.1016/j.tree.2010.01.010.

Ghazoul, J. and Chazdon, R. (2017) 'Degradation and Recovery in Changing Forest Landscapes : A Multiscale Conceptual Framework', *Annual Review of Environment and Resources*, 42, pp. 161–88.

Gray, J. M., Bishop, T. F. A. and Wilson, B. R. (2016) 'Factors Controlling Soil Organic Carbon Stocks with Depth in Eastern Australia', *Soil Science Society of America Journal*, 79, pp. 1741–1751. doi: 10.2136/sssaj2015.06.0224.

Grime, J. P. (1974) 'Vegetation classification by reference to strategies', *Nature*, 250(5461), p. 26. doi: 10.1038/250026a0.

Grime, J. P. *et al.* (1996) 'Evidence of a Causal Connection between Anti-Herbivore Defence and the Decomposition Rate of Leaves', *Nordic Society Oikos*, 77(3), pp. 489–494. Available at: https://www.jstor.org/stable/3545938.

Grimm, R. *et al.* (2008) 'Soil organic carbon concentrations and stocks on Barro Colorado Island — Digital soil mapping using Random Forests analysis', *Geoderma*, 146(1–2), pp. 102–113. doi: 10.1016/j.geoderma.2008.05.008.

Gross, C. D. and Harrison, R. B. (2019) 'The Case for Digging Deeper: Soil Organic Carbon Storage, Dynamics, and Controls in Our Changing World', *Soil Systems*, 3(2), p. 28. doi: 10.3390/soilsystems3020028.

Guo and Gifford (2002) 'Soil carbon stocks and land use change : a meta analysis', *Global Change Biology*, 8, pp. 345–360.

Hanson, P. J. *et al.* (2000) 'Separating Root and Soil Microbial Contributions to Soil Respiration : A Review of Methods and Observations', *Biogeochemistry*, 48(1), pp. 115–146. doi: 10.1023/A:1006244819642.

van Haren, J. L. M. *et al.* (2010) 'Do plant species influence soil CO 2 and N 2 O fluxes in a diverse tropical forest ?', *Journal of Geophysical Research*, 115, pp. 1–9. doi: 10.1029/2009JG001231.

Hattenschwiler, S. *et al.* (2011) 'Leaf traits and decomposition in tropical rainforests: Revisiting some commonly held views and towards a new hypothesis', *New Phytologist*, 189(4), pp. 950–965. doi: 10.1111/j.1469-8137.2010.03483.x.

Hättenschwiler, S., Tiunov, A. V. and Scheu, S. (2005) 'Biodiversity and Litter Decomposition in Terrestrial Ecosystems', *Annual Review of Ecology, Evolution, and Systematics*, 36(1), pp. 191–218. doi: 10.1146/annurev.ecolsys.36.112904.151932.

Heiberger, R. M. (2020) 'HH: Statistical Analysis and Data Display: Heiberger and Holland. R package version 3.1-40'.

Holdridge, L. R. and Budowski, H. (1956) 'Report of an ecological survey of the Republic of Panama.', *Caribbean Forester*, pp. 17:92-110.

Hubau, W. *et al.* (2020) 'Asynchronous carbon sink saturation in African and Amazonian tropical forests', *Nature*, 579, pp. 80–87. doi: 10.1038/s41586-020-2035-0.

Jewell, M. D. *et al.* (2017) 'Partitioning the effect of composition and diversity of tree communities on leaf litter decomposition and soil respiration', *Oikos*, 126(7), pp. 959–971. doi: 10.1111/oik.03868.

Jobbágy, E. G. and Jackson, R. B. (2000) 'The vertical distribution of soil organic carbon and its relation to climate and vegetation', *Ecological Applications*, 10(2), pp. 423–436. doi: 10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2.

Johnson, C. M. *et al.* (2000) 'Post-Disturbance Aboveground Biomass Accumulation in Global Secondary Forests', *Ecology*, 81(5), pp. 1395–1401.

Jones, I. L. *et al.* (2019) 'Above- and belowground carbon stocks are decoupled in secondary tropical forests and are positively related to forest age and soil nutrients respectively', *Science of the Total Environment*, 697, p. 133987. doi: 10.1016/j.scitotenv.2019.133987.

Kaspari, M. *et al.* (2008) 'Multiple nutrients limit litterfall and decomposition in a tropical forest', *Ecology Letters*, (11), pp. 35–43.

Kattge, J. *et al.* (2011) 'TRY - a global database of plant traits', *Global Change Biology*, 17(9), pp. 2905–2935. doi: 10.1111/j.1365-2486.2011.02451.x.

Keller, A. B. *et al.* (2013) 'Effects of canopy tree species on belowground biogeochemistry in a lowland wet tropical forest', *Soil Biology and Biochemistry*. Elsevier Ltd, 58, pp. 61–69. doi: 10.1016/j.soilbio.2012.10.041.

Kerdraon, D. *et al.* (2019) 'Litter traits of native and non-native tropical trees influence soil carbon dynamics in timber plantations in Panama', *Forests*, 10(3). doi:

10.3390/f10030209.

Kerdraon, D. *et al.* (2020) 'Litter Inputs, but Not Litter Diversity, Maintain Soil Processes in Degraded Tropical Forests—A Cross-Continental Comparison', *Frontiers in Forests and Global Change*, 2(90), pp. 1–14. doi: 10.3389/ffgc.2019.00090.

Kramer-Walter, K. R. *et al.* (2016) 'Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum', *Journal of Ecology*, 104(5), pp. 1299–1310. doi: 10.1111/1365-2745.12562.

Krishna, M. P. and Mohan, M. (2017) 'Litter decomposition in forest ecosystems: a review', *Energy, Ecology and Environment*. Joint Center on Global Change and Earth System Science of the University of Maryland and Beijing Normal University, 2(4), pp. 236–249. doi: 10.1007/s40974-017-0064-9.

Kutsch, W. L., Bahn, M. and Heinemeyer, A. (eds) (2009) *Soil carbon dynamics: An integrated methodology*. Cambridge University Press. doi: 10.1017/CBO9780511711794.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017) 'lmerTest Package : Tests in Linear Mixed Effects Models', *Journal of Statistical Software*, 82(13). doi: 10.18637/jss.v082.i13.

Laganière, J., Angers, D. A. and Paré, D. (2010) 'Carbon accumulation in agricultural soils after afforestation: A meta-analysis', *Global Change Biology*, 16(1), pp. 439–453. doi: 10.1111/j.1365-2486.2009.01930.x.

Laird-Hopkins, B. C. *et al.* (2017) 'Tree functional diversity affects litter decomposition and arthropod community composition in a tropical forest', *Biotropica*, 49(6), pp. 903–911. doi: 10.1111/btp.12477.

Lauber, L. *et al.* (2008) 'Stoichiometry of soil enzyme activity at global scale', *Ecology Letters*, 11, pp. 1252–1264. doi: 10.1111/j.1461-0248.2008.01245.x.

Lebrija-Trejos, E. *et al.* (2010) 'Functional traits and environmental filtering drive community assembly in a species-rich tropical system', *Ecology*, 91(2), pp. 386–398.

Leigh., E. G. *et al.* (2004) 'Barro Colorado Island Forest Dynamics Plot, Panama', in *Tropical forest diversity and dynamism: Findings from a large-scale plot network*, pp. 451–463.

Leigh, E. G., Rand, A. S. and Windsor, D. M. (1983) *The ecology of a tropical forest: seasonal rhythms and long-term changes*. Edited by D. M. Leigh, E.G., Rand, A.S. and Windsor. Smithsonian Institution Press, Washington, DC, US.

Lewis, S. L. *et al.* (2019) 'Regenerate natural forests to store carbon', *Nature*, 568, pp. 25–28.

Li, D., Niu, S. and Luo, Y. (2012) 'Global patterns of the dynamics of soil carbon and nitrogen stocks following afforestation: A meta-analysis', *New Phytologist*, 195(1), pp. 172–181. doi: 10.1111/j.1469-8137.2012.04150.x.

Liang, C., Schimel, J. P. and Jastrow, J. D. (2017) 'The importance of anabolism in microbial control over soil carbon storage', *Nature Microbiology*, 2(8), pp. 1–6. doi: 10.1038/nmicrobiol.2017.105.

Lohbeck, M. *et al.* (2012) 'Functional diversity changes during tropical forest succession', *Perspectives in Plant Ecology, Evolution and Systematics*, 14(2), pp. 89–96. doi: 10.1016/j.ppees.2011.10.002.

Mackey, B. *et al.* (2020) 'Understanding the importance of primary tropical forest protection as a mitigation strategy', *Mitigation and Adaptation Strategies for Global Change*. doi: 10.1007/s11027-019-09891-4.

Malhi, Y. (2010) 'The carbon balance of tropical forest regions , 1990 – 2005', *Current Opinion in Environmental Sustainability*, 2(4), pp. 237–244. doi: 10.1016/j.cosust.2010.08.002.

Marìn-Spiotta, E. *et al.* (2008) 'Trends in above and belowground carbon with forest regrowth after agricultural abandonment in the neotropics', in Myster, R. W. (ed.) *Post-Agricultural Succession in the Neotropics*. Springer US, pp. 22–72. doi: 10.1007/978-0-387-33642-8_2.

Marín-Spiotta, E. and Sharma, S. (2013) 'Carbon storage in successional and plantation forest soils: A tropical analysis', *Global Ecology and Biogeography*, 22(1), pp. 105–117. doi: 10.1111/j.1466-8238.2012.00788.x.

Martin, P., Bullock, J. and Newton, A. (2013) 'Carbon pools recover more rapidly than plant biodiversity in secondary tropical forests', *Philosophical Transactions of the Royal Society B*, 280, pp. 1–8. doi: 10.1098/rspb.2013.2236.

Mascaro, J. *et al.* (2012) 'Scale-dependence of aboveground carbon accumulation in secondary forests of Panama: A test of the intermediate peak hypothesis', *Forest Ecology and Management*, 276, pp. 62–70. doi: 10.1016/j.foreco.2012.03.032.

McGuire, K. L. *et al.* (2012) 'Fungal Community Composition in Neotropical Rain Forests: The Influence of Tree Diversity and Precipitation', *Microbial Ecology*, 63(4), pp. 804–812. doi: 10.1007/s00248-011-9973-x.

Metcalfe, D. B., Fisher, R. A. and Wardle, D. A. (2011) 'Plant communities as drivers of soil respiration: Pathways, mechanisms, and significance for global change', *Biogeosciences*, 8(8), pp. 2047–2061. doi: 10.5194/bg-8-2047-2011.

Mommer, L. and Weemstra, M. (2012) 'The role of roots in the resource economics spectrum', *New Phytologist*, 195(4), pp. 725–727. doi: 10.1111/j.1469-8137.2012.04247.x.

Norden, N. *et al.* (2015) 'Successional dynamics in Neotropical forests are as uncertain as they are predictable', *Proceedings of the National Academy of Sciences of the United States of America*, 112(26), pp. 8013–8018. doi: 10.1073/pnas.1500403112.

Nottingham, A. T. *et al.* (2013) 'Root and arbuscular mycorrhizal mycelial interactions with soil microorganisms in lowland tropical forest', *FEMS Microbiology Ecology*, 85(1), pp. 37–50. doi: 10.1111/1574-6941.12096.

Oksanen, J. *et al.* (2018) 'vegan: Community Ecology Package. R package version 2.5-2.' Available at: https://cran.r-project.org/package=vegan.

Orihuela-Belmonte, D. E. *et al.* (2013) 'Carbon stocks and accumulation rates in tropical secondary forests at the scale of community, landscape and forest type', *Agriculture, Ecosystems and Environment*. Elsevier B.V., 171, pp. 72–84. doi: 10.1016/j.agee.2013.03.012.

Ostertag, R. *et al.* (2008) 'Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico', *Ecosystems*, 11(5), pp. 701–714. doi: 10.1007/s10021-008-9152-1.

Pan, Y. *et al.* (2011) 'A large and persistent carbon sink in the world's forests', *Science*, 333, pp. 988–993. doi: 10.1126/science.1201609.

Pausch, J. and Kuzyakov, Y. (2018) 'Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale', *Global Change Biology*, 24(1), pp. 1–12. doi: 10.1111/gcb.13850.

Paz, C. P. *et al.* (2016) 'Soil types influence predictions of soil carbon stock recovery in tropical secondary forests', *Forest Ecology and Management*, 376, pp. 74–83. doi: 10.1016/j.foreco.2016.06.007.

Pendrill, F. *et al.* (2019) 'Agricultural and forestry trade drives large share of tropical deforestation emissions', *Global Environmental Change*, 56, pp. 1–10. doi: 10.1016/j.gloenvcha.2019.03.002.

Poorter, L. *et al.* (2016) 'Biomass resilience of Neotropical secondary forests', *Nature*, 530(7589), pp. 211–214. doi: 10.1038/nature16512.

Powers, J. S. *et al.* (2009) 'Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient', *Journal of Ecology*, 97(4), pp. 801–811. doi: 10.1111/j.1365-2745.2009.01515.x.

Powers, J. S. and Marín-Spiotta, E. (2017) 'Ecosystem Processes and Biogeochemical Cycles in Secondary Tropical Forest Succession', *Annual Review of Ecology, Evolution, and Systematics*. Annual Reviews, 48(1), pp. 497–519. doi: 10.1146/annurev-ecolsys-110316-022944.

Prescott, C. E. (2010) 'Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils?', *Biogeochemistry*, 101(1), pp. 133–149. doi: 10.1007/s10533-010-9439-0.

Prescott, C. E. and Grayston, S. J. (2013) 'Forest Ecology and Management Tree species influence on microbial communities in litter and soil : Current knowledge and

research needs', *Forest Ecology and Management*, 309, pp. 19–27. doi: 10.1016/j.foreco.2013.02.034.

Pugh, T. A. M. *et al.* (2019) 'Role of forest regrowth in global carbon sink dynamics', *Proceedings of the National Academy of Sciences of the United States of America*, 116(10), pp. 4382–4387. doi: 10.1073/pnas.1810512116.

Qie, L. *et al.* (2017) 'Long-term carbon sink in Borneo's forests halted by drought and vulnerable to edge effects', *Nature Communications*, 8(1). doi: 10.1038/s41467-017-01997-0.

R Core Team (2018) 'R: A Language and Environment for Statistical Computing.' Vienna: R Foundation for Statistical Computing. Available online at: https:// www.R-project.org/

Reich, P. B. (2014) 'The world-wide " fast – slow " plant economics spectrum : a traits manifesto', *Journal of Ecology*, 102, pp. 275–301. doi: 10.1111/1365-2745.12211.

Romijn, E. *et al.* (2019) 'Land restoration in Latin America and the Caribbean : An overview of recent , ongoing and planned restoration initiatives and their potential for climate change mitigation', *Forests*, 10(6). doi: 10.3390/f10060510.

Rousk, J. *et al.* (2008) 'Examining the fungal and bacterial niche overlap using selective inhibitors in soil', *FEMS Microbiology Ecology*, 63(3), pp. 350–358. doi: 10.1111/j.1574-6941.2008.00440.x.

Rousk, J. *et al.* (2010) 'Soil bacterial and fungal communities across a pH gradient in an arable soil', *ISME Journal*, 4(10), pp. 1340–1351. doi: 10.1038/ismej.2010.58.

Rousk, J. and Bååth, E. (2007) 'Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique', *Soil Biology and Biochemistry*, 39(8), pp. 2173–2177. doi: 10.1016/j.soilbio.2007.03.023.

Rousk, J., Brookes, P. C. and Bååth, E. (2009a) 'Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization', *Applied and Environmental Microbiology*, 75(6), pp. 1589–1596. doi: 10.1128/AEM.02775-08.

Rousk, J., Brookes, P. C. and Bååth, E. (2009b) 'Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization', *Applied and Environmental Microbiology*, 75(6), pp. 1589–1596. doi: 10.1128/AEM.02775-08.

Rozendaal, D. M. A. *et al.* (2019) 'Biodiversity recovery of Neotropical secondary forests', *Science Advances*, 5(3). doi: 10.1126/sciadv.aau3114.

Rüger, N. *et al.* (2009) 'Response of recruitment to light availability across a tropical lowland rain forest community', *Journal of Ecology*, 97(6), pp. 1360–1368. doi:

10.1111/j.1365-2745.2009.01552.x.

Rüger, N. *et al.* (2020) 'Demographic trade-offs predict tropical forest dynamics', *Science*, 368(6487), pp. 165–168. doi: 10.1126/science.aaz4797.

Russell, A. E. *et al.* (2010) 'Impacts of individual tree species on carbon dynamics in a moist tropical forest environment', *Ecological Applications*, 20(4), pp. 1087–1100.

Santiago, L. S. (2007) 'Extending the leaf economics spectrum to decomposition: Evidence from a tropical forest', *Ecology*, 88(5), pp. 1126–1131. doi: 10.1890/06-1841.

Sayer, E. J. (2006) 'Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems', *Biological Reviews*, 81(1), pp. 1–31. doi: 10.1017/S1464793105006846.

Sayer, E. J. *et al.* (2011) 'Soil carbon release enhanced by increased tropical forest litterfall', *Nature Climate Change*, 1(9), pp. 304–307. doi: 10.1038/nclimate1190.

Sayer, E. J. and Tanner, E. V. J. (2010) 'A new approach to trenching experiments for measuring root-rhizosphere respiration in a lowland tropical forest', *Soil Biology and Biochemistry*. Elsevier Ltd, 42(2), pp. 347–352. doi: 10.1016/j.soilbio.2009.11.014.

Sayer, Emma J. and Tanner, E. V. J. (2010) 'Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling', *Journal of Ecology*, 98(5), pp. 1052–1062. doi: 10.1111/j.1365-2745.2010.01680.x.

Schimel, J. P. and Schaeffer, S. M. (2012) 'Microbial control over carbon cycling in soil', *Frontiers in Microbiology*, 3(SEP), pp. 1–11. doi: 10.3389/fmicb.2012.00348.

Schlessinger, W. and Andrews, J. A. (2000) 'Soil respiration and the global carbon cycle', *Biogeochemistry 48:*, 48, pp. 7–20. doi: https://doi-org.ezproxy.lancs.ac.uk/10.1023/A:1006247623877.

Schwartz, N. B. *et al.* (2017) 'Land-use dynamics influence estimates of carbon sequestration potential in tropical second-growth forest', *Environmental Research Letters*, 12(7). doi: 10.1088/1748-9326/aa708b.

Simpson, A. J. *et al.* (2007) 'Microbially Derived Inputs to Soil Organic Matter : Are Current Estimates Too Low ?', *Environmental Science & Technology*, 41(23), pp. 8070–8076. doi: 10.1021/es071217x.

Singh, B. K. *et al.* (2010) 'Microorganisms and climate change: Terrestrial feedbacks and mitigation options', *Nature Reviews Microbiology*, 8(11), pp. 779–790. doi: 10.1038/nrmicro2439.

Van Soest, P. J., Robertson, J. B. and Lewis, B. A. (1992) 'Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition', *Journal of Dairy Science*. Elsevier, 74(10), pp. 3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2.

Sokol, N. W. and Bradford, M. A. (2019) 'Microbial formation of stable soil carbon is more

efficient from belowground than aboveground input', *Nature Geoscience*. Springer US, 12(1), pp. 46–53. doi: 10.1038/s41561-018-0258-6.

Strickland, M. S. *et al.* (2009) 'Litter quality is in the eye of the beholder: Initial decomposition rates as a function of inoculum characteristics', *Functional Ecology*, 23(3), pp. 627–636. doi: 10.1111/j.1365-2435.2008.01515.x.

Szefer, P. *et al.* (2017) 'Determinants of litter decomposition rates in a tropical forest: functional traits, phylogeny and ecological succession', *Oikos*, 126(8), pp. 1101–1111. doi: 10.1111/oik.03670.

Townsend, A. R. *et al.* (2011) 'Multi-element regulation of the tropical forest carbon cycle', *Frontiers in Ecology and the Environment*, 9(1), pp. 9–17. doi: 10.1890/100047.

Tripathi, B. M. *et al.* (2016) 'Distinctive tropical forest variants have unique soil microbial communities, but not always low microbial diversity', *Frontiers in Microbiology*, 7(APR), pp. 1–11. doi: 10.3389/fmicb.2016.00376.

Turner, B. L. and Wright, S. J. (2014) 'The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen , phosphorus , and potassium addition in a lowland tropical rain forest', *Biogeochemistry*, 117, pp. 115–130. doi: 10.1007/s.

Urbanová, M., Šnajdr, J. and Baldrian, P. (2015) 'Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees', *Soil Biology and Biochemistry*, 84, pp. 53–64. doi: 10.1016/j.soilbio.2015.02.011.

Vitousek, P. . M. . and Sanford, R. . L. . J. . (1986) 'Nutrient Cycling in Moist Tropical Forest', *Annual Review of Ecology and Systematics*, 17, pp. 137–167.

Waldrop MP , Balser TC, F. M. (2000) 'Linking microbial community composition to function in a tropical soil', *Soil Biology and Biochemistry*, (32), pp. 1837–1846. doi: 10.1016/S0038-0717(00)00157-7.

Waring, B. G., Averill, C. and Hawkes, C. V. (2013) 'Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis and theoretical models', *Ecology Letters*, 16(7), pp. 887–894. doi: 10.1111/ele.12125.

Whitfeld, T. J. S. *et al.* (2014) 'Species richness, forest structure, and functional diversity during succession in the New Guinea lowlands', *Biotropica*, 46(5), pp. 538–548. doi: 10.1111/btp.12136.

Wieder, K. and Wright, J. (1995) 'Tropical Forest Litter Dynamics and Dry Season Irrigation on Barro Colorado Island , Panama', *Ecology*, 76(6), pp. 1971–1979.

Wiesmeier, M. *et al.* (2019) 'Geoderma Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales', *Geoderma*. Elsevier, 333, pp. 149–162. doi: 10.1016/j.geoderma.2018.07.026.

Windsor, D. M. (1990) *Climate and moisture variability in a tropical forest: Long-term*

Records from BCI. Available at: http://www.sil.si.edu/smithsoniancontributions/EarthSciences/pdf_hi/sces-0029.pdf (Accessed: 23 October 2015).

Wright, I. J. *et al.* (2004) 'The worldwide leaf economics spectrum', *Nature*, 12(428:), pp. 821–827.

Yang, Y., Luo, Y. and Finzi, A. C. (2011) 'Carbon and nitrogen dynamics during forest stand development: A global synthesis', *New Phytologist*, 190(4), pp. 977–989. doi: 10.1111/j.1469-8137.2011.03645.x.

Yao, H. *et al.* (2000) 'Microbial Biomass and Community Structure in a Sequence of Soils with Increasing Fertility and Changing Land Use', *Microbial Ecology*, 40, pp. 223–237. doi: 10.1007/s002480000053.

Yavitt, J. B. (2000) 'Nutrient Dynamics of Soil Derived from Different Parent Material on Barro Colorado Island, Panama'', *Biotropica*, 32(2), pp. 198–207.

Yavitt, J. B. *et al.* (2011) 'Soil fertility and fine root dynamics in response to 4 years of nutrient (N , P , K) fertilization in a lowland tropical moist forest , Panama', *Australian Ecology*, 36, pp. 433–445. doi: 10.1111/j.1442-9993.2010.02157.x.

Yavitt, J. B. and Kelman Wieder, R. (1988) 'Nitrogen, Phosphorus, and Sulfur Properties of Some Forest Soils on Barro Colorado Island, Panama', *Biotropica*, 20(1), pp. 2–10.

Yin, S., Wu, W. and Li, X. (2018) 'Comparison of temporal and spatial changes in three major tropical forests based on MODIS data', *Journal of Forestry Research*. doi: 10.1007/s11676-018-0695-5.

Zhang, Z. D., Zang, R. G. and Qi, Y. D. (2008) 'Spatiotemporal patterns and dynamics of species richness and abundance of woody plant functional groups in a tropical forest landscape of Hainan Island, South China', *Journal of Integrative Plant Biology*, 50(5), pp. 547–558. doi: 10.1111/j.1744-7909.2008.00663.x.

Zhou, Z. *et al.* (2017) 'Trends in soil microbial communities during secondary succession', *Soil Biology and Biochemistry*. Elsevier Ltd, 115, pp. 92–99. doi: 10.1016/j.soilbio.2017.08.014.

Zhou, Z., Wang, C. and Luo, Y. (2018) 'Effects of forest degradation on microbial communities and soil carbon cycling: A global meta-analysis', *Global Ecology and Biogeography*, 27(1), pp. 110–124. doi: 10.1111/geb.12663.

Zhu, K. *et al.* (2018) 'Limits to growth of forest biomass carbon sink under climate change', *Nature Communications*, 9(1), p. 2709. doi: 10.1038/s41467-018-05132-5.

Zhu, Z. *et al.* (2017) 'Fate of rice shoot and root residues, rhizodeposits, and microbial assimilated carbon in paddy soil - part 2: turnover and microbial utilization', *Plant and Soil*. Plant and Soil, 416(1–2), pp. 243–257. doi: 10.1007/s11104-017-3210-4.

7 Appendices

7.1 Appendix A – Supplementary Materials to Chapter 2

Appendix A.1 Supplementary Tables

In Chapter 2 I characterised tree community functional composition in five age classes in a chronosequence of 10 tropical forest stands in the Barro Colorado Nature Monument (BCNM), Panama. I calculated stand basal area (BA; m^2 ha^{-1}) and the relative influence (RI; %) of trees in three functional groups (ACC, DEC and NEG; Chapter 3) using species-specific growth response to increasing light values (Rüger *et al.*, 2009) as a proxy for tree community shade-tolerance. I assessed the relationship between the RI of tree functional groups and forest age using mean values from two stands per age class. Table S2.1 shows the mean and standard deviation for stand BA and the RI each tree functional group and the RI of trees not assigned to a functional group. I assessed the relationship between stand age and soil C content (%) and stocks (Mg/ha^{-1}) using mean soil C values from two stands per age class. Table S2.2 shows the mean and standard error for soil C stocks at 0-10 cm and 10-20 cm for n = 8 samples from two stands in each of five age categories.

Table S2.1 Relative proportions of three functional groups of trees and tree basal area (BA) in each age class of a chronosequence of naturally regenerating tropical forest in Panama, Central America. Values are mean and standard deviation for *n* = 2 stands per age class. RI ACC = accelerating growth species, DEC = decelerating growth species, RI NEG = negative growth species, RI NA = species not assigned to a functional group, see Table 3.1 for tree functional group descriptions.

Forest age class	40Y	60Y	90Y	120Y	OG
Tree functional group					
RI ACC (%)	35.7 ± 7.7	24.6 ± 1.6	18.9 ± 0.8	24.5 ± 4	13.8 ± 3.7
RI DEC (%)	46 ± 3.3	55.6 ± 0.5	67.4 ± 1.31	59.3 ± 3.9	73.9 ± 1.3
RI NEG (%)	0.6 ± 0.4	4.4 ± 2.6	4.3 ± 2.8	3.9 ± 2.2	4.7 ± 0.4
RI NA (%)	17.7 ± 4	15.4 ± 4.7	9.3 ± 5	12.4 ± 5.7	7.6 ± 2
Basal area (m^2 ha^{-1})	21.1 ± 0.5	22.26 ± 0.2	35.7 ± 4.6	28.9 ± 0.5	25.5 ± 0.5

Table S2.2 Soil C and N stocks at two depth intervals (0-10 and 10-20 cm) across five age classes of forest stands in a chronosequence of naturally regenerating tropical forest in Panama, Central America. Means and standard errors are given for *n* = 8 (4 x replicate blocks per stand, 2 x stands per age class). Different super-script letters indicate significant differences among forest age classes at $p < 0.05$ determined by ANOVAS with Turkey post-hoc comparisons and correction for multiple comparisons.

Forest age class		40Y	60Y	90Y	120Y	OG
Soil C stocks (Mg/ha^{-1}) with depth (cm)	0-10	64.6^{a} (± 5.20)	60.5^{ab} (± 2.19)	48.8^{b} (± 2.76)	63.9^{a} (± 3.95)	47.0^{b} (± 1.78)
	10-20	38.2^{ab} (± 4.80)	47.5^{a} (± 3.45)	30.1^{b} (± 1.42)	43.7^{a} (± 2.20)	39.1^{ab} (± 1.67)
Soil N stocks (Mg/ha^{-1}) with depth (cm)	0-10	5.25 (± 0.54)	5.26 (± 0.28)	3.86 (± 0.35)	5.29 (± 0.36)	4.38 (± 0.21)
	10-20	2.54^{ab} (± 0.44)	3.52^{a} (± 0.37)	1.52^{b} (± 0.23)	3.30^{a} (± 0.30)	3.35^{a} (± 0.25)

Appendix A.1 Supplementary References

Rüger, N. *et al.* (2009) 'Response of recruitment to light availability across a tropical lowland rain forest community', *Journal of Ecology*, 97(6), pp. 1360–1368. doi: 10.1111/j.1365-2745.2009.01552.x.

7.2 Appendix B – Supplementary Materials to Chapter 3

Appendix B.1 Supplementary Methods

In Chapter 3 I selected a subset of four stands to represent a successional gradient of naturally recovering secondary forest and an old-growth (OG) stand (Figure S3.1). Ideally, when substituting space-for-time in a chronosequence study such as this, all environmental factors across the stands would be equal with the only variable being forest age. However, the real world rarely provides such 'clean' and predicable conditions for field experiments, as demonstrated with the Barro Colorado Nature Monument (BCNM) chronosequence which overlies four geological formations (Baillie *et al.*, 2007). As such it was not possible to select a suitable subset of stands overlying the same geological formation. Although existing research indicates that in general, chemical properties of soils derived from different parent materials do not differ significantly from one another (Yavitt and Kelman Wieder, 1988; Yavitt, 2000; Barthold, Stallard and Elsenbeer, 2008), I wanted to reduce potential variability as much as possible. Therefore, I conducted a site-specific literature review and characterised the soils across the chronosequence stands to select a suitable subset of stands to use in Chapter 3 and 4.

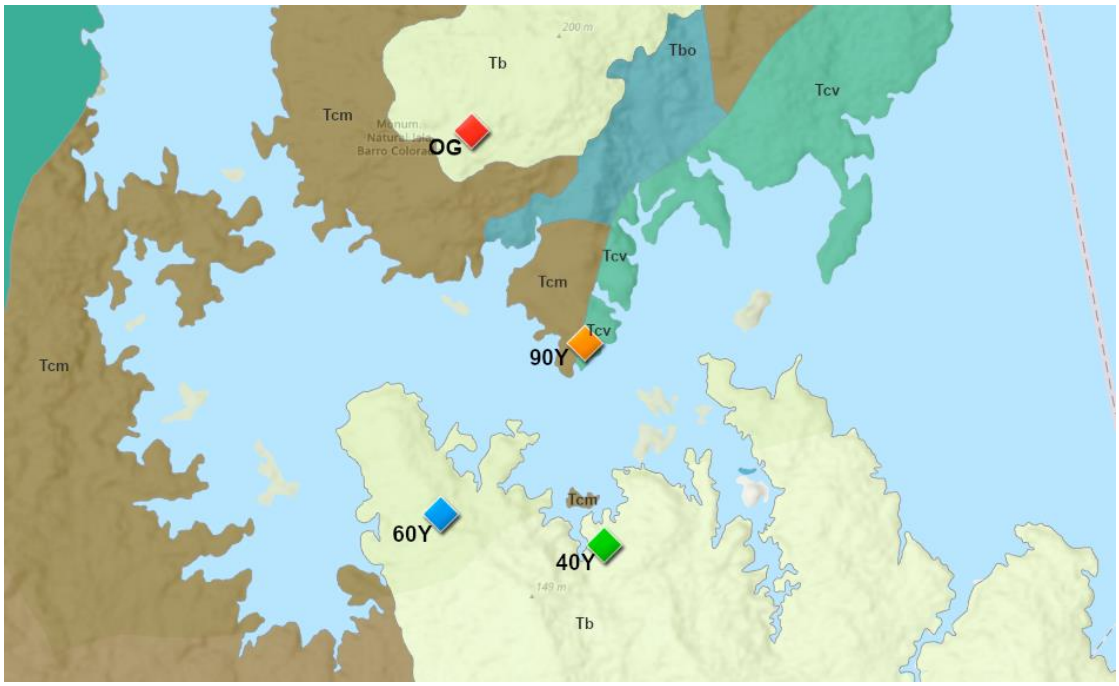


Figure S3.1 Geological map of the Barro Colorado Nature Monument (BCNM) in Panama showing the underling geology of the subset of four forest stands used in a litter decomposition experiment; OG = old-growth (red diamond), 90Y = 90 year old stand (orange diamond), 60Y = 60 year old stand (blue diamond) and 40Y = 40 year old stand (green diamond). Map layer is 'The geological composition of the Panama Canal Watershed' from the Monitoring Project Canal Basin (PMCC), created in 1998. Source: STRI GIS Laboratory. Tb = Volcanic (intrusive and extrusive andesite), Tcm = Sedimentary marine (tuffaceous sandstone, tuffaceous siltstone, tuff and foraminiferal limestone), Tcv = Sedimentary volcanic (agglomerate and tuffaceous graywacke). Location of four forest stands determined from GPS coordinates.

Appendix B.2 Supplementary Table

Table S3.2 Litter properties from five single species used to create three functional litter treatment mixtures and a standard litter treatment in a decomposition experiment in the BCNM, Panama, Central America. ACC = light-demanding (accelerating growth) species, DEC = shade-tolerant (decelerating growth) species, STD = non-forest standard litter treatment. MICOAR = *Miconia argentea,* LUEHSE = *Luehea seemannii*, TET2PA = *Tetragastris panamensis*, and PROTPA = *Protium panamense*. ADF = acid detergent fibre, NDF = neutral detergent fibre, TC = total carbon, TN = total nitrogen, CN ratio = carbon:nitrogen, L:N ratio = lignin:nitrogen. Litter properties given as means and standard errors for *n* =

5 (TC, TN, C:N ratio), *n* = 3 (P, K, Mg, Na, Ca, Fe, Zn), *n* = 2 (ADF, NDF, S) and *n* = 1 (lignin, L:N ratio). See table 4.2 for species and treatment descriptions.

Treatment	*ACC*		*DEC*		*STD*
Species	MICOAR	LUEHSE	TET2PA	PROTPA	PB
ADF (%)	40.67 (±3.90)	52.50 (±2.27)	50.44 (±1.26)	64.17 (±1.62)	51.13 (±4.23)
NDF (%)	34.10 (±1.00)	44.35 (±0.65)	39.90 (±0.00)	53.40 (±1.10)	75.10 (±0.20)
Lignin (%)	15.00	26.00	14.85	30.00	4.00
TC (%)	41.95 (±0.21)	44.44 (±0.12)	38.79 (±0.31)	40.48 (± 0.50)	42.16 (±0.11)
TN (%)	1.04 (±0.07)	1.26 (±0.12)	1.07 (±0.04)	0.95 (±0.05)	1.68 (±0.05)
C.N Ratio	40.99 (±2.88)	36.54 (±2.96)	36.44 (±1.60)	42.92 (±2.14)	25.20 (±0.82)
L:N Ratio	14.37	20.70	13.85	31.51	2.38
P (mg/g)	0.36 (±0.01)	0.79 (±0.03)	0.28 (±0.01)	0.37 (±0.04)	0.94 (±0.03)
K (mg/g)	3.51 (±0.30)	7.87 (±0.21)	1.61 (±0.18)	1.13 (±0.25)	15.37 (±0.47)
Mg mg/g	2.85 (±0.19)	4.04 (±0.06)	2.83 (±0.17)	2.36 (±0.12)	0.99 (±0.10)
Na (mg/g)	1.80 (±0.08)	1.30 (±0.07)	0.87 (±0.21)	0.97 (±0.28)	0.59 (±0.07)
S (mg/g)	5.69 (±0.69)	0.74 (±0.10)	0.81 (±0.04)	1.26 (±0.12)	2.32 (±0.05)
Ca (mg/g)	16.52 (±0.40)	16.52 (±0.86)	13.12 (±0.23)	15.59 (±0.51)	2.08 (±0.20)
Cu (mg/g)	6.16 (±0.06)	9.91 (±0.50)	3.90 (±0.18)	5.28 (±0.80)	8.91 (±0.22)
Fe (mg/Kg)	53.90 (±5.92)	73.77 (±8.03)	75.04 (±10.76)	76.34 (±16.36)	120.72 (±14.50)
Zn (mg/Kg)	64.79 (±5.17)	19.07 (±0.79)	8.35 (±1.13)	8.11 (±0.72)	17.15 (±0.93)

Appendix B.2 Supplementary References

Baillie, I. *et al.* (2007) *Semi-Detailed Soil Survey of Barro Colorado Island, Panama*, *Smithsonian Tropical Research Institute*. Available at: http://biogeodb.stri.si.edu/bioinformatics/bci_soil_map/documentation/BCI_soil_report_complete.pdf.

Barthold, F. K., Stallard, R. F. and Elsenbeer, H. (2008) 'Soil nutrient-landscape relationships in a lowland tropical rainforest in Panama', *Forest Ecology and Management*, 255(3–4), pp. 1135–1148. doi: 10.1016/j.foreco.2007.09.089.

Rüger, N. *et al.* (2009) 'Response of recruitment to light availability across a tropical lowland rain forest community', *Journal of Ecology*, 97(6), pp. 1360–1368. doi: 10.1111/j.1365-2745.2009.01552.x.

Yavitt, J. B. (2000) 'Nutrient Dynamics of Soil Derived from Different Parent Material on Barro Colorado Island, Panama'', *Biotropica*, 32(2), pp. 198–207.

Yavitt, J. B. and Kelman Wieder, R. (1988) 'Nitrogen, Phosphorus, and Sulfur Properties of Some Forest Soils on Barro Colorado Island, Panama', 20(1), pp. 2–10.

7.3 Appendix C – Supplementary Materials to Chapter 4

Appendix C.1 Supplementary Methods

In chapter 4 I assessed the relationship between soil microbial community composition, tree community shade-tolerance and soil C turnover using block mean data in the subset of four stands. Before combining PLFA data from four soil samples in each replicate block per stand, I first tested the influence of litter treatment within individual experimental mesocosms (Chapter 3) on soil microbial community metrics after four months of active litter decomposition. I performed multivariate analyses using the vegan package (Oksanen *et al.*, 2018) in R version 3.4.0 (R Core Team 2018) to compare soil microbial communities among litter treatments. I used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of the relative abundances of PLFA biomarkers (MetaMDS function) to visualise the data and tested the influence of litter treatment by permutational analysis of variance (PERMANOVA; *adonis* function), using 99999 permutations constrained within replicate blocks to generate significance values. I then tested the influence of litter treatment on microbial biomass and biomarker functional groups using linear mixed effects models (*lmer* function) and one-way ANOVAs (*lm* function) to ensure there was no significant influence of litter treatment on the soil microbial community before combining PLFA data by replicate block.

Table S4.1 Model results from PERMANOVA (*adonis* function in R) testing the influence of forest stand and litter treatment (and their interaction) on soil microbial community composition after four months of a litter decomposition experiment across a successional gradient of four tropical forest stands in Panama, Central America.

Model terms	Degrees of freedom	Sum of squares	Mean of squares	*F*	R^2	*P*
Forest stand	3	0.46914	0.156379	10.6812	0.36709	< 0.001
Litter treatment	3	0.01048	0.003494	0.2386	0.00820	0.9656
Stand * Litter treatment	9	0.09561	0.010623	0.7256	0.07481	0.7405
Residuals	48	0.70275	0.014641		0.54989	
Total	63	1.27798			1.00000	

Appendix C.2 Supplementary Results

NMDS ordination and PerMANOVA revealed that microbial community composition did not differ among the four litter treatments ($F_{3,63}$ = 0.24, R^2 = 0.008, p = 0.966; Figure S4.1; Table S4.1) and there was no effect of litter treatment on total soil microbial biomass (p = 0.986; Figure S4.2a), total fungal biomass (p = 0.88; Figure S4,2b), the ratios of fungi:bacteria (p = 0.19; Figure S4.2d) and Gpos:Gneg bacteria (p = 0.997; Figure S4.2e) or the relative abundance of AM fungi (p = 0.594; Figure S4.2c), Gneg bacteria (p = 0.944; Figure S4.2f) and Gpos bacteria (p = 0.921; Figure S4.2g) in any stand.

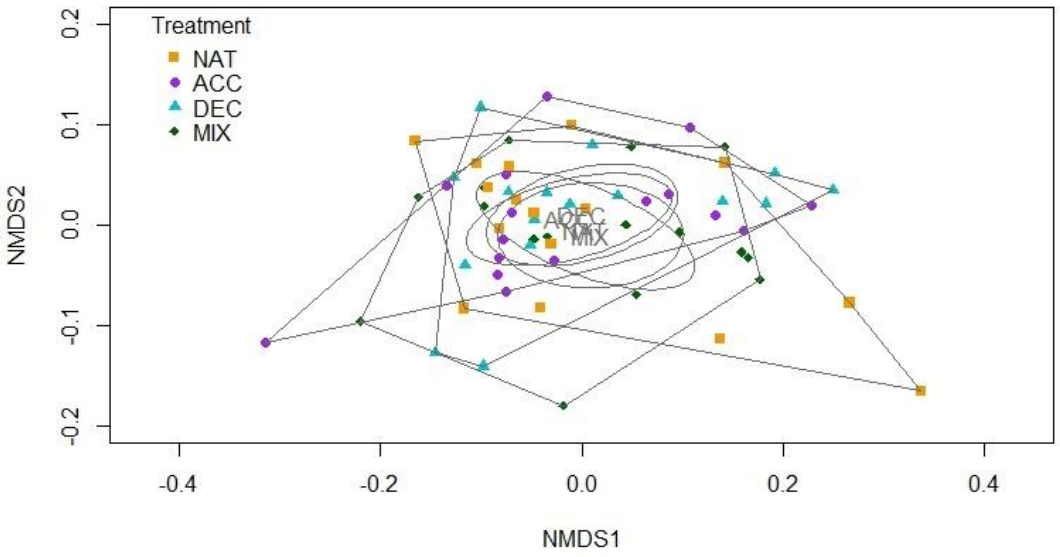
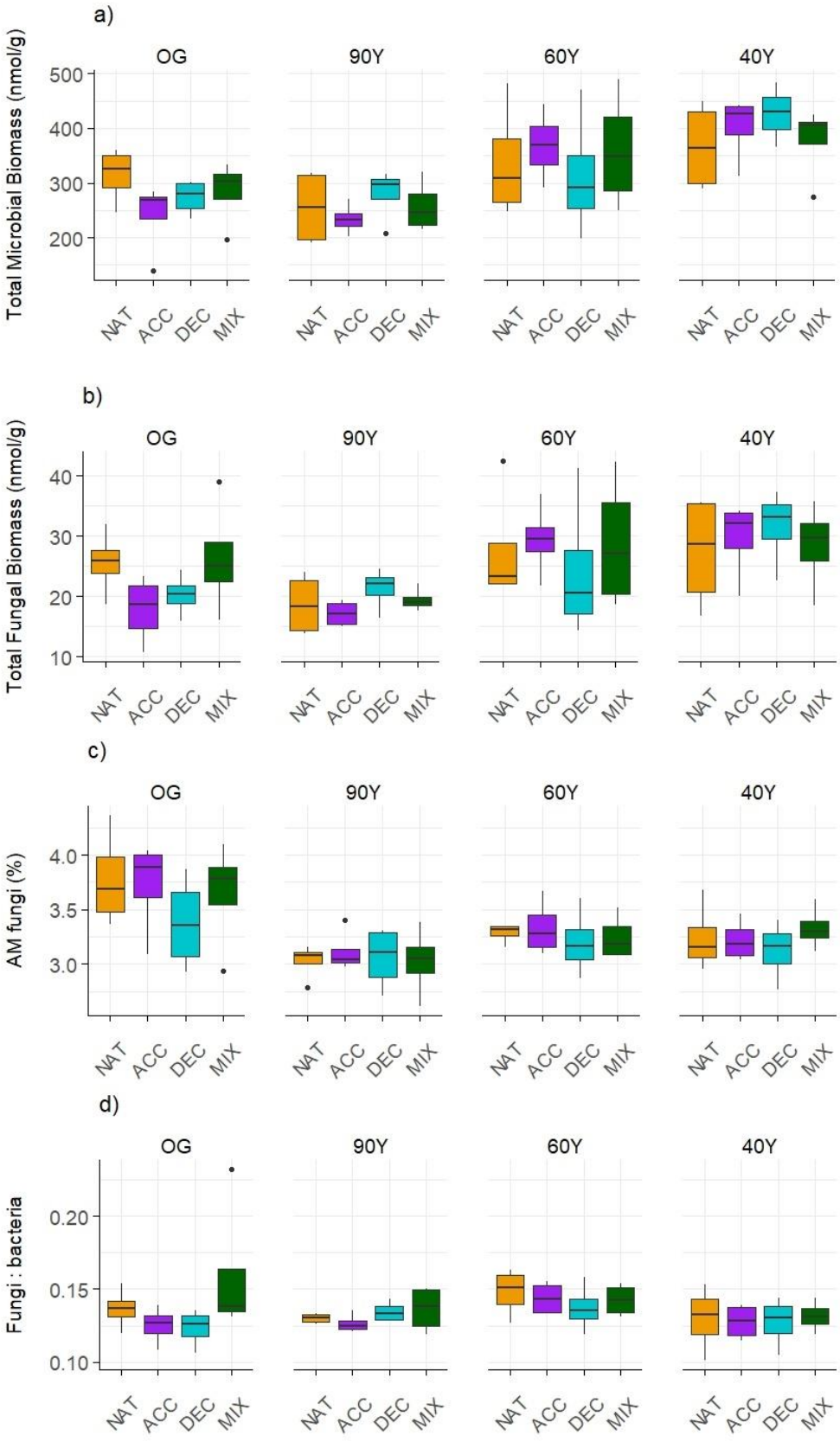


Figure S4.2 NMDS representation of soil microbial community composition from PLFA analysis from soil: Samples collected from 0-5 cm in four blocks in each of four stands along an age gradient of naturally regenerating tropical forest stands, from four experimental litter treatments; NAT = natural litter (orange squares), ACC = light-demanding, accelerating growth species (purple circles), DEC = shade-tolerant, decelerating growth species (blue triangles) and MIX = a mixture of light-demanding and shade-tolerant species (dark green diamonds) after five months of litter decomposition in the BCNM, Panama, Central America. Ordinations were based on Bray–Curtis dissimilarities.


a)
OG
90Y
60Y
40Y
Total Microbial Biomass (nmol/g)
500
400
300
200
NAT
ACC
DEC
MIX
b)
Total Fungal Biomass (nmol/g)
40
30
20
10
c)
AM fungi (%)
4.0
3.5
3.0
d)
Fungi : bacteria
0.20
0.15
0.10

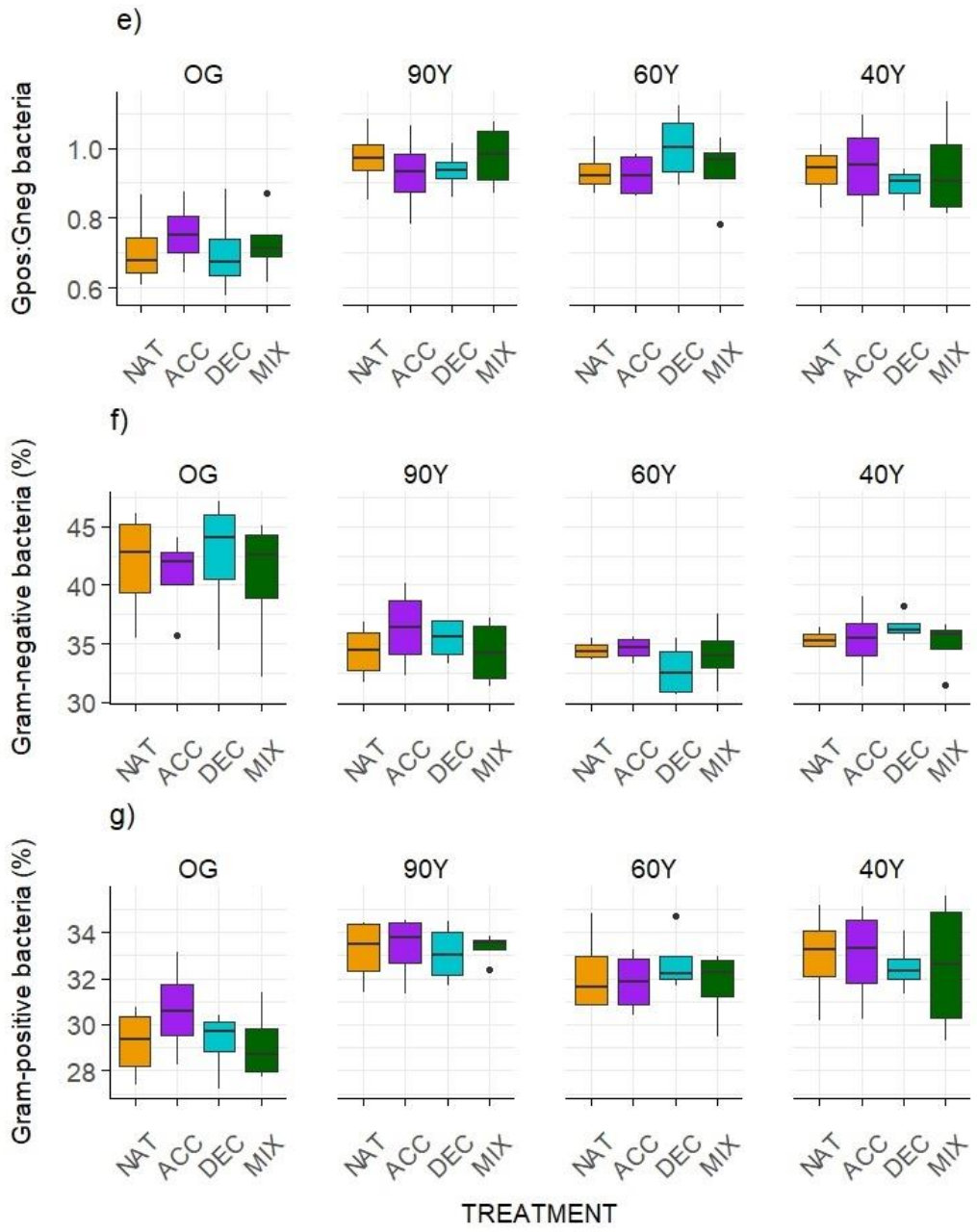


Figure S4.3 Soil microbial biomarker groups from PLFA analysis in four experimental litter treatments (where NAT = natural litter, ACC = light-demanding, accelerating growth species, DEC = shade-tolerant, decelerating growth species and MIX = a mixture of light-demanding and shade-tolerant species) along a successional gradient of four forest stands for a) total microbial biomass, b) total fungal biomass, c) the relative abundance of arbuscular mycorrhizal fungi (AM fungi), d) the ratio between fungi and bacteria, e) the ratio between gram-positive and gram-negative bacteria, f) the relative abundance of gram-negative bacteria, and g) the relative abundance of gram-positive bacteria. Soil was sampled from 0-5 cm depth. Boxes denote the 25th and 75th percentiles and median lines are given for n = 4, whiskers indicate

values up to 1.5 x the interquartile range, and dots indicate outliers. See Chapter 4, Table 4.2 for treatment descriptions. 40Y, 60Y and 90Y refer to stand ages (years since last disturbance), OG refers to old growth, undisturbed forest.

Appendix C.3 Supplementary Tables

Table S4.2 Phospholipid fatty acid biomarkers used to represent microbial functional groups in soils along a successional gradient of tropical forest stands, Panama, Central America. The nomenclature is based on Bartelt-Ryser et al. (2005) and the classification follows Zelles et al. (1999), Bartelt-Ryser et al. (2005), and Ruess & Chamberlain (2010), using branched chain fatty acids (iso, anteiso) as indicators for gram-positive bacteria, and monounsaturated, hydroxy, and cyclopropyl fatty acids for gram-negative bacteria (Kerger et al., 1986; Frostegård et al., 1993; Zelles, 1999).

Biomarker group	Peaks			
AM Fungi	16:1 w5c			
Gram-negative bacteria	10:0 2OH	10:0 3OH	12:1 w8c	12:1 w5c
	13:1 w5c	13:1 w4c	13:1 w3c	12:0 2OH
	14:1 w9c	14:1 w8c	14:1 w7c	14:1 w5c
	15:1 w9c	15:1 w8c	15:1 w7c	15:1 w6c
	15:1 w5c	14:0 2OH	16:1 w9c	16:1 w7c
	16:1 w6c	16:1 w4c	16:1 w3c	17:1 w9c
	17:1 w8c	17:1 w7c	17:1 w6c	17:1 w5c
	17:1 w4c	17:1 w3c	16:0 2OH	17:0 cyclo w7c
	18:1 w8c	18:1 w7c	18:1 w6c	18:0 cyclo w6c
	18:1 w3c	19:1 w9c	19:1 w8c	18:1 w5c
	19:1 w6c	19:0 cyclo w9c	19:0 cyclo w7c	9:1 w17c
	20:1 w9c	20:1 w8c	20:1 w6c	19:0 cyclo w6c
	20:1 w4c	20:0 cyclo w6c	21:1 w9c	21:1 w8c
	21:1 w6c	21:1 w5c	21:1 w4c	21:1 w3c
	22:1 w9c	22:1 w8c	22:1 w6c	22:1 w5c
	22:1 w3c	22:0 cyclo w6c	24:1 w9c	24:1 w7c
	11:0 iso 3OH	14:0 iso 3OH		
Gram-positive bacteria	11:0 iso	11:0 anteiso	12:0 iso	12:0 anteiso
	13:0 iso	13:0 anteiso	14:1 iso w7c	14:0 iso
	14:0 anteiso	15:1 iso w9c	15:1 iso w6c	15:1 anteiso w9c
	15:0 iso	15:0 anteiso	16:0 iso	16:0 anteiso
	17:1 iso w9c	17:0 iso	17:0 anteiso	18:0 iso
	17:1 anteiso w9c	17:1 anteiso w7c	20:0 iso	22:0 iso
	19:0 iso	19:0 anteiso		

Table S4.3 Model results from stepwise linear regressions, assessing the relationship between biomarker functional groups and tree functional parameters and soil characteristics at the block-level in four tropical forest stands in Panama, Central America. Biomarker functional groups (from PLFA analyses) were response variables and soil or tree functional characteristics were explanatory variables. Initial models included all explanatory variables (RI.DEC + P + K + pH + mean b). Models compared with forward and backward selection of variables using AIC values to assess each model fit until a minimum adequate model was reached (stepAIC function). Where RI.DEC = the relative influence of shade-tolerant tree species (Chapter 3), P = soil phosphorus (%), K = soil potassium (%), pH = soil pH, and mean b = mean species growth response (Ruger et al., 2009; Chapter 3).

Initial model: Total Biomass ~ RI.DEC + P + K + pH + mean *b*

Final model: Total Biomass ~ K + pH + mean *b*
F = 8.531, Adjusted R-squared = 0.6, *p* = 0.002

Explanatory variable	t-value	p-value
K	-2.195	0.049
pH	2.119	0.056
Mean *b*	3.399	0.005

Initial model: Fungal Biomass ~ RI.DEC + P + K + pH + mean *b*

Final model: Fungal Biomass ~ K + pH + mean *b*
F = 4.268, Adjusted R-squared = 0.3, p = 0.029

Explanatory variable	t-value	p-value
K	-1.399	0.187
pH	1.502	0.159
Mean *b*	2.428	0.032

Initial model: AM fungi ~ RI.DEC + P + K + pH + mean *b*

Final model: AM fungi ~ K + pH
F = 4.662, Adjusted R-squared = 0.3, p = 0.030

Explanatory variable	t-value	p-value
K	2.251	0.042
pH	-2.701	0.018

Initial model: Gneg Bacteria ~ RI.DEC + P + K + pH + mean *b*

Final model: Gneg Bacteria ~ pH
F = 25.45, Adjusted R-squared = 0.6, p < 0.001

Explanatory variable	t-value	p-value
pH	-5.045	< 0.001

Initial model: Gpos:Gneg Bacteria ~ RI.DEC + P + K + pH + mean *b*

Final model: Gpos:Gneg Bacteria ~ pH
F = 10.95, Adjusted R-squared = 0.4, p = 0.005

Explanatory variable	t-value	p-value
pH	3.308	0.005

Table S4.4 Model results from stepwise linear regressions, assessing the relationship between soil C turnover (litter decay rate and soil respiration) and microbial biomarker functional groups, tree functional parameters and soil characteristics at the block-level in four tropical forest stands in Panama, Central America. Litter decay rate (*k*) and total soil respiration (SR) were response variables and biomarker functional groups (from PLFA analysis), soil or tree functional characteristics were explanatory variables. Initial models included all explanatory variables (Total Fungal Biomass + Gpos:Gneg Bacteria + Fungi:Bacteria + RI.DEC + P + K + pH + mean b). Models compared with forward and backward selection of variables using AIC values to assess each model fit until a minimum adequate model was reached (stepAIC function). Where Decay rate = block mean litter decay rate (*k*) and MEAN SR = total soil respiration data, calculated as the mean for five litter treatments per replicate block in each of the four forest stands from a four month decomposition experiment (Chapter 3), Gpos:Gneg = ratio of Gram-positive:Gram-negative bacteria, RI.DEC = the relative influence of shade-tolerant tree species (Chapter 4), P = soil phosphorus (%), K = soil potassium (%), pH = soil pH, and mean b = mean species growth response (Ruger et al., 2009; Chapter 3).

Initial model: Decay rate ~ Total Fungal Biomass + Gpos:Gneg Bacteria + Fungi:Bacteria + RI.DEC + P + K + pH + mean *b*

Final model: Decay rate ~ Total Fungal Biomass + Gpos:Gneg + K + pH
F = 10.47, Adjusted R-squared = 0.7, p < 0.001

Explanatory variable	t-value	p-value
Total Fungal Biomass	1.494	0.163
Gpos:Gneg Bacteria	-3.770	0.003
K	3.201	0.008
pH	2.394	0.036

Initial model: Decay rate ~ Total Microbial Biomass + Gpos:Gneg Bacteria + Fungi:Bacteria + RI.DEC + P + K + pH + mean *b*

Final model: Decay rate ~ Total Microbial Biomass + Gpos:Gneg + K + pH
F = 8.52, Adjusted R-squared = 0.7, p = 0.001

Explanatory variable	t-value	p-value
Total Microbial Biomass	1.501	0.002
Gpos:Gneg Bacteria	-4.120	0.203
K	3.171	0.010
pH	1.976	0.076

Initial model: Mean SR ~ Total Microbial Biomass + Gpos:Gneg Bacteria + Fungi:Bacteria + RI.DEC + P + K + pH + mean *b*

Final model: Mean SR ~ Total Microbial Biomass + Gpos:Gneg + Fungi:Bacteria + P +K
F = 8.51, Adjusted R-squared = 0.7, p = 0.002

Explanatory variable	t-value	p-value
Total Microbial Biomass	3.778	0.005
Gpos:Gneg Bacteria	-2.101	0.062
Fungi:Bacteria	-3.895	0.003
P	4.775	< 0.001
K	3.798	0.003

Initial model: Mean SR ~ Total Fungal Biomass + Gpos:Gneg Bacteria + Fungi:Bacteria + RI.DEC + P + K + pH + mean *b*

Final model: Mean SR ~ Total Fungal Biomass + Gpos:Gneg + Fungi:Bacteria + P + K
F = 8.37, Adjusted R-squared = 0.7, p = 0.002

Explanatory variable	t-value	p-value
Total Fungal Biomass	3.579	0.005
Gpos:Gneg Bacteria	-1.663	0.127
Fungi:Bacteria	-4.372	0.001
P	4.606	< 0.001
K	3.856	0.003

Appendix C.4 Supplementary References

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., & Balser, T. (2005). Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology, Evolution and Systematics*, *7*, 27-49.

Frostegård, Å, Bååth, E., Tunlid, A., (1993). Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, 25, 723–730.

Kerger, B.D., Nichols, P.D., Antworth, C.P., Sand, W., Bock, E., Cox, J.C., Langworthy, T.A., White, D.C., (1986). Signature fatty acids in the polar lipids of acid-producing Thiobacillus spp.: methoxy, cyclopropyl, alpha-hydroxy-cyclopropyl, branched and normal monoenoic fatty acids. FEMS Microbiology Ecology 38, 67e77.

Oksanen, J. et al. (2018) 'vegan: Community Ecology Package. R package version 2.5-2.' Available at: https://cran.r-project.org/package=vegan.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ruess, L., & Chamberlain, P. M. (2010). The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, *42*, 1898-1910.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, *29*, 111-129.

7.4 Appendix D – Published work

Kerdraon, D., Drewer, J., Castro-Trujillo, B., Wallwork, A., Hall, J. and Sayer, E.J., 2019. Litter traits of native and non-native tropical trees influence soil carbon dynamics in timber plantations in Panama, *Forests*, 10 (3), 209.

Kerdraon, D., Drewer, J., Chung, A.Y., Majalap, N., Slade, E.M., Bréchet, L., Wallwork, A., Castro-Trujillo, B. and Sayer, E.J., 2020. Litter inputs, but not litter diversity, maintain soil processes in degraded tropical forests—a cross-continental comparison. *Frontiers in Forests and Global Change,* 2, p.90.

My contribution to the studies comprised participation in the fieldwork, discussion of the experimental design and interpretation of the results.-