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Controls on carbon cycling in tropical soils from the Amazon to the Andes

The influence of climate, plant inputs, nutrients and soil organisms

Lettice Cricket Hicks

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Controls on carbon cycling in tropical soils from the Amazon to the Andes
The influence of climate, plant inputs, nutrients and soil organisms

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Abstract

Tropical soils are a globally important store of terrestrial carbon (C) and source of atmospheric carbon dioxide (CO₂), regulated by the activity of soil microorganisms, through the mineralisation of plant residues and soil organic matter (SOM). Climatic warming will influence microbial activity, and this may accelerate the rate of C release from soils as CO₂, contributing to alterations in current atmospheric composition, and generating feedbacks to climate change. Yet the magnitude of C loss from tropical soils remains uncertain, partly because we do not fully understand how non-climatic factors – including the chemistry of plant inputs, the availability of soil nutrients and the composition of the decomposer community – will interact to determine the response to changes in temperature. This thesis examines how these factors together regulate the rate of C cycling in contrasting soils across a 3400 m tropical elevation gradient in the Peruvian Andes, spanning a 20 ºC range (6.5 – 26.4 ºC) in mean annual temperature. Large-scale field-based manipulation experiments, translocating leaves and soil-cores across the elevation gradient (to impose an in-situ experimental warming treatment), were combined with controlled laboratory studies to examine the microbial-scale mechanisms which underlie the processes of decomposition and soil respiration observed in-situ.

Results show that, across the gradient, rates of leaf-decomposition were determined principally by temperature and foliar chemical traits, while soil fertility had no significant influence. The effect of temperature was, however, stronger across higher-elevation sites, suggesting a greater vulnerability of the C-rich soils in montane systems to increased C loss under climatic warming. In lowland forests, the presence of invertebrate macrofauna also accelerated rates of decomposition, but leaf chemistry explained the greatest proportion of the observed variance, with a strong role for leaf chemical traits also identified under controlled conditions. Despite marked differences in microbial abundance and community composition among soils, these metrics were not associated with observed rates of decomposition. These results suggest that climate-related changes to plant species distributions (with associated changes to the chemistry of leaf-inputs), and upslope extension of macrofaunal ranges, could strongly
influence future rates of leaf decomposition, independently of the direct response to warming.

From the soil translocation study, root-soil interactions stimulated substantial net C loss from montane soils following translocation downslope (experimental warming treatment), indicating that warming-related changes to root productivity, exudation and/or species-composition could represent an important mode of future C loss from these soils. To examine more closely how inputs of plant-derived C influence the turnover of pre-existing SOM, and whether soil nutrient availability modulates the response, soils were amended with simple and complex $^{13}$C-labelled substrates in combination with inorganic nutrient treatments. Isotopic partitioning was used to determine the degree to which C and nutrient inputs accelerated (positive priming) or retarded (negative priming) the decomposition of SOM. Amendment of upper montane forest and montane grassland soils with nitrogen (N; alone and in combination with C) substantially retarded the decomposition of SOM, suggesting that microbial demand for N strongly regulates the turnover of organic matter in these soils. In contrast, amendment of lower montane and lowland forest soils with C stimulated positive priming of SOM, which was strongest in response to the simple C substrate and was not influenced by nutrient treatments, suggesting that microorganisms in these soils are primarily constrained by availability of labile C. Functional differences among microbial groups were also evident, with gram-negative bacteria and fungi using more labile sources of C while gram-positive bacteria used more complex C.

Together, results from these studies considerably advance our understanding of soil C dynamics across lowland and montane systems, painting a rich picture of interacting processes which will determine the future soil C balance in tropical ecosystems. They show that the influence of temperature on the rate of soil C cycling is strongly affected by the nature and composition of plant-derived and atmospheric inputs, the principal additional constraints varying with elevation, leading to both opposing and reinforcing effects on rates of decomposition. The greater observed temperature sensitivity of decomposition at higher elevations is coupled with high microbial demand for N which regulates the turnover of SOM, whereas at lower elevations leaf decomposition is accelerated by active macrofaunal breakdown, while microbial decomposition of SOM is constrained by the availability of labile C. Under
a global change scenario of increased temperature and N deposition, results therefore suggest that: (i) modified chemistry of plant inputs will influence rates of decomposition, independently of climate; (ii) increased availability of labile C will lead to more rapid decomposition of SOM at lower elevations; (iii) greater root productivity (associated with warming and plant-community shifts) will stimulate soil C loss across montane regions; but (iv) at higher elevations, a possible countervailing effect may be imposed on rapid warming-accelerated decomposition if increased N availability reduces microbial mineralisation of SOM. The net effect on the ecosystem C budget will depend on the balance of C gain from primary productivity and C loss from soils. Overall, however, the results presented here suggest that the large soil C stores in higher-elevation montane regions are particularly vulnerable to substantial reductions under exposure to short- and medium-term climatic warming.
Lay summary

Globally, more carbon is stored by soils than by vegetation and the atmosphere combined, with tropical forests accounting for a third of soil carbon stocks worldwide despite representing only 15% of land surface area. These large carbon stocks have accumulated because decomposition rates lag behind the supply of organic material from plants to soil. However, decomposition rates tend to increase under elevated temperature, meaning that soils are vulnerable to increased carbon loss in response to climatic warming (with losses occurring as emissions of carbon dioxide). With so much carbon in tropical soils, small increases in the rate of decomposition could substantially alter the amount of carbon dioxide in the atmosphere, with implications for future climate change. Yet the magnitude of net carbon loss from soils, and the resulting carbon dioxide emission in response to climatic warming remains uncertain, because we lack understanding of how multiple biophysical factors, in addition to temperature, also regulate rates of decomposition. To resolve this uncertainty, we urgently need a better understanding of how the chemistry of plant inputs to soil, the availability of soil nutrients, and the composition of the microbial ‘decomposer’ community will interact to determine the overall response in tropical soils to changes in temperature.

Here I used a large elevation gradient in natural forest and grassland, traversing 3400 m in elevation from the top of the Peruvian Andes to the Amazon lowlands, to investigate how temperature, soil nutrients, soil microbes and plant species composition affect rates of decomposition. The sites along the transect vary strongly in mean annual temperature (ranging from 6.5 to 26.4 ºC), offering a valuable opportunity to examine the effect of temperature under field conditions. Large-scale manipulation experiments were conducted, moving leaves and soil-cores across this natural temperature gradient, thereby imposing warming or cooling (by movement down or upslope) to assess the impact on decomposition. Controlled laboratory studies were also undertaken, to more closely examine how the quality of organic matter inputs (i.e. chemically simple or complex carbon compounds), the availability of soil nutrients and the microbial community influence rates of decomposition, independently of climate.
Results show that several interacting processes will have an important influence on the future soil carbon balance in tropical ecosystems. In addition to temperature, the chemical composition of leaves strongly regulated rates of decomposition across the elevation gradient. Invertebrate macrofauna (for example termites and ants) accelerated rates of leaf-decomposition in lowland forests, but not at higher elevation montane sites where their natural abundance is low. The composition of the microbial community did not influence rates of decomposition, despite marked differences across the elevation gradient. However, different types of microbes were associated with the decomposition of chemically simple and more complex carbon. These results suggest that warming-related changes to plant species distributions (which would modify the chemistry of leaf inputs to soil), and upslope extension of macrofaunal ranges, could therefore influence future rates of leaf decomposition, independently of the direct response to elevated temperature.

Warming-related changes to tree-species composition and root productivity could also represent an important mode of future soil carbon loss across the elevation gradient, as root-soil interactions stimulated substantial loss of carbon from montane forest and grassland soils following movement of soils downslope (experimental warming treatment). Evidence from a controlled laboratory study suggested that this loss of carbon might have been caused by the new inputs of chemically simple carbon compounds, from roots, stimulating microbial decomposition of ‘old’ soil carbon. Under controlled conditions, inputs of simple carbon compounds accelerated the decomposition of soil organic matter, increasing emissions of carbon dioxide, particularly from lower montane and lowland forest soils. However, in the case of upper montane forest and grassland soils, extra inputs of nitrogen (both alone and in combination with added carbon) reduced microbial decomposition of old soil carbon. This suggests that elevated nitrogen availability (from atmospheric deposition) could reduce carbon loss and stabilise soil carbon stocks at higher elevations. Overall, these findings suggest that future rates of soil carbon cycling will not only be affected by temperature changes, but decomposition rates will also be strongly influenced by the composition of plant-derived and atmospheric nutrient inputs, consideration of which will be critical in predicting responses of the soil carbon cycle to future climatic warming.
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<td>Autotrophic respiration</td>
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<td>Decomposition coefficient</td>
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<td>Dry weight</td>
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Declaration

I declare that I composed this thesis; the work presented is entirely my own (except where otherwise acknowledged) and has not been submitted, either in whole or in part, for any other degree or qualification.

Lettice Hicks
February 2017
Acknowledgements

The research in this thesis contributes to the work carried out by the Andes Biodiversity and Ecosystem Research Group. This project was supported by a grant from the Natural Environment Research Council (NERC), and an additional grant-in-kind from the NERC Stable Isotope Facility. I thank Derek and Maureen Moss for their generous financial support, which enabled me to undertake more fieldwork than would have otherwise been possible. I also thank ACCA for the use of the Wayqecha field station, the Explorers Inn for the use of the Tambopata field station, and the Manu National Park and Tambopata National Reserve Authorities.

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Chapter 1

Introduction

Across the globe, soils play a crucial role in regulating the concentration of atmospheric carbon dioxide (CO$_2$), storing an estimated 2000 Pg of carbon (C) in soil organic matter (Janzen, 2004) and releasing 98 ± 12 Pg C to the atmosphere each year as a product of soil respiration (Bond-Lamberty and Thomson, 2010). Tropical soils in particular make a substantial contribution to the terrestrial C budget, accounting for a third of global soil C stocks (Jobbágy and Jackson, 2000). Yet the fate of these large soil C stocks in response to global change remains uncertain. Climatic warming of up to 5 °C is projected to occur across the globe over the course of this century (IPCC, 2013), resulting in novel temperature regimes in the tropics under which no closed canopy forest exists today (Wright et al., 2009). Warming is likely to stimulate microbial activity and this may accelerate the rate of C release from soils (Conant et al., 2011, Lu et al., 2013), altering atmospheric composition and generating feedbacks to climate change (Davidson and Janssens, 2006, Friedlingstein et al., 2006). However, the potential magnitude of soil C loss is subject to great uncertainty (Wood et al., 2012, Wieder et al., 2013, Bradford et al., 2016a), partly because we do not fully understand how non-climatic factors – including the chemistry of plant inputs, the availability of soil nutrients, and the composition of the decomposer community – will interact to determine the response to changes in temperature. Improved understanding of the factors which regulate soil C dynamics in the tropics is therefore crucial, in order to more comprehensively predict responses to global change.

In this chapter, I provide the scientific background for my thesis. The global importance of tropical ecosystems and their sensitivity to climatic warming is first outlined, followed by an overview of the terrestrial C cycle and the principal factors which regulate the rate of C turnover in soils. The value of using elevation gradients, to investigate the influence of temperature on soil C cycling in the tropics, is also introduced (with further discussion of translocation experiments provided in Chapter 2). An overview of this thesis follows, including the overarching aim and fundamental science questions addressed by the four independent papers presented in Chapters 3-6.
1.1 Scientific background

1.1.1 Tropical ecosystems; importance and vulnerability

Tropical forests represent only 15% of terrestrial surface area (Pan et al., 2013), yet account for over two-thirds of terrestrial plant biomass (Pan et al., 2011, Saatchi et al., 2011) and a third of global soil C (Jobbágy and Jackson, 2000, Kauffman et al., 2009). Lowland Amazonian rainforests and the adjacent tropical Andes (montane forests and grasslands) are often described as the most dynamic, species-rich and ecologically diverse terrestrial ecosystems in the world (Myers et al., 2000, Dirzo and Raven, 2003, Hoorn et al., 2010), playing an important role in the provision of global ecosystem services (Foley et al., 2007, Anderson et al., 2011), including C sequestration in plant biomass and soils (Malhi et al., 2006, Davidson et al., 2012a) and regulation of water balance, by promoting cloud formation and regional rainfall (Bala et al., 2007, Spracklen et al., 2012). Changes in such functions across tropical ecosystems could therefore have profound consequences for global biogeochemical and hydrological cycles, and consequently for climate (Meir et al., 2006, Bonan, 2008, Grace et al., 2014).

Global climate change, in addition to land use change, represents one of the greatest threats to tropical ecosystems (Thomas et al., 2004, Malhi et al., 2008, Cusack et al., 2016). Since the mid-1970s, the tropics have warmed at a rate of 0.26 ± 0.05 °C per decade, with this warming trend attributed primarily to the anthropogenic greenhouse effect (Malhi and Wright, 2004). The rate of climatic warming is projected to increase, with a temperature rise of 1.8-5.1 °C estimated across the tropical Andes during this century (Urrutia and Vuille, 2009), the wide variance dependent on greenhouse gas emissions scenarios and climate model uncertainty, especially in mountainous regions. Over recent decades, reduced precipitation across the Amazon basin has also resulted in a general drying trend (Marengo, 2004, Marengo and Espinoza, 2015), although the signal is spatially variable, with intensification of the hydrological cycle and substantial wetting also reported (Gloor et al., 2013). Future changes to precipitation regimes in the tropics, projected by global climate models, remain subject to great uncertainty (Jupp et al., 2010, Huang et al., 2013), though reduced rainfall over parts of Amazonia is likely (Joetzjer et al., 2013, Cai et al., 2014,
Duffy et al., 2015). Reduced water availability, from increased evaporation in response to warming even if rainfall is unchanged (Corlett, 2011), may influence soil processes and vegetation dynamics. However, given the wide spatial consistency in predictions of warming, and its fundamental influence on biochemical processes, I focus here on temperature as the principal and most widely-felt climatic driver of 21st century change in tropical ecosystems.

Evidence from long-term studies suggest that climatic warming, together with increased concentrations of atmospheric CO₂, is changing the ecology of tropical systems, increasing primary productivity, tree growth and recruitment, as well as mortality (Phillips et al., 2004, Lewis et al., 2009, Brienen et al., 2015). Temperature and CO₂-related increases in primary productivity will, in turn, increase the quantity of C inputs to soil (Cernusak et al., 2013, Giardina et al., 2014), while climate-related shifts in distributions of plant species, observed across gradients of tropical lowland to montane forests (Chen et al., 2011, Feeley et al., 2011, Duque et al., 2015), will also modify the chemical composition of plant inputs (Hättenschwiler et al., 2008). Coupled climate-C cycle models indicate that C acquisition by terrestrial ecosystems will be increased as a result of enhanced plant photosynthesis in response to a higher CO₂-atmosphere, yet increasing temperature will also decrease C storage, owing to greater rates of C loss from soil and plant respiration (Friedlingstein et al., 2006, Cox et al., 2013). The balance between C uptake (by plants) and release (from soils and plants) in the tropics, however, represents a major uncertainty in predicting future responses to warming in tropical ecosystems (Meir et al., 2006, Meir et al., 2008, Galbraith et al., 2010, Wood et al., 2012, Huntingford et al., 2013, Friedlingstein et al., 2014, Cusack et al., 2016).

Given the large amount of C stored by tropical soils (Jobbágy and Jackson, 2000, Kauffman et al., 2009), particularly in tropical montane regions (Gibbon et al., 2010, Zimmermann et al., 2010b), relatively small changes to the rate of soil C cycling could have a globally important influence on atmospheric composition. However, we lack understanding of the complex direct and indirect factors which interact to determine the long-term sensitivity of soil C loss in the tropics in response to elevated temperature (Nottingham et al., 2015c). In addition to the direct effect of temperature on soil C loss, we must also consider how parallel warming-related changes to the
quantity and chemistry of plant derived C inputs will modulate this response. Our understanding of soil C dynamics in tropical systems is further complicated because soils vary widely in factors such as fertility (see Box 1) and microbial community composition (Townsend et al., 2008, Whitaker et al., 2014b), known to influence the rate of C turnover. A clear, mechanistic understanding of the factors which regulate rates of C cycling in tropical lowland forest, montane forest and montane grassland soils is therefore a priority for research, in order to resolve uncertainty and more comprehensively predict the fate of tropical soils in response to increasing temperature.

**Box 1**

**Soil fertility; nutrient availability to soil microorganisms**

Traditionally, soil fertility refers to the ability of soil to sustain plant growth. As plants are only able to take up inorganic nutrients, soils with higher concentrations of essential nutrients in plant-available, inorganic forms are therefore described as more fertile. However, when considering the influence of soil fertility on soil C cycling, a process regulated by the activities of soil microorganisms, soil fertility must refer to the ability of soil to sustain microbial growth and function. In addition to inorganic soil nutrients immediately available for microbial uptake, microbes may also be able to access organically-bound nutrients by the synthesis of extracellular enzymes, especially when provided with sufficient energy (labile carbon) from fresh plant-inputs (Caldwell, 2005, Nanniperi et al., 2011). Consequently, the total pool of soil nutrients (organic + inorganic) may be a better indicator of soil fertility – the ability of soil to sustain microbial growth and function – in this case. Henceforth, throughout this thesis, soil fertility is considered in terms of nutrient availability to soil microbes.

### 1.1.2 The terrestrial carbon cycle

Carbon is first assimilated from the atmosphere by plants through photosynthesis, during primary production (Figure 1.1). The gross uptake of C by terrestrial plants across the globe is estimated as 123 ± 8 Pg C each year, representing a large flux of C into the terrestrial biosphere, of which c. 60 % of this uptake occurs in the tropics (Beer et al., 2010). In lowland tropical forests, gross primary production (GPP) is estimated
as 30-40 Mg C ha⁻¹ year⁻¹ (Malhi, 2012), with lower rates of GPP (c. 16 Mg C ha⁻¹ year⁻¹) estimated for tropical montane forests (van de Weg et al., 2014). A large fraction of this GPP (c. 70 %) is however used for the plant’s metabolic needs (Chambers et al., 2004, Malhi et al., 2009), resulting in rapid return of CO₂ to the atmosphere as a product of autotrophic respiration (Ra). Net primary productivity (NPP) represents the amount of C assimilated into plant biomass (c. 30% of GPP), after the loss of C from Ra, whereby C is allocated to biomass aboveground (leaf canopy and woody biomass), as well as belowground (roots and root exudates) (Malhi et al., 2011).

**Figure 1.1:** carbon pathways through the terrestrial biosphere, whereby arrow thickness represents the relative size of the carbon flux (Trumbore, 2006). Large carbon uptake by plants through photosynthesis, and carbon loss from soils through microbial decomposition of plant-residues and soil organic matter (SOM), in addition to autotrophic respiration losses.

Organic C enters the soil from leaf and woody litterfall, as well as belowground from dead roots and root exudates. These sources of plant-derived C together with soil organic matter (SOM) are used by soil microorganisms and detritivore invertebrate macrofauna as a source of energy, whereby organic C is mineralised and CO₂ is returned to the atmosphere (Conrad, 1996), as the product of heterotrophic
respiration (Rh). Microbial decomposition of plant-residues and SOM (catalysed by enzymatic activity) is therefore an important ecosystem process, regulating rates of C and nutrient release from organic matter (Swift et al., 1979, Tiessen et al., 1994). Autotrophic respiration by roots also contributes to the total CO₂ efflux derived from soil, yet this flux is difficult to partition from that associated with microbial mineralisation of labile root exudates in the rhizosphere (Kuzyakov, 2006, Jones et al., 2009). Consequently, the CO₂ efflux derived from autotrophic root respiration and heterotrophic respiration of root exudates is often measured together, and referred to as root-rhizosphere respiration (Rr) (Sayer and Tanner, 2010). The contribution of Rr to the total CO₂ efflux derived from soil (Rs) however is highly variable, with estimates varying markedly among studies (Hanson et al., 2000). For example, Rr was reported to account for 24-35 % Rs in one lowland Amazonian study (Silver et al., 2005) but up to 61 % in another (Metcalfe et al., 2007a). Fresh inputs of labile plant-derived C to soils can also enhance microbial mineralisation of pre-existing SOM (Fontaine et al., 2007, Bird et al., 2011, Cheng et al., 2014, Finzi et al., 2015), stimulating additional release of CO₂ to the atmosphere (positive priming). Soil respiration therefore represents a complex, multi-source flux (Figure 1.2), the dynamics and determinants of which remain poorly resolved for tropical systems.

Figure 1.2; schematic diagram illustrating the five main sources which contribute to the total CO₂ efflux measured from soils (Kuzyakov, 2006).
To predict how the rate of C release from tropical soils will be influenced by global change, it is first necessary to understand the factors which regulate rates of C cycling. Rates of decomposition can be influenced by climate (temperature, moisture) (Liski et al., 2003, Davidson and Janssens, 2006, Conant et al., 2011), the chemical composition of organic matter (Hättenschwiler and Jørgensen, 2010, Waring, 2012), the availability of soil nutrients (Cleveland et al., 2006, Cusack et al., 2011, Dale et al., 2015) and the composition of the decomposer community (Strickland et al., 2009a, Whitaker et al., 2014b). Yet the extent to which these factors interact to determine rates of C cycling in tropical soils remains poorly resolved. The potential role of each of these factors in regulating rates of decomposition independently will briefly be reviewed in turn, before also considering plant-soil interactions, in each case highlighting the key unknowns which currently limit our ability to predict how soil C cycling in the tropics will be influenced by future global change.

**Climatic controls on decomposition and soil respiration**

Climate is a dominant, coarse-scale control determining rates of C cycling in soils (Lavelle et al., 1993, Liski et al., 2003), whereby on a global scale soil respiration fluxes are positively correlated with temperature across both space (Raich and Schlesinger, 1992) and time (Bond-Lamberty and Thomson, 2010). Precipitation can also influence the rate of plant-residue and SOM decomposition (Austin and Vitousek, 2000, Reichstein et al., 2003, Powers et al., 2009), with a parabolic relationship between soil moisture content and soil respiration widely reported, whereby soil respiration is optimised at intermediate levels of soil moisture (Meir et al., 1996, Davidson et al., 2000, Sotta et al., 2004). Soil moisture content may therefore most strongly influence soil C dynamics in dry tropical systems, where seasonal droughts (low soil moisture availability) constrain microbial activity (Chen et al., 2002, Curiel Yuste et al., 2007), whereas in wet tropical forests where soil moisture availability is near-optimal for microbial activity, temperature is likely the principal climatic control (Prescott, 2010, Zimmermann et al., 2010a, Salinas et al., 2011).

Given the likelihood of future climatic warming (IPCC, 2013), understanding the temperature dependence (see Box 2) and sensitivity (see Box 3) of decomposition
and soil respiration has been a priority for research (Davidson and Janssens, 2006, Conant et al., 2011). However, the majority of studies investigating the temperature sensitivity of soil respiration have been conducted in temperate or boreal regions (Wood et al., 2012) and to date in-situ soil warming experiments are yet to be implemented in the tropics (Cavaleri et al., 2015, Nottingham et al., 2015c). Controlled incubation experiments, under optimal soil moisture and non-limiting substrate supply, have reported that higher temperatures enhance microbial activity and accelerate the decomposition of SOM (Holland et al., 2000, Fang et al., 2005, Balser and Wixon, 2009, Cusack et al., 2010). Studies conducted across natural temperature gradients in the tropics have also reported faster decomposition of plant-material (Coûteaux et al., 2002, Salinas et al., 2011, Bothwell et al., 2014) and SOM (Zimmermann et al., 2009a) at warmer sites, indicating the potential for increased C loss from soils in response to future climatic warming. However, although large-scale in-situ soil warming experiments (in temperate-regions) have reported soil respiration to initially increase under elevated temperature, with prolonged warming the response is often transient, and not sustained with time (Luo et al., 2001, Kirschbaum, 2004, Melillo et al., 2011). The long-term sensitivity of soil C loss to elevated temperature may therefore be influenced by several indirect and confounding factors, consideration of which will be important for predicting long-term responses to climatic warming.

Firstly, experimental and theoretically-based studies have suggested that the observed response to warming will be strongly influenced by the size of the labile C pool providing an unconstrained supply of C to decomposer organisms (Knorr et al., 2005, Gershenson et al., 2009, Billings and Ballantyne, 2013). Once this pool is depleted, microbial activity will be constrained by the supply of labile C, reducing soil respiration rates (irrespective of temperature) and hence dampening the observed temperature sensitivity of decomposition (Curiel Yuste et al., 2010, Fissore et al., 2013). Laboratory-based studies, or those which only examine the temperature sensitivity of heterotrophic respiration with exclusion of plant-derived C inputs (Zimmermann et al., 2009a, Luan et al., 2014) are therefore likely to become confounded by reduced substrate supply. Furthermore, the reductive nature of studies which exclude plant communities generates further uncertainty regarding the long-term temperature sensitivity of soil respiration to warming, as the root-rhizosphere
component of soil respiration may exhibit a different sensitivity to temperature compared to heterotrophic respiration processes alone (Boone et al., 1998, Hartley et al., 2007, Zhu and Cheng, 2011). Understanding how temperature-related changes to plant communities and plant-derived C inputs will influence the observed temperature sensitivity of decomposition and soil respiration, will therefore be important for predicting long-term responses to climatic warming.

Microbial responses to temperature, whether through changes at the community-level or through physiological acclimation (Zogg et al., 1997, Allison et al., 2010), may also reduce the long-term sensitivity of soil respiration to elevated temperature (compensatory response) (Bradford et al., 2008, Crowther and Bradford, 2013). Yet microbial-community responses to warming have also been reported to have an enhancing effect, increasing the long-term sensitivity of soil respiration to temperature (Hartley et al., 2008, Karhu et al., 2014). Results from a modelling-based study suggested that temperature-related changes to microbial growth efficiency (proportion of C assimilated into biomass per unit C consumed) (Frey et al., 2013, Geyer et al., 2016) will be important in determining the magnitude of future soil C loss in response to warming, as if microbial growth efficiency declines with increasing temperature then this will reduce microbial biomass and consequently reduce rates of decomposition (Wieder et al., 2013). Examining the sensitivity of soil respiration to long-term changes in temperature will therefore be important, given that instantaneous responses to temperature may not reflect the long-term response.

While temperature will be important in determining future soil C dynamics, a recent study across a latitudinal gradient of temperate forest revealed that our understanding of decomposition, typically based upon mean decomposition rates (local-scale variance aggregated into mean values), may have masked and underestimated the importance of local-scale factors such as soil fertility and biotic composition (Bradford et al., 2014). Moreover, a meta-analysis of tropical-region studies found leaf-decomposition across wet montane forests (MAT < 20 ºC) to be more sensitive to temperature compared to wet lowland forests (MAT > 20 ºC) where instead the chemistry of leaf-inputs most strongly influenced the rate of decomposition (Waring, 2012). There is therefore a need to more closely examine how non-climatic factors (leaf chemical traits, soil fertility, and the microbial community), together with
temperature, interact to determine rates of C cycling (Bradford et al., 2016b), and potential differences in the relative importance of different controls across tropical lowland and montane systems.

**Box 2**

**Temperature dependence of soil respiration**

Many equations have been developed to describe the temperature dependence of soil respiration (Lloyd and Taylor, 1994, Kätterer et al., 1998). The most widely applied equation, the van’t Hoff equation (Eq. 1.1), describes the relationship between soil respiration ($R_s$) and temperature ($T$) using a simple first-order exponential function, where $a$ and $b$ are fitted parameters (Davidson et al., 2006), assuming moisture availability and substrate supply do not constrain microbial activity.

$$R_s = ae^{bT}$$

(Eq. 1.1)

Alternative functions for the temperature dependence of soil respiration have been investigated. For example, Lloyd and Taylor (1994) evaluated several competing functions including the van’t Hoff equation, Arrhenius equation (which incorporates the activation energy required to trigger chemical reactions), and other more complex, empirically developed functions. Yet, despite increasing the model complexity, the improvement in model-fit was relatively minor ($r^2 = 0.70$ to $r^2 = 0.79$). Furthermore, another study evaluating a suite of different temperature-soil respiration functions reported that more complex functions did not significantly improve model fit over the simple exponential equation (Fang and Moncrieff, 2001). As soil respiration does not describe a single biochemical reaction (with single activation energy, single substrate, and single enzyme) and uncertainty remains as to the underlying processes which together operate to determine the response to temperature, a perfect mechanistic equation is unlikely. Therefore, whilst simple, the van’t Hoff equation (Eq. 1.1) may be sufficient to describe the temperature dependence of soil respiration, especially for experimentally derived data for which the signal may be inherently noisy.
Intrinsic and apparent temperature sensitivities

The temperature sensitivity ($Q_{10}$) is defined as the factor by which a process rate ($R$; decomposition, soil respiration) increases with a 10 °C increase in temperature ($T$), with the $Q_{10}$ calculated according to equation 1.2 (assuming first-order exponential temperature dependence; see Box 2).

$$Q_{10} = \left[ \frac{R_2}{R_1} \right]^{\frac{10}{(T_2-T_1)}}$$  (Eq. 1.2)

The temperature sensitivity of soil respiration takes into account a wide range of kinetic properties involved in the decomposition of SOM, including microbial and enzymatic activity, substrate availability and complexity (Davidson et al., 2006). According to kinetic theory, and supported by experimental evidence, more chemically recalcitrant organic matter exhibits a greater intrinsic sensitivity to temperature (because of the greater activation energy required to trigger a reaction) (Fierer et al., 2005, Conant et al., 2008, Craine et al., 2010). However, the $Q_{10}$ may be suppressed if SOM is physically or chemically protected against microbial decomposition (Zimmermann et al., 2012), or if microbial activity is constrained by the availability of moisture (Davidson et al., 2006) or labile C (Billings and Ballantyne, 2013).

Experimentally observed $Q_{10}$ values may be better defined as an apparent $Q_{10}$, rather than intrinsic $Q_{10}$, as they are often confounded by parallel temperature effects on other interacting processes which together contribute to the observed temperature sensitivity (Nottingham et al., 2015c). For example, the temperature sensitivity of soil respiration will be influenced by the chemical composition of SOM, plant productivity and community composition (determining the supply of plant-derived C to soil), microbial community composition, enzyme activity, soil nutrient and moisture availability. Although understanding the intrinsic temperature sensitivity of individual processes will be important in building a fundamental mechanistic model of decomposition (Ise and Moorcroft, 2006, Davidson et al., 2012b), in practice it is difficult to differentiate between and measure all the individual processes which contribute to an observed temperature response. By estimating the apparent temperature sensitivity of decomposition and soil respiration, and critically evaluating how other factors contribute to the overall, observed response, we will however advance our understanding of the key controls on these processes under warming. Furthermore, this approach might reveal whether temperature alone is sufficient to understand and predict changes in soil CO$_2$ efflux and decomposition rates in response to future warming, or whether other factors will be important in modulating the response.
The chemical composition of plant inputs has been found to strongly influence the rate of decomposition (Aerts, 1997, Waring, 2012). Indeed, from global datasets, leaf chemical traits (total nutrient concentration and C:N ratio) account for more variation in decomposition rates compared to climate (Zhang et al., 2008), likely because of high interspecies-based variation in leaf chemistry (Cornwell et al., 2008, Hättenschwiler et al., 2008). Typically, plant material rich in nutrients (Bakker et al., 2011, Waring, 2012) and easily accessible C compounds, such as cellulose (Loranger et al., 2002, Bachega et al., 2016), are degraded most readily, whereas those rich in chemically complex compounds, such as lignin, decompose more slowly (Hättenschwiler and Jørgensen, 2010, Freschet et al., 2012). Yet the chemical traits which best explain rates of decomposition vary among studies, limiting our ability to predict how changes in the chemistry of plant inputs to soil, whether in response to temperature (Ford et al., 1979, Tully and Lawrence, 2010) or through warming-related shifts in plant-species distributions (Chen et al., 2011), will influence future rates of decomposition in tropical systems. Moreover, results from a pan-tropical study suggest that site-dependent factors (including soil fertility, and decomposer community composition) might interact with leaf-chemistry to determine the rate at which plant material is broken down (Powers et al., 2009). A more integrated understanding of how the chemical composition of plant inputs to soil, together with soil fertility and decomposer community composition, regulate rates of decomposition is therefore required.

The chemical composition of plant inputs to soil might also influence the mineralisation of pre-existing SOM, through a process known as priming (Kuzyakov et al., 2000, Fontaine et al., 2007, Sayer et al., 2011). Labile C inputs, for example from root exudation (Grayston et al., 1997, Jones et al., 2009), can stimulate microbial and enzymatic activity and enhance the mineralisation of SOM (positive priming) (Carney et al., 2007, Cheng et al., 2014, Whitaker et al., 2014a) leading to net C loss (Dijkstra and Cheng, 2007, Bird et al., 2011, Wang et al., 2016). One temperate-region study reported that the degree of root-mediated soil C loss was positively related to plant productivity (Dijkstra et al., 2006), suggesting that increased inputs of labile C belowground, as a consequence of climatic warming (Cernusak et al., 2013, Giardina
et al., 2014), could stimulate C loss from soils, independently of the direct response to increased temperature. This mechanism may explain why studies investigating ecosystem responses to warming and elevated atmospheric CO\textsubscript{2} often observe no change to soil C stocks, despite increased primary productivity increasing the quantity of C inputs to soil (Hungate et al., 1997, Lichter et al., 2005, Van Groenigen et al., 2014). Understanding of how warming-related changes to plant (root) communities and productivity will influence rates of soil C cycling, and how this may influence the observed temperature sensitivity of soil respiration is therefore required.

**Nutrient constraints to microbial activity in tropical soils**

Nitrogen (N) and phosphorus (P) are essential nutrients required by microorganisms to build and maintain cellular structures, and as such the availability of N and P is critical for microbial growth and activity (Sterner and Elser, 2002, Hartman and Richardson, 2013). As the stoichiometry (C:N:P) of organic matter (Hättenschwiler et al., 2008) is typically wider than that of microbial biomass (Cleveland and Liptzin, 2007), additional N and/or P must often be acquired from soil to meet microbial metabolic demands. Low concentrations of soil nutrients can therefore constrain microbial activity and hence rates of C cycling (Finzi et al., 2011). A pan-tropical study reported faster rates of leaf-decomposition in soils with a high total N and P concentration (Cleveland et al., 2011), with differences in decomposition rates also observed across natural soil fertility gradients (Dent et al., 2006, Dale et al., 2015). However, the different sources from which N and P are derived in terrestrial ecosystems means that either element may become limiting relative to the other and constrain microbial activity in tropical soils. Nitrogen is acquired from the atmosphere via biological fixation, where atmospheric N is converted to usable forms of ammonium and nitrates through nitrification (Vitousek et al., 2010). Accumulation of N in soils over time therefore means that young soils often contain low concentrations of inorganic bio-available N. By contrast, P is derived from bedrock and made available through weathering and erosion, meaning that over time soil P can become depleted as P is leached and not replenished (Walker and Syers, 1976). Old, strongly weathered and leached tropical lowland soils may therefore be deficient in rock-
derived P (Reed et al., 2011a) while N is relatively high due to accumulation over time and rapid rates of internal N cycling (Templer et al., 2008). Younger, tropical montane forest and grassland soils, in contrast, may be more deficient in N because of slow accumulation via biological N-fixation (due to cooler temperatures; Tanner et al., 1998) while P remains abundant due to landslide events (Clark et al., 2016) and near-surface weathering (Porder et al., 2007).

A change in nutrient acquisition investment by soil microorganisms along an elevation gradient in the Peruvian Andes suggested a shift with elevation in the relative demand for P and N by microbes (Nottingham et al., 2015a). Furthermore, fertilisation with N and P, in different montane and lowland forests respectively, has been shown to increase microbial respiration (Cleveland and Townsend, 2006, Fisher et al., 2013) and growth of microbial biomass (Cusack et al., 2011, Liu et al., 2013, Turner and Wright, 2014), consistent with the prevailing theory. However, in other studies of lowland and montane forest no difference has been reported in leaf decomposition rates among control and fertilised plots (Hobbie and Vitousek, 2000, McGroddy et al., 2004) leading to uncertainty regarding how microbial demand for nutrients influences soil C dynamics in different tropical systems. Moreover, although theories of soil development and some experimental studies suggest that biological activity in tropical montane forests is constrained by the availability of N (Tanner et al., 1998, Cusack et al., 2011, Fisher et al., 2013), a meta-analysis across Central America and the Caribbean observed high nitrate concentrations in montane forest streams, suggesting open nutrient cycles with abundant N available relative to biotic demand (Hedin et al., 2009, Brookshire et al., 2012). External supply of N is increasing in some areas of the tropics as a result of biomass burning (Hietz et al., 2011), with high N deposition reported even across remote Andean systems (Fabian et al., 2005, Boy et al., 2008). Aeolian sources of P, originating from North Africa, will also likely increase the future availability of P across the Amazon basin (Okin et al., 2004). Consideration for how the availability of N and P currently modulates rates of C cycling in tropical soils, and how rates of C cycling may be influenced by future nutrient deposition is therefore important as, despite the potential for strong regulatory effects, our understanding of the relationship between soil nutrient availability, microbial activity and hence rates of decomposition across lowland and montane systems remains limited.
Microbial community composition and function

Functional dissimilarity among microbial communities may also influence rates of soil C cycling (Heemsbergen et al., 2004, Strickland and Rousk, 2010). Bacteria and fungi are the principal microbial agents involved in the decomposition of plant-derived and soil organic matter, with gram-negative (GN) and gram-positive (GP) bacteria reported to use labile and more recalcitrant sources of C respectively (Waldrop and Firestone, 2004, Fierer et al., 2007, Kramer and Gleixner, 2008, De Vries and Shade, 2013). Fungi have diverse capabilities in degrading a wide range of simple to complex substrates (Kjøller and Struwe, 2002, van der Wal et al., 2013) but are most often associated with the degradation of more recalcitrant C fractions (de Boer et al., 2005, Cusack et al., 2011). As such, several subtropical studies have reported faster rates of leaf-decomposition in soils with greater fungal dominance (García-Palacios et al., 2013b, He et al., 2016), with a tropical study also finding the ratio of fungi:bacteria together with the ratio of GP:GN bacteria important in explaining soil respiration responses following amendment with a range of different C substrates (Whitaker et al., 2014b). Recent statistical analysis, using 82 global datasets, revealed that including data on microbial community structure strengthened predictions of soil C cycling, compared to the explanatory power of environmental variables alone (Graham et al., 2016). Yet few models explicitly consider the effect of microbial community composition (Treseder et al., 2012, Todd-Brown et al., 2012), in part because of uncertainty regarding the generality of relationships between microbial composition and function (Allison and Martiny, 2008), and of how microbial communities will be influenced by future global change (Zogg et al., 1997, Castro et al., 2010). To resolve uncertainty, more studies are required to evaluate how microbial community composition affects rates of C cycling, especially for the tropics where empirical evidence is lacking.

In addition to soil microorganisms, detritivore macrofauna can make an important contribution to the rate at which organic matter is broken down (Powers et al., 2009, Dahlsjö et al., 2014), with a meta-analysis of studies from across the globe reporting that macrofaunal activity consistently accelerated rates of leaf-decomposition (García-Palacios et al., 2013a). However, marked variation in macrofaunal abundance has been observed across gradients of lowland to montane...
forests (Palin et al., 2011, Werenkraut and Ruggiero, 2014), likely strongly influenced by climate (Wall et al., 2008). Understanding the role of invertebrate macrofauna, as well as soil microorganisms, in regulating rates of decomposition across lowland and montane systems will therefore be important, especially given the potential for climate-related shifts in macrofaunal ranges (Colwell et al., 2008, Moret et al., 2016).

**Plant-soil interactions**

While plant inputs and soil properties (fertility and microbial community) can influence soil C dynamics independently, there is increasing evidence for plant-soil interactions, which consequently determine rates of decomposition (Bardgett and Wardle, 2010). The degree to which plant-derived C inputs stimulate the mineralisation of SOM (Fontaine et al., 2007, Bird et al., 2011) may be strongly influenced by soil nutrient availability and microbial demand for nutrients (Nottingham et al., 2012b, Dijkstra et al., 2013). In soils where nutrient availability is low, microorganisms may use the labile C as a source of energy and co-mineralise SOM to liberate and acquire ‘limiting’ nutrients from soil (nutrient-mining) (Craine et al., 2007, Chen et al., 2014, Rousk et al., 2016). This mechanism may also increase the availability of soil nutrients for uptake by plants (Bengtson et al., 2012, Murphy et al., 2015, Nie and Pendall, 2016), such that plant-soil interactions could strongly influence future responses to global change. For example, an experimental study of temperate forest under elevated atmospheric CO₂ reported an increase in the flux of plant-derived C belowground which stimulated microbial activity and resulted in increased microbial decomposition of SOM (Drake et al., 2011). The associated release of N from organic matter was, in turn, taken up by plants and used to support increased primary productivity, contributing to a positive feedback which sustained the elevated soil CO₂ efflux with time. However, despite the potential importance of plant-soil interactions in regulating the efflux of CO₂ from soils, our understanding of these interactions in tropical systems remains limited.

Plant-soil interactions may also modify microbial community composition and hence function, as soil microorganisms are inextricably linked to above-ground plant communities (Wardle et al., 2004, Bardgett et al., 2008). Some tropical studies have
reported microbial composition to vary dependent on plant diversity (Carney and Matson, 2005) and leaf-litter inputs (Leff et al., 2012), with differences in microbial composition, in turn, found to influence the mineralisation of different C substrates. Long-term plant-soil interactions may mean that over time, soil microorganisms ‘adapt’ to more efficiently break-down plant-material most frequently encountered. The theory of home-field advantage (HFA) states that leaves decompose more rapidly on soil where the plant originates (home) relative to other soils (away) (Ayres et al., 2009). Whereas the theory of functional breadth (Van Der Heijden et al., 2008, Fanin et al., 2015a) states that where plant inputs are diverse and more recalcitrant, for example in forests, microorganisms will have a wide functional ability (functionally broad), whereas where plant inputs are more labile, for example in grasslands, microorganisms may be constrained in their ability to degrade more chemically complex leaves (functionally narrow). As such, past resource history may influence the contemporary ability of soil microorganisms to degrade novel plant-inputs, although evidence from tropical studies remains limited. Home-field advantage has been supported by some tropical forest studies (de Toledo Castanho and de Oliveira, 2008, Vivanco and Austin, 2008), but not others (Gießelmann et al., 2011, Bachega et al., 2016), while the theory of microbial functional breadth, to date, has only been investigated comparing rates of leaf-decomposition among forests and grasslands in temperate regions (Keiser et al., 2011, Fanin et al., 2015a).

1.1.3 Elevation gradients to investigate responses to climatic warming

From the preceding discussion, it is evident that the response of soil C to future climatic warming will be more complex than that currently represented in mechanisms and predicted by Earth systems models, which have been primarily parameterised around responses to temperature and soil moisture availability (Todd-Brown et al., 2013). To resolve uncertainty regarding the gaps in understanding described above, a more comprehensive insight is required into how plant-derived C inputs, the availability of soil nutrients and microbial community composition will interact to determine the response of soil C to elevated temperature. As in-situ warming
experiments are yet to be implemented in the tropics (Cavaleri et al., 2015, Nottingham et al., 2015c), elevation gradients provide a valuable opportunity to examine the influence of temperature change on ecosystem processes and function (Körner, 2007, González et al., 2013, Sundqvist et al., 2013). The use of tropical elevation gradients is long-established; historically, Alexander von Humboldt (1849) observed and examined changing biodiversity and species distributions along the slopes of Mount Chimborazo in the Ecuadorean Andes as a function of climatic and edaphic factors. The use of natural environmental gradients continues to be a valuable tool for the study of ecosystem ecology, to assess environmental controls on ecosystem functions, in order to better understand ecosystem responses to global change (Kitayama, 2006, Becker et al., 2007, Malhi et al., 2010). Translocation experiments are a particularly powerful methodological approach in order to investigate the temperature dependence of decomposition, as experimental warming can be imposed by movement of organic matter (plant material, or soil) across a natural temperature gradient (Vitousek et al., 1994, Coûteaux et al., 2002, Zimmermann et al., 2009a, Salinas et al., 2011, Bothwell et al., 2014). The use of elevation gradients and translocation experiments is evaluated further in Chapter 2.

1.2 Thesis overview

The overarching aim of this thesis is to contribute towards a better understanding of the factors which regulate rates of C cycling in tropical lowland forest, montane forest and montane grassland soils. We know that climatic warming will influence microbial activity and the kinetics of decomposition, and this may accelerate the rate of C release from soils (Davidson and Janssens, 2006, Conant et al., 2011, Lu et al., 2013, Wieder et al., 2013). However, the extent to which other factors – including the chemical composition of plant-derived C inputs to soil, the availability of soil nutrients and the community composition of decomposer soil-organisms – also regulate C cycling in tropical soils, and will interact to determine the response to changes in temperature remains largely unresolved. A more integrated understanding of these factors is required in order to more comprehensively evaluate future responses to global change.
Specifically, this thesis asks the following overarching questions:

(1) How does the chemical composition of plant-inputs to soil influence soil C dynamics?

(2) Is there a shift with elevation in the most limiting nutrient to microbial activity and hence decomposition, from P in lowland forests to N in montane forest and grassland soils?

(3) Do differences in microbial community composition among soils (with elevation) influence soil C dynamics?

(4) How may these factors together influence the observed, apparent temperature sensitivity of soil C cycling to climatic warming?

Using findings from two in-situ translocation experiments conducted across a 3400 m elevation gradient in the Peruvian Andes, together with results from controlled-environment laboratory experiments, this thesis is composed of four independent research papers, each investigating a subset of these questions (Figure 1.3). An overview of the studied Kosñipata elevation gradient and the five individual sites that comprise the study-transect used throughout this thesis follows in Chapter 2, including further discussion of translocation experiments, as the methodological approach which underpins the studies presented in Chapters 3 and 5. Further study-specific methods are provided within respective chapters 3-6.
Figure 1.3; conceptual diagram of the four studies presented in this thesis (boxes; L and F denote laboratory- or field-based approach respectively) and the different factors (ovals) which are considered by each of these studies. The study presented in Chapter 6 more closely examines the microbial mechanisms underlying the ecosystem-scale processes (decomposition, soil respiration) observed in Chapters 3 and 5.
Chapters 3 and 4 examine the relative importance of different abiotic and biotic controls on leaf decomposition. The field-based study presented in Chapter 3 investigates how climate (temperature, precipitation) together with the chemical composition of leaves and soil fertility regulates decomposition by invertebrate macrofauna and soil microorganisms. Leaves from eight different species of plant were translocated among five sites across a 3400 m elevation gradient, spanning 20 °C (6.5-26.4 °C) in mean annual temperature (MAT). Litterbags with different mesh size were used to selectively exclude or enable access by invertebrate macrofauna. A statistical modelling approach was employed to identify the relative importance of climate, leaf traits, soil fertility and macrofaunal activity in explaining rates of decomposition across the gradient. The statistical analysis was repeated separately for sites where MAT < 20 °C (montane forests and grasslands) and MAT > 20 °C (lowland forests) to assess whether there were differences in the factors which most strongly regulated rates of decomposition across higher- and lower-elevation systems. The apparent temperature sensitivity ($Q_{10}$) of decomposition was also derived, using the temperature variation across the elevation gradient as a whole, and separately across ecotones to assess differences in the temperature sensitivity of decomposition with elevation. To complement this in-situ study (Chapter 3), a laboratory experiment under controlled climatic conditions was also conducted, to further investigate how leaf chemical traits, soil fertility and microbial community composition interact to determine rates of decomposition (Chapter 4). Temperature- and moisture-controlled microcosms were constructed, to measure the rate of mass loss for four different species of leaves on contrasting fertility soils from the same 3400 m elevation gradient. To evaluate the functional significance of the microbial community, soil microbial abundance and community composition (relative abundance of different functional groups) was determined by analysis of microbial phospholipid fatty acids (PLFAs; where different chemical structures are indicative of specific groups of microorganisms).

Using results from a large-scale soil translocation experiment across the 3400 m elevation gradient, Chapter 5 investigates how differences in root productivity and altered root-communities, as a consequence of climatic warming, might influence soil C dynamics. To do so, intact soil cores were reciprocally translocated among four
different sites spanning 20 °C (6.5-26.4 °C) in MAT. Soil cores were divided into two treatments: cores where root growth was permitted (root community-soil interaction) and cores where root growth was excluded (controls), with effluxes of CO₂ measured over 24 months following translocation. These different treatments enabled partitioning of soil CO₂ effluxes into heterotrophic (Rh) and root-rhizosphere (Rr) derived components by a mass balance approach, in order to examine how root-soil interactions influenced soil C dynamics (CO₂ effluxes, total soil C content). Using among-site temperature variation, imposed by translocation of soil cores across the gradient, Q₁₀ coefficients were also derived to evaluate whether the inclusion of roots influenced the observed sensitivity of soil respiration to temperature. This study was therefore analogous to the leaf-translocation study presented in Chapter 3, however, instead of moving leaves across the gradient (to interact with different soils), here intact soil cores were reciprocally translocated and allowed to interact with new plant (root) communities.

The controlled laboratory study presented in **Chapter 6** more closely examines the mechanistic interactions between the chemical complexity of plant-inputs, soil nutrient availability and microbial activity. By using isotopically labelled ¹³C-substrates (as surrogates of plant-derived C), sources of respired CO₂ (substrate- or soil-derived) could be identified, to examine whether the mineralisation of added C substrates and pre-existing SOM was influenced by the availability of N and P in montane and lowland soils respectively. The incorporation of ¹³C was also traced into microbial PLFAs to further probe whether specific microbial groups were differentially constrained by N and P in their mineralisation of labile and more recalcitrant C.

Finally, **Chapter 7** provides a synthesis of results from Chapters 3-6, and discusses the key findings in relation to the principal science questions identified here. The wider implications of these findings are also evaluated in the context of global change, including recommendations for future research to address additional questions raised by Chapters 3-6, and concluding remarks.
Chapter 2

Study sites and use of elevation gradients

This chapter provides an overview of the Kosñipata elevation gradient, located in Andean Peru, and the five field sites used in the studies presented throughout this thesis. The value of conducting translocation experiments across elevation gradients to investigate the influence of temperature on soil C cycling is also discussed, as the methodological approach which underpins the studies presented in Chapters 3 and 5. The fieldwork conducted for this thesis occurred during three field campaigns, July-October 2013, May-August 2014 and September-October 2015. Study-specific methods are detailed in each individual paper (Chapters 3-6).

2.1 Kosñipata transect and history

The Kosñipata transect is a 3400 m elevation gradient, spanning 20 °C (6.5 – 26.4 °C) in mean annual temperature (MAT), situated along the eastern flanks of the Peruvian Andes and adjacent Amazonian lowlands, South Eastern Peru (Figure 2.1). The Kosñipata transect is the focus of the interdisciplinary Andes Biodiversity and Ecosystem Research Group (ABERG; www.andesconservation.org), with the overarching aim to understand the distribution of biodiversity and ecosystem functioning of tropical Andean montane systems in relation to past, current and future climates (Malhi et al., 2010).

In 2003, a series of 1 ha permanent study plots were established along the Kosñipata Valley, with montane forest plots located in or close to the Manu National Park and additional lowland forest plots located in the adjoining Tambopata National Reserve (Figure 2.2). To date, studies across these sites have been central in developing our understanding of carbon (C) cycling at the stand scale (Girardin et al., 2010, Malhi et al., 2014) and of plant-species distributional ecology and tree growth (Feeley et al., 2011, Rapp et al., 2012). Studies across the montane forest sites have yielded important insights into plant ecophysiology (van de Weg et al., 2012), and the environmental factors which control gross primary productivity (by a processed-based modelling approach, the first of its kind for tropical montane forests; van de Weg et
al., 2014), while remotely sensed measurements across the gradient have been used to evaluate the spatial distribution of above-ground biomass in Amazonian forests (Saatchi et al., 2007), forest structure at the landscape scale (Asner et al., 2014a) and how canopy traits vary with elevation (Asner et al., 2015). Ecosystem C storage, both above and belowground, across the montane forest-grassland transition has also been quantified (Gibbon et al., 2010, Zimmermann et al., 2010b), with soils and belowground ecosystem processes across the gradient continuing to receive considerable focus over recent years. Soil abiotic and biotic properties, and their elevational trends have been characterised (Zimmermann et al., 2012, Whitaker et al., 2014b, Nottingham et al., 2015a), along with studies investigating temporal variation and the climate dependence of soil respiration (Zimmermann et al., 2009b, Zimmermann et al., 2010a), as well as elevational and seasonal variation in soil methane and nitrous oxide fluxes (Teh et al., 2014, Jones et al., 2016). Large-scale manipulation experiments (translocation experiments, further discussed in section 2.3) across the gradient have also been conducted to estimate the temperature sensitivity of soil respiration (Zimmermann et al., 2009a) and leaf-litter decomposition (Salinas et al., 2011), with findings indicating that soils across the gradient may be vulnerable to increased C loss in response to future climatic warming.

Figure 2.1: the Kosñipata transect, situated on the eastern flanks of the Andes; red stars illustrate key study sites along the transect (Tres Cruces; T.C., Wayqecha; WAY., San Pedro; S.P. and Tambopata; TAM.). Adapted from image produced by J. Rapp.
2.2 Study site characteristics

Location

Five sites along the elevation transect have been the focus of this thesis (Figure 2.2a). The two lowest elevation, lowland rainforest sites (194 m and 210 m above sea level; asl) are located in the Tambopata National Reserve, lowland Amazonian basin (240 km to the east of the main Kosñipata transect; Figure 2.2b). The three higher elevation sites are located in the Manu National Park, with sites representative of lower tropical montane forest (1500 m asl), upper tropical montane forest (3025 m asl) and montane puna-grassland (3644 m asl) above the treeline, which occurs at approximately 3500 m asl.

All forest sites were established where there was closed canopy primary forest with relatively homogeneous stand structure, topography and no sign of human interference. An overview of site characteristics is presented in Table 2.1. Further descriptions of climate, vegetation and soil characteristics across the sites are provided in the following sections. Despite the close proximity of the two lower elevation study sites (194 m and 210 m), both were included in the in-situ leaf decomposition study (Chapter 3) as they differ markedly in terms of underlying geology and soil fertility (Quesada et al., 2010, see following soils section). For Chapters 4-6, only one of the lower elevation sites was included (210 m site), in addition to the three higher elevation sites (1500 m, 3025 m and 3644 m) used for all studies (Chapters 3-6), to enable comparison of soil C dynamics among lowland tropical rainforest, lower montane forest, upper montane forest, and montane grassland systems (Figure 2.3).
Figure 2.2: (a) map locating the five study sites in south-east Peru as the focus of this thesis; three higher elevation sites, located in the Manu National Park (MANU) and the two lower elevation sites located in the Tambopata National Reserve (TAM.; only one star visible) (source Google Maps, 2016), (b) map illustrating the extent of the Manu National Park and Tambopata National Reserve (source World Map, 2016), with Manu and Tambopata study regions demarcated by dash lines and study sites marked by stars.
Table 2.1: Site characteristics (mean annual temperature; MAT, mean annual precipitation; MAP).

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Elevation (m asl)</th>
<th>Latitude (° S)</th>
<th>Longitude (° W)</th>
<th>MAT (°C)</th>
<th>MAP (mm year⁻¹)</th>
<th>Geological Substrate</th>
<th>Soil Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata-6</td>
<td>194</td>
<td>12° 49' 48&quot;</td>
<td>69° 16' 16&quot;</td>
<td>26.4</td>
<td>1900</td>
<td>Holocene alluvial terrace</td>
<td>Haplic Alisol</td>
</tr>
<tr>
<td>Tambopata-5</td>
<td>210</td>
<td>12° 50' 11&quot;</td>
<td>69° 16' 45&quot;</td>
<td>26.4</td>
<td>1900</td>
<td>Pleistocene alluvial terrace</td>
<td>Haplic Cambisol</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>13° 02' 56&quot;</td>
<td>71° 32' 13&quot;</td>
<td>17.4</td>
<td>5302</td>
<td>Plutonic Intrusion (granite)</td>
<td>Cambisol</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>13° 11' 24&quot;</td>
<td>71° 35' 13&quot;</td>
<td>11.1</td>
<td>1560</td>
<td>Palaeozoic shales-slates</td>
<td>Umbrisol</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>13° 07' 29&quot;</td>
<td>71° 36' 86&quot;</td>
<td>6.5</td>
<td>*760</td>
<td>Palaeozoic shales-slates</td>
<td>Umbrisol</td>
</tr>
</tbody>
</table>

MAT and MAP determined over five years (2006-2011) by the Andes Biodiversity and Ecosystem Research Group

*MAP measured at Ajanaco, 3450 m asl (Oliveras et al., 2014)
Figure 2.3; photographs illustrating study sites characteristic of (a) montane grassland (Tres Cruces; 3644 m), (b) upper montane forest (Wayqecha; 3025 m), (c) lower montane forest (San Pedro; 1500 m) and (d) lowland rainforest (Tambopata; 210 m). Photographs courtesy of A. Griffiths, J. Michel and A. Nottingham.
Climate

Across the gradient, climate is seasonal, with a relatively warm-wet season (November-March) and a relatively cool-dry season (May-September). Mean annual temperature decreases with elevation, from 26.4 °C (at 194 m) to 6.5 °C (at 3644 m), with low temperature variation exhibited over the seasonal cycle (Girardin et al., 2014, Malhi et al., 2014). Although temperature fluctuates over the diurnal cycle, diurnal temperature ranges are relatively consistent across the gradient, with more marked diurnal variation occurring above the canopy than the understory (Rapp and Silman, 2012). Mean annual precipitation (MAP) does not vary linearly with elevation, and peaks at mid-elevations (5302 mm year\(^{-1}\) at 1500 m; Table 2.1). However, despite wet and dry seasons, there is little seasonal variation in soil moisture (Girardin et al., 2013), in part, as montane sites undergo frequent periods of cloud immersion, resulting in low evapotranspiration (Grubb, 1977). As such, evidence to date suggests that plants and soils across the gradient are rarely moisture limited over the seasonal cycle (van de Weg et al., 2009, van de Weg et al., 2014, Zimmermann et al., 2010a).

Vegetation

Across the Kosñipata transect there are marked transitions in vegetation structure and taxonomic composition. Trees in montane forests are typically of lower stature compared to lowland rainforests (Bruijnzeel and Veneklaas, 1998), with canopy height decreasing with increasing elevation along the gradient (Asner et al., 2014a). Aboveground biomass and net primary productivity (NPP) typically decrease with increasing elevation (Girardin et al., 2010, Huasco et al., 2014), with this trend largely attributed to cooler temperatures and lower photosynthetic active radiation at higher elevations (van de Weg et al., 2014). Belowground C allocation tends to increase with elevation (Gibbon et al., 2010, Moser et al., 2011), with greater fine root biomass at higher elevations along the Kosñipata transect (Girardin et al., 2013). Leaves from montane forest trees tend to be smaller and tougher, with lower foliar nutrient concentrations compared to those from lowland forests (Fahey et al., 2015). Across the Kosñipata transect, leaf mass per area (LMA; g m\(^{-2}\)) and leaf nitrogen (N) concentration decreased with elevation, whereas leaf phosphorus (P) concentration showed no
significant elevational trend (van de Weg et al., 2009). However, despite lower foliar N at higher elevation sites across the Kosñipata transect, foliar N concentrations were higher compared to that measured at other tropical montane forest sites (in Hawaii and Borneo) (Vitousek et al., 1992, Tanner et al., 1998, Kitayama and Aiba, 2002), potentially a consequence of relatively high atmospheric N deposition across Andean forests (Fabian et al., 2005, Boy et al., 2008) or alternatively a consequence of high N demand to maintain metabolism at lower average temperatures (van de Weg et al., 2012, Bahar et al., 2016).

Across the elevation gradient, tree (> 10 cm DBH- diameter breast height) species diversity is high, with over 1000 different species identified across the 1 ha inventory plots (Feeley et al., 2011). Diversity is particularly high at lower elevations, with c.150-170 species ha\(^{-1}\) up to 1750 m asl elevation, above which diversity declines linearly with elevation to c.40 species ha\(^{-1}\) at 3300 m asl (Jankowski et al., 2013). Dominant tree families Leythidaceae, Bixacea, Moraceae and Fabaceae occur at the lower rainforest sites (194 m and 210 m), shifting to Euphorbiaceae, Fabaceae and Lauraceae in the lower montane forest (1500 m) and Clusiaceae, Cunoniaceae and Rosaceae in the upper montane forest (3025 m) (Girardin et al., 2010, Salinas et al., 2011). Above the treeline, tussock forming grasses dominate, from the genera *Calamagrostis*, *Scirpus* and *Festuca* (Oliveras et al., 2014). These shifts in vegetation composition are largely associated with decreasing temperature, higher precipitation and greater cloud immersion at higher elevations.

Climatic warming is driving changes in the distribution of tree species across the gradient. Mean tree species ranges are estimated to have moved upslope at a rate of 2.5-3.5 vertical m each year in response to warming (Feeley et al., 2011). However, a recent study has suggested that this is the result of contraction of species ranges at the warm trailing edge, rather than systematic movement of maintained species ranges upslope (Duque et al., 2015). Furthermore, although the treeline at the Kosñipata forest-grassland transition has been relatively stable over the last four decades, there is some evidence for warming-driven treeline advancement upslope into the grassland, especially in areas protected from grazing (Lutz et al., 2013). The extent to which the treeline will advance or remain stationary under future climatic warming may depend on other factors, including solar radiation, modes of seed dispersal and germination
success, and human activities, in addition to temperature (Rehm and Feeley, 2015). Nonetheless, warming-driven changes to tree species distributions, and potential advancement of trees into the grassland, would result in marked changes to the quantity and chemistry of plant inputs to soil across this gradient.

**Soils**

The montane grassland and upper montane forest soils have developed on Palaeozoic sedimentary mudstones while the lower montane forest soils are underlain by plutonic (granite) intrusions (Clark et al., 2013). The lowland forest soils are situated on alluvial terraces, with Tambopata-5 (210 m) situated on a relatively infertile Pleistocene terrace whereas, by contrast, Tabopata-6 (194 m) is situated on a more recent Holocene floodplain (Quesada et al., 2010). Soils at higher elevation sites are typically shallower, but have a deeper organic horizon (Table 2.2).

Concentrations of total C, N and P in soils generally increase with increasing elevation (Table 2.2), where cooler temperatures at higher elevations have constrained the turnover of organic matter and nutrient cycling (Salinas et al., 2011). Soil C (0-10 cm depth) is lowest (1.70 ± 0.3 %) at 210 m and highest (46.5 ± 2.1 %) at 3025 m, in part due to deeper organic horizons at higher elevation sites (Figure 2.4a). Total N concentration varies between 0.23 ± 0.03 % (at 210 m) and 2.39 ± 0.12 % (at 3025 m), and total P concentration varies between 0.18 ± 0.03 mg g⁻¹ (at 210 m) and 1.36 ± 0.37 mg g⁻¹ (at 1500 m; Table 2.2). Despite typically higher concentrations of total N and P at higher elevations, a function of greater accumulation of organic matter due to slower rates of decomposition in cooler-montane sites (Bruijnzeel and Veneklaas, 1998), these concentrations do not reflect the availability of inorganic N and P across the gradient. While resin-extractable inorganic P (PO₄) typically increases with elevation, mineralised N (NH₄ + NO₃) decreases with increasing elevation (Teh et al., 2014, Nottingham et al., 2015a; Figure 2.4b). In the montane grassland soil, however, the concentration of resin-extractable inorganic P and rate of N mineralisation are both very low (Table 2.2). Soils across the gradient are strongly acidic: soil pH does not vary markedly among the forest sites (pH 3.8 - 4.6) but is higher in grassland soil above the treeline (pH 4.9).
Table 2.2; soil properties (0-10 cm depth). Data represent mean (1SE), n = 5.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Elevation (m asl)</th>
<th>Organic Horizon Depth (cm)</th>
<th>Total C (% dwt)</th>
<th>Total N (% dwt)</th>
<th>Total P (mg g⁻¹)</th>
<th>PO₄-P (mg P kg⁻¹)</th>
<th>NH₄ + NO₃ (µg N g⁻¹ resin day⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata-6</td>
<td>194</td>
<td>1</td>
<td>2.38 (0.3)</td>
<td>0.35 (0.03)</td>
<td>0.49 (0.07)</td>
<td>3.3 (0.8)</td>
<td>-</td>
<td>4.6 (0.1)</td>
</tr>
<tr>
<td>Tambopata-5</td>
<td>210</td>
<td>2</td>
<td>1.70 (0.3)</td>
<td>0.23 (0.03)</td>
<td>0.18 (0.03)</td>
<td>2.7 (0.2)</td>
<td>27.6 (3.4)</td>
<td>3.8 (0.1)</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>16</td>
<td>10.3 (1.8)</td>
<td>0.91 (0.12)</td>
<td>1.36 (0.37)</td>
<td>44.7 (20.1)</td>
<td>27.2 (3.9)</td>
<td>4.0 (0.1)</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>23</td>
<td>46.5 (2.1)</td>
<td>2.39 (0.12)</td>
<td>1.09 (0.08)</td>
<td>82.0 (23.3)</td>
<td>12.3 (1.1)</td>
<td>4.1 (0.1)</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>4</td>
<td>16.9 (1.1)</td>
<td>1.44 (0.07)</td>
<td>0.92 (0.13)</td>
<td>2.5 (0.5)</td>
<td>10.7 (0.8)</td>
<td>4.9 (0.1)</td>
</tr>
</tbody>
</table>

a Extractable P measured as phosphate recovered from anion-exchange membranes by shaking for 1 hour in 50 ml 0.25 M H₂SO₄

b Mineralised N (NH₄ + NO₃) determined using in-situ cation and anion-exchange resins, extracted using 2 M KCl

c pH measured in water (soil:H₂O, 1:2 w:v)

Nottingham et al. (2015a and unpublished data for Tres Cruces)
Figure 2.4; (a) soil carbon stocks (kg C m\(^{-2}\)) measured to 50 cm depth (Nottingham et al., 2015c), and (b) bio-available inorganic nitrogen (mineralised N; NO\(_3\) + NH\(_4\); \(\mu\)g N g\(^{-1}\) resin day\(^{-1}\)) and phosphorus (resin extractable PO\(_4\)-P; mg P kg\(^{-1}\) soil dwt) determined 0-10 cm depth (Nottingham et al., 2015a) along the Kosñipata transect. Note log\(_{10}\) scale to more clearly illustrate elevational trends.
Soil microbial abundance and community composition also changes distinctly with elevation (Table 2.3). Microbial abundance (total phospholipid fatty acids; PLFAs, a proxy for microbial biomass) increases with increasing elevation along the transect, as does the ratio of fungi: bacteria (F:B) in soils, while the ratio of gram positive: gram negative (GP:GN) bacteria decreases with increasing elevation (Whitaker et al., 2014b). The activity of hydrolytic enzymes (determined at standardised temperature) decreases with increasing elevation across the gradient (Nottingham et al., 2015a), posited as a result of lower N-availability at higher elevations, because of N required for enzyme synthesis (Allison and Vitousek, 2005), rather than a direct temperature effect. The ratio of enzymes involved in the acquisition of N and P, however, increases with increasing elevation, suggesting a shift in the relative demand by microorganisms for N compared to P with increasing elevation (Nottingham et al., 2015a). In addition to soil microorganisms, invertebrate macrofauna (dominated by termites) also contribute to decomposition of organic matter and C cycling at the lowland forest sites (Dahlsjö et al., 2014). Termite diversity and abundance decreases with increasing elevation, with the upper range-limit of termites occurring at 925-1500 m (soil-feeding termites) and 1550-1850 m (wood-feeding termites) (Palin et al., 2011).

Table 2.3; soil microbial abundance (total phospholipid fatty acid; PLFA) and community composition (ratio of fungi:bacteria; F:B and ratio of gram positive:gram negative bacteria; GP:GN) (0-10 cm depth). Data represents mean (1SE), n = 5.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Elevation (m asl)</th>
<th>Total PLFA (nmol g⁻¹ dwt)</th>
<th>F:B Ratio</th>
<th>GP:GN bacteria Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata-6</td>
<td>194</td>
<td>94.9 (6.9)</td>
<td>0.11 (0.00)</td>
<td>1.37 (0.05)</td>
</tr>
<tr>
<td>Tambopata-5</td>
<td>210</td>
<td>54.3 (7.7)</td>
<td>0.11 (0.01)</td>
<td>1.23 (0.04)</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>323.6 (73.8)</td>
<td>0.21 (0.01)</td>
<td>0.91 (0.06)</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>473.7 (49.3)</td>
<td>0.33 (0.02)</td>
<td>0.97 (0.10)</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>474.2 (62.6)</td>
<td>0.35 (0.01)</td>
<td>0.73 (0.03)</td>
</tr>
</tbody>
</table>

Whitaker et al. (2014b and unpublished data for Tres Cruces)
2.3 Elevation gradients as natural laboratories

The Kosñipata transect (> 3400 m elevation) is the largest tropical forest elevation-temperature gradient studied to date. Spanning a 20 °C range in temperature (6.5-26.4 °C MAT) it offers a unique combination of environmental factors to examine the influence of temperature on ecosystem functioning (Malhi et al., 2010, Sundqvist et al., 2013). In contrast to latitudinal gradients, tropical elevation gradients such as the Kosñipata transect do not experience a dormant (winter) season and low variation in temperature occurs over the seasonal cycle (Rapp and Silman, 2012, Girardin et al., 2014, Malhi et al., 2014). Furthermore, important co-variants of precipitation and soil pH do not vary linearly with elevation (Tables 2.1 and 2.2), and despite marked variation in MAP among sites, findings to date suggest that soil moisture content remains high (Rapp and Silman, 2012) and plants and soils at all sites are rarely moisture limited over the seasonal cycle (van de Weg et al., 2009, Zimmermann et al., 2010a, van de Weg et al., 2014), providing the opportunity to evaluate the effect of temperature independently from soil moisture. While differences in the receipt of solar radiation across the Kosñipata transect may contribute to differences in gross primary productivity with elevation (van de Weg et al., 2014), temperature remains the principal environmental control across the gradient, especially in relation to soil processes. The Kosñipata transect is therefore a model ecosystem to examine the role of temperature, and the interacting influence of other edaphic factors (Table 2.2 and 2.3), in regulating rates of soil C cycling across tropical lowland and montane systems.

Translocation experiments

Translocation experiments are a particularly useful approach for studying the temperature dependence of soil C cycling in tropical forests where in-situ warming experiments are yet to be implemented (Cavaleri et al., 2015, Nottingham et al., 2015c), as experimental warming can be imposed by movement of organic matter across a natural temperature gradient. In an early example of a translocation experiment in the tropics, leaf-litter from one native tree species, and other common C-substrates (filter paper and wooden sticks) were translocated across elevational
(temperature) gradients in Hawaii (Vitousek et al., 1994). This study yielded particularly high temperature sensitivities for decomposition ($Q_{10} = 4.0-6.2$ for leaves and 5.6-11.0 for the wooden sticks) compared to the $Q_{10}$ of 2 typically assumed for biological processes (Kätterer et al., 1998). However, it has since been proposed that systematic variation in nutrient availability with elevation may have increased the observed sensitivity to temperature (Scowcroft et al., 2000), as soil fertility was greater at lower (warmer) elevations (Vitousek et al., 1992). $Q_{10}$ coefficients estimated by translocation experiments across temperature gradients are therefore best described as observed, *apparent* temperature sensitivities, because in addition to the direct effect of temperature they will be a function of other factors which differ among sites and co-vary with temperature (Nottingham et al., 2015c).

The temperature sensitivity of decomposition has also been evaluated by a few other translocation-studies across tropical elevation gradients. Translocation of a standard plant-substrate across an elevation gradient in the Venezuelan Andes revealed that decomposition of the most labile C-fraction was rapid and independent of temperature, whereas decomposition of more recalcitrant C was strongly related to differences in temperature across the gradient (Coûteaux et al., 2002). However, decomposition of the recalcitrant C fraction was also significantly correlated to metrics of soil fertility (C:N ratio, calcium and magnesium concentrations), further in support that soil nutrient availability together with temperature regulates the rate of decomposition.

In a study along the Kosñipata transect in Peru, leaf-litter from 15 different tree-species were translocated among five sites, spanning 2800 m in elevation and 12.8 °C (11.1- 23.9 °C) in MAT (Salinas et al., 2011). In this study, differences in the rate of decomposition among sites was assumed to be driven by temperature, from which the temperature sensitivity of decomposition ($Q_{10} = 3.1 ± 0.3$) was derived. However, this study did not consider how differences in soil fertility and the relative availability of inorganic N and P, varying markedly across the gradient (Nottingham et al., 2015a), may have contributed to the observed temperature-response. Moreover, rates of decomposition were evaluated using the litterbag technique (Bärlocher, 2005) whereby the chosen mesh size enabled access by detritivore invertebrate macrofauna. As such, differences in macrofaunal abundance across the gradient may have also
contributed to the high-apparent temperature sensitivity of decomposition, because termite abundance is greatest at the warmest-lowest elevation site and decreases with increasing elevation (decreasing temperature), reaching an upper range limit at 925-1500 m (Palin et al., 2011). In a more recent study across a montane forest in Hawaii, spanning only 800 m in elevation (5.2 °C range in MAT), the effect of temperature on decomposition was isolated because other factors including vegetation, soil chemistry and soil moisture did not vary across the relatively small-gradient (Bothwell et al., 2014). While the temperature sensitivity of leaf decomposition derived from this Hawaiian study was lower ($Q_{10} = 2.2$) than $Q_{10}$ coefficients previously reported from other tropical elevation gradients, it was only determined for a single species of leaf-litter of Hawaiian endemic (Metrosideros polymorpha). The temperature sensitivity of decomposition estimated for this one species may not therefore be representative of other species of leaves, especially given that leaf-chemistry (varying widely among species) can strongly influence rates of decomposition (Zhang et al., 2008, Waring, 2012) and the sensitivity to temperature (Fierer et al., 2005). Indeed, in the study by Salinas et al. (2011) decomposition varied five-fold among the 15 different species of leaves (at standardised temperature). The specific leaf-trait explaining the interspecies variance in decomposition were not however identified by this Peruvian study. Knowledge of the chemical traits which drive differences in rates of decomposition will be important for predicting responses to global change, if, as seems likely, the chemistry of leaf inputs were to change differentially through responses to temperature or other climate-related change to tree-species distributions (Feeley et al., 2011, Duque et al., 2015).

Although less prevalent, soil translocation experiments have also been conducted across elevation gradients, to evaluate the temperature dependence of soil respiration. In a study across the Kosñipata elevation gradient in Peru, 50 cm deep soil monoliths were reciprocally translocated among four sites spanning a 14 °C (12.5 – 26.4 °C) range in MAT, to examine the temperature dependence of heterotrophic soil respiration (Zimmermann et al., 2009a). In this study, the difference in temperature among sites was the dominant factor influencing soil CO$_2$ effluxes, because above- and belowground plant inputs were excluded. However, reduced substrate supply due to the exclusion of plant derived-C inputs may have confounded
findings and contributed to the relatively low $Q_{10}$ values (1.2-2.5) for heterotrophic respiration reported by this study (Zimmermann et al., 2009a). In contrast, $Q_{10}$ values for total soil respiration (including the contribution from roots) derived in-situ using intra-annual temperature variation were higher (2.1-6.9), but also more variable among soils (Zimmermann et al., 2010a). A similar soil translocation approach was employed by a temperate-region study, where soil cores were reciprocally translocated among two forest sites, with 3.3 °C difference in temperature (Luan et al., 2014). In this study, experimental warming, imposed by moving soils from the high to the lower elevation site, increased the soil CO$_2$ efflux by 44 % compared to the CO$_2$ efflux measured from the soil at its native higher-elevation site (equivalent to a $Q_{10}$ of 3.4). However, again this study only considered the heterotrophic component of soil respiration, as plant inputs were excluded. The extent to which belowground C-supply from roots influences soil C dynamics, and the implications for the observed temperature sensitivity of soil respiration therefore warrants further investigation. Improved understanding of how root activity and root-soil interactions influence soil C cycling will be important in predicting responses to global change, as climatic warming (together with elevated concentrations of atmospheric CO$_2$) will likely increase plant productivity (Lewis et al., 2009, Cernusak et al., 2013) and alter plant species ranges (Chen et al., 2011), in turn modifying the quantity and chemistry of root-derived C inputs to soils (Giardina et al., 2014).

While our understanding of the temperature sensitivity of decomposition (Vitousek et al., 1994, Coûteaux et al., 2002, Salinas et al., 2011, Bothwell et al., 2014) and soil respiration (Zimmermann et al., 2009a, Luan et al., 2014) has been greatly advanced by translocation studies, and despite consensus that climatic warming will accelerate these processes, evidence from the tropics remains very limited and estimations of the temperature sensitivity vary markedly among studies. Moreover, while there is evidence that the chemistry of plant-inputs (Zhang et al., 2008, Hättenschwiler and Jørgensen, 2010, Waring, 2012) soil fertility (Cleveland et al., 2011, Dale et al., 2015), microbial composition (Whitaker et al., 2014b, He et al., 2016) and macrofaunal abundance (García-Palacios et al., 2013a, Dahlsjö et al., 2014) can influence rates of decomposition (see Chapter 1 for an overview), these factors have largely been investigated individually such that the relative importance of each
and their interaction with temperature remains poorly understood. For this thesis, both leaf (Chapter 3) and soil (Chapter 5) translocation approaches were employed to investigate how temperature, together with other abiotic and biotic factors determine rates of leaf-decomposition and soil respiration, and how these factors may influence and contribute to the observed temperature sensitivity under future climatic warming.
Chapter 3

Leaf traits modulate the effect of temperature on decomposition along a tropical elevation gradient

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Author Contribution

LH led and implemented the study, with assistance from AN and PM in experimental design, and AC in the field. LH analysed the data with YS, AN and PM. LH wrote the chapter with contributions from PM, AN and all authors.

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Abstract

Climatic warming is predicted to increase decomposition rates with consequences for global carbon cycling. Yet, because we do not fully understand how multiple interacting abiotic and biotic factors determine decomposition rates, especially in tropical ecosystems, we cannot accurately predict the magnitude of this response to increased temperature.

To investigate how the effect of temperature on decomposition is modulated by leaf chemical traits, soil properties and invertebrate macrofauna, leaves from 8 different plant species were translocated among 5 sites across a 3400 m tropical elevation gradient in the Peruvian Andes. Macrofaunal access was manipulated using litterbags with different mesh-sizes.

Across the gradient, decomposition rates were determined principally by temperature, leaf carbon and leaf nitrogen concentration, with a secondary influence of leaf carbon chemistry (cellulose and lignin content), irrespective of differences in soil fertility. Invertebrate macrofauna accelerated decomposition at lower elevations, but had a negligible effect above 1500 m.

Decomposition was more temperature-sensitive across higher elevation sites, suggesting greater vulnerability to increased carbon loss from montane systems under climatic warming. Findings also suggest that climate-related shifts in macrofaunal abundance and plant species distributions (with associated changes to the chemistry of leaf inputs) have the potential to alter rates of decomposition across tropical ecosystems.
3.1 Introduction

Decomposition is a fundamental ecological process and component of terrestrial biogeochemical cycles, regulating the rate of carbon (C) and nutrient release from plant-residues (Swift et al., 1979). Climatic warming will stimulate microbial activity, and this may accelerate rates of decomposition (Conant et al., 2011, Lu et al., 2013), contributing to alterations in atmospheric composition and generating feedbacks to climate change (Davidson and Janssens, 2006, Friedlingstein et al., 2006). While climate largely drives decomposition on regional to global scales (Meentemeyer, 1978, Aerts, 1997, Liski et al., 2003) there is increasing evidence that leaf chemical traits (Zhang et al., 2008, Waring, 2012), soil fertility (Cleveland et al., 2011, Dale et al., 2015) and the decomposer community (García-Palacios et al., 2013a, Cleveland et al., 2014) also influence rates of decomposition. However, these climatic, abiotic and biotic controls are rarely examined together, such that the relative importance of each remains poorly resolved, especially for tropical ecosystems. Improved understanding of how multiple factors together determine rates of decomposition in the tropics is therefore required, in order to better predict responses to global change.

Climatic drivers (temperature and moisture) of decomposition have been long-established (Meentemeyer, 1978, Aerts, 1997, Davidson and Janssens, 2006), and a few such relationships have been shown in tropical forests (Meir et al., 1996, Powers et al., 2009, Wieder et al., 2009, Salinas et al., 2011, Bothwell et al., 2014). However, evidence that decomposition is predominantly determined by climate is not unequivocal. Global-scale analyses have suggested a stronger role for leaf chemical traits than for climate (Cornwell et al., 2008, Zhang et al., 2008), and more recent wide-ranging synthesis studies from ecosystems spanning the sub-arctic to tropics have only reported a strong temperature dependence of decomposition at cooler sites (Waring, 2012, Bradford et al., 2016b). These recent examples support the existence of climatic thresholds (Prescott, 2010), where low temperatures at cooler sites may strongly constrain decomposition, irrespective of other factors. In contrast, where climate is more favourable for decomposition, other factors including leaf traits, soil fertility and the active decomposer community may modulate the effects of climate to determine rates of decomposition. To accurately predict responses to climatic warming, we must therefore assess the role of interacting non-climatic controls on decomposition, and
consider the influence of these controls above and below potential temperature thresholds.

High biodiversity in tropical forests gives rise to large interspecific variation in leaf chemical traits among plant species (Hättenschwiler et al., 2008). Yet the traits which best explain decomposition have been found to vary among studies. In one lowland forest study, foliar C fractions (non-structural carbohydrates, phenolics, condensed tannins and lignin) explained most variation in decomposition rate, while foliar nutrient concentration was not significant (Hättenschwiler and Jørgensen, 2010). In contrast, foliar nutrient contents (N, P, K, Ca and Mg) were shown to be good predictors of mass loss in wet tropical forests across the globe (Waring, 2012). However, a pan-tropical study, comparing the decomposition of leaf-litter from two different species, found that the type of leaf which decomposed fastest differed among sites (Powers et al., 2009), indicating that local-scale site-dependent factors (for example, soil fertility and decomposer community composition) may have interacted with leaf chemistry to determine the rate of decomposition.

Soil fertility may regulate rates of decomposition (Scowcroft et al., 2000, Dent et al., 2006, Dale et al., 2015), as biogeochemical cycles are closely coupled to the nutrient demands of microorganisms which degrade organic matter (Finzi et al., 2011). Low concentrations of essential soil nutrients, primarily nitrogen (N) and phosphorus (P), can constrain microbial activity and consequently rates of decomposition (Cleveland et al., 2011). However, the different sources from which N and P are derived means that either element may become limiting relative to the other in different tropical soils. Strongly weathered and leached lowland soils may be more deficient in rock-derived P (Walker and Syers, 1976, Vitousek, 1984). In contrast, montane soils may be more deficient in N due to slower accumulation via biological fixation at cooler temperatures (Tanner et al., 1992), while P is more frequently replenished and abundant in these montane environments due to landslide events (Clark et al., 2016) and near-surface weathering (Porder et al., 2007). Though there is some support for this transition in P-N limitation with elevation (Nottingham et al., 2015a), findings are relatively limited and inconsistent among studies. Fertilisation with P enhanced decomposition in some lowland forests (Cleveland et al., 2002, Chen et al., 2015), but had no apparent effect in another (McGroddy et al., 2004), with leaf N concentration
(rather than P) most strongly influencing rates of decomposition in lowland Bolivia (Bakker et al., 2011). In separate montane forest studies, fertilisation with N increased microbial respiration (Fisher et al., 2013) and growth of microbial biomass (Cusack et al., 2011). However, in another montane forest study, higher concentrations of N in leaves and soil had no significant influence on leaf decomposition rates (Hobbie and Vitousek, 2000). More studies are therefore required to better assess the role of soil fertility and the potential shift from a P to an N constraint on decomposition with elevation.

The composition of the decomposer community can also influence rates of decomposition. A synthesis of soil fauna exclusion experiments revealed that at global and tropical biome scales, exclusion of macrofauna consistently reduced rates of decomposition (García-Palacios et al., 2013a). However, macrofaunal abundance can vary markedly across gradients of lowland to montane forests (Palin et al., 2011, Werenkraut and Ruggiero, 2014), potentially leading to differences with elevation in the contribution of macrofauna to decomposition. Furthermore, the chemical traits which determine rates of leaf-decomposition may also differ dependent on the composition of the decomposer community, as in a temperate forest study foliar N content drove decomposition in the presence of macrofauna but when macrofauna were excluded decomposition was more dependent on foliar P content (Vos et al., 2013). In lowland French Guiana, leaf mass loss was best explained by leaf lignin concentration in the presence of macrofauna, and total phenolics when macrofauna were excluded (Hättenschwiler and Jørgensen, 2010). However, this study was atypical of other tropical forest studies as leaf nutrient concentration did not affect decomposition. The interacting influences of macrofaunal abundance and foliar chemistry on decomposition rates in tropical ecosystems therefore requires further examination.

In a study of leaf-litter decomposition along an elevation gradient in the Peruvian Andes, temperature explained much variation in the overall mean rate of decomposition across 15 plant species (Salinas et al., 2011), but large variation among species, for example, five-fold inter-species variance in decomposition (at standardised temperature) was also reported. This would suggest, consistent with the preceding discussion, that factors in addition to temperature also regulate the rate of
decomposition at more local scales (Bradford et al., 2014). To investigate how foliar chemistry and soil properties, in addition to temperature, regulate the decomposition of leaves by microorganisms and invertebrate macrofauna, an *in-situ* leaf decomposition experiment was conducted, measuring rates of decomposition for leaves from eight different species at five sites across the same 3400 m temperature (and soil fertility) gradient in Peru. The following hypotheses were tested:

H1. Leaf chemical traits, soil fertility and the decomposer community, in addition to climate, will exert a significant influence on rates of decomposition, although there will be differences in the relative importance of these factors with elevation. Cool temperatures will most strongly constrain decomposition at higher elevation sites, while other factors such as leaf traits will be more important at warmer lowland sites, where temperature does not impose an overriding constraint. Invertebrate macrofauna will accelerate decomposition in lowland forests, but not at higher elevation sites where their abundance is low.

H2. Foliar and soil P and N concentration will most strongly influence leaf decomposition in lowland and montane systems respectively, due to a transition with elevation from a P to an N constraint on the decomposer community.

### 3.2 Materials and Methods

**Study sites**

Leaves from eight different plant species, representing the dominant taxonomic groups and a range of chemical traits, were translocated among five sites along a 3400 m tropical elevation gradient in Peru. The three highest elevation sites were located in or close to the Manu National Park, centred on the Kosñipata Valley on the eastern slopes of the Andes (3644 m, 3025 m and 1500 m elevation above sea level; asl). The two lower elevation sites were in the lowland Amazon basin, Tambopata Reserve, southeastern Peru, 240 km east of the main Kosñipata transect (210 m and 194 m asl). All sites from lowland Amazonian rainforest to upper montane cloud forest have continuous closed canopy forest cover, with montane-grassland above the treeline which is situated at approximately 3500 m asl. Site characteristics are summarised in Table 3.1. Mean annual temperature (MAT) decreases with increasing elevation along
the transect, from 26.4 to 6.5 °C, whereas mean annual precipitation (MAP) does not change linearly with elevation, and peaks at the mid-elevation site (5302 mm year\(^{-1}\) at 1500 m). Despite marked variation in MAP among sites, and distinct wet (November-March) and dry (May-September) seasons (Rapp and Silman, 2012), there is little variation in soil moisture content over the seasonal cycle at all sites, and evidence to date suggests that plants and soils are rarely moisture limited (Zimmermann et al., 2010a, van de Weg et al., 2014).

Along the elevation gradient there are large shifts in vegetation taxonomic composition, with dominant tree families Leythidaceae, Bixacea, Moraceae and Fabaceae at the lower rainforest sites (194 m and 210 m), shifting to Euphorbiaceae, Fabaceae and Lauraceae in the lower montane forest (1500 m) and Clusiaceae, Cunoniaceae and Rosaceae in the upper montane forest (3025 m) (Girardin et al., 2010, Salinas et al., 2011). Above the treeline, tussock-forming grasses dominate, from the genera *Calamagrostis*, *Scirpus* and *Festuca* (Oliveras et al., 2014).

Soil properties for the five sites included in the study are summarised in Table 3.2. Total C, N and P concentrations in surface soils (0-10 cm depth) generally increase with elevation across the forest sites, with lower concentrations measured in the grassland soil above the treeline. Soil pH shows little variation among the forest sites (pH 3.6-4.4), but is higher above the treeline (pH 4.9). Microbial abundance and the ratio of fungi:bacteria (F:B) in soils also generally increases with elevation (Whitaker et al., 2014b).

Despite the close proximity of the two lowland plots, Tambopata-5 (210 m asl) and Tambopata-6 (194 m asl), both were included in the study as they differ in underlying geology and soil fertility (Malhi et al., 2014). Tambopata-5 is situated on a relatively infertile Pleistocene terrace (< 100,000 years old) where exchangeable base cations are very low and exchangeable aluminium concentrations are high (Quesada et al., 2010). By contrast Tambopata-6 is situated on a more recent Holocene floodplain (< 10,000 years old), and the soil is more fertile (compared to Tambopata-5 and soils common to much of Eastern Amazonia), with higher concentrations of P, base cations (Ca, Mg, K, Na) and aluminium (Quesada et al., 2010).
Table 3.1: summary of site characteristics (mean annual temperature; MAT, mean annual precipitation; MAP).

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Elevation (m asl)</th>
<th>Latitude (S)</th>
<th>Longitude (W)</th>
<th>MAT (°C)</th>
<th>MAP (mm yr⁻¹)</th>
<th>Soil Classification</th>
<th>Dominant Plant Species (leaves used for experiment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata-6</td>
<td>194</td>
<td>12° 49' 48&quot;</td>
<td>69° 16' 16&quot;</td>
<td>26.4</td>
<td>1900</td>
<td>Haplic Alisol</td>
<td>-</td>
</tr>
<tr>
<td>Tambopata-5</td>
<td>210</td>
<td>12° 50' 11&quot;</td>
<td>69° 16' 45&quot;</td>
<td>26.4</td>
<td>1900</td>
<td>Haplic Cambisol</td>
<td>Calophyllum brasiliense (CB), Bixa arborea (BX)</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>13° 02' 56&quot;</td>
<td>71° 32' 13&quot;</td>
<td>17.4</td>
<td>5302</td>
<td>Cambisol</td>
<td>Alchornea latifolia (AL), Vismia sp. (VS)</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>13° 11' 24&quot;</td>
<td>71° 35' 13&quot;</td>
<td>11.1</td>
<td>1560</td>
<td>Umbrisol</td>
<td>Weimannia crassifolia (WC), Clusia alata (CA)</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>13° 07' 29&quot;</td>
<td>71° 36' 86&quot;</td>
<td>6.5</td>
<td>*760</td>
<td>Umbrisol</td>
<td>Calamagrostis grass (CTS), Moss</td>
</tr>
</tbody>
</table>

*Measured at Acjanaco, 3450 m asl (Oliveras et al., 2014)

Soil classification from Quesada et al. (2010), dominant plant species from Salinas et al. (2011) and Oliveras et al. (2014)

Table 3.2: soil characteristics (0-10 cm depth), where data represents mean (1SE) measured from 8 subplots at each elevation site (n = 8).

<table>
<thead>
<tr>
<th>Elevation</th>
<th>aSoil Temp (°C)</th>
<th>aSoil Moisture (%)</th>
<th>Total C (% dwt)</th>
<th>Total N (% dwt)</th>
<th>C:N</th>
<th>bTotal P (mg g⁻¹)</th>
<th>bC:P</th>
<th>bN:P</th>
<th>pH</th>
<th>cMicrobial PLFA (PLFA nmol g⁻¹)</th>
<th>cFungi: Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata-6</td>
<td>24.9 (0.04)</td>
<td>30.1 (1.9)</td>
<td>3.8 (0.9)</td>
<td>0.4 (0.06)</td>
<td>9.4 (0.8)</td>
<td>0.49</td>
<td>48.6</td>
<td>7.1</td>
<td>4.4 (0.06)</td>
<td>94.9</td>
<td>0.105</td>
</tr>
<tr>
<td>Tambopata-5</td>
<td>24.5 (0.07)</td>
<td>18.3 (1.6)</td>
<td>3.8 (0.6)</td>
<td>0.3 (0.03)</td>
<td>11.9 (0.8)</td>
<td>0.18</td>
<td>94.4</td>
<td>12.8</td>
<td>3.6 (0.08)</td>
<td>54.3</td>
<td>0.107</td>
</tr>
<tr>
<td>San Pedro</td>
<td>18.6 (0.06)</td>
<td>44.4 (4.8)</td>
<td>46.8 (4.4)</td>
<td>2.9 (0.18)</td>
<td>16.1 (0.8)</td>
<td>1.36</td>
<td>76.0</td>
<td>6.7</td>
<td>4.0 (0.06)</td>
<td>323.6</td>
<td>0.211</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>11.4 (0.04)</td>
<td>18.0 (1.1)</td>
<td>56.4 (0.7)</td>
<td>2.3 (0.10)</td>
<td>24.7 (1.4)</td>
<td>1.09</td>
<td>427.0</td>
<td>21.9</td>
<td>3.9 (0.08)</td>
<td>473.7</td>
<td>0.328</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>10.0 (0.03)</td>
<td>69.0 (0.7)</td>
<td>35.4 (1.5)</td>
<td>1.8 (0.17)</td>
<td>19.5 (1.2)</td>
<td>0.92</td>
<td>184.0</td>
<td>15.7</td>
<td>4.9 (0.04)</td>
<td>474.2</td>
<td>0.350</td>
</tr>
</tbody>
</table>

a soil temperature and moisture measured over 10 months during decomposition experiment (September 2013-June 2014)
b determined by Nottingham et al. (2015a and unpublished data for Tres Cruces)
c determined by Whitaker et al. (2014b and unpublished data for Tres Cruces)
In-situ litterbag experiment

Rates of leaf decomposition were evaluated using the litterbag technique (Bärlocher, 2005) with litterbags installed at the end of the dry season in September 2013 and harvested in June 2014. Leaves from eight different plant species were included in the study (six species of trees, plus one grass and one moss), chosen as leaves from two dominant species occurring at each of the lowland forest, lower montane forest, upper montane forest and grassland sites (Table 3.1). Fresh leaves were used for this study, collected directly from trees to enable clear identification of species and to ensure that leaves had not been partly decomposed before the start of the experiment, such that the large volume of leaves required for each species (400 g dwt) was as homogenous as possible. Leaves were cut into 3 x 3 cm pieces to homogenise surface area among species, and oven dried at 40 °C to constant mass to standardise the initial mass whilst minimising potential changes to foliar chemistry. Exactly 5.00 g dry leaf material was weighed separately by species into 20 x 20 cm nylon litterbags, with mesh size of either 0.5 mm or 2.0 mm. Mesh size was chosen to either exclude or enable access by invertebrate macrofauna (0.5 mm or 2.0 mm mesh respectively), where for this study macrofauna were defined as larger than 0.5 mm, encompassing mesofauna and macrofaunal groups (Bradford et al., 2002). Decomposition of leaves within 0.5 mm mesh litterbags was defined as being primarily from soil microorganisms (bacteria and fungi) and microfauna (such as nematodes and protozoa) (Swift et al., 1979).

Litterbags were installed at five study sites (Table 3.1). Eight subplots were located at each site, around the perimeter of pre-existing 1 ha permanent study plots of the Andes Biodiversity and Ecosystem Research Group (ABERG; Malhi et al. 2010), with the exception of the 3644 m site where the eight subplots were enclosed in a smaller area (approximately 8 x 8 m) constrained by a fence to protect from disturbance. One replicate litterbag for each plant species and mesh size was installed in each of the eight subplots at every elevation site, resulting in eight individual replicates. High within-site replication was intended to capture local-scale variation, and associated biophysical controls on decomposition (Bradford et al., 2016b).

In total, 640 litterbags were distributed (5 elevation sites x 8 leaf species x 2 mesh sizes x 8 replicates). Each litterbag was marked with a metal tag for identification.
and secured in place by long wire pins to aid retrieval at the end of the experiment. Litterbags were placed in direct contact with the soil surface, gently removing the natural litter layer where necessary without disturbing the underlying soil. Finally, four cuts of 4 cm length were made in the upper side of the 2.0 mm mesh litterbags to enable access by larger invertebrate macrofauna whilst limiting loss of decomposing leaf fragments from the underside. At the time of litterbag installation and every 2-3 months following installation until harvesting, soil temperature and soil moisture were measured in each subplot at 0-10 cm depth using a thermometer and soil moisture probe (Delta-T Devices Ltd., United Kingdom) (Table 3.2).

Litterbags were harvested in June 2014, 265-300 days after they were first installed (depending on site). Out of 640 litterbags first installed, only one was not retrieved, such that the total number of litterbags processed was 639. All remaining leaf material within each litterbag was carefully separated from ingrowing roots, soil and other debris and oven dried at 40 °C to constant mass before being weighed. The remaining mass of leaves in each litterbag was used to calculate the decomposition coefficient $k$ (see Data Analysis).

**Leaf analyses**

At the time of litterbag construction, a random subsample of each species of leaf was retained for analysis, to characterise a suite of chemical traits. Dried and ground homogenous samples were analysed for total C and N content using a TruSpec CN elemental analyser (LECO, USA). Total P content was measured by sulphuric acid-hydrogen peroxide digestion with phosphate detection by automated molybdate colorimetric analysis (Bran Luebbe AutoAnalyser 3, Germany). Leaf samples were also analysed for cellulose, non-cellulosic polysaccharides and lignin content. Briefly, samples of ball-ground dry plant material were first washed in ethanol and then acetone to remove low molecular weight compounds and lipids, while leaving polymers insoluble (polysaccharides, lignin, proteins, RNA, DNA). Lignin content was measured using the acetyl bromide method, described by Hatfield and Fukushima (2005). Non-cellulosic polysaccharides (TFA-hydrolysable, alcohol-insoluble polymers, including hemicelluloses, pectins, and any starch and fructans) were
measured as monosaccharides obtained after hydrolysis in 2 M TFA at 120 °C for 1 hour by the \( p \)-hydroxybenzoic acid hydrazide (PAHBAH) assay for reducing sugars (Lever, 1972). Cellulose content was determined in the TFA residue by the anthrone/H\(_2\)SO\(_4\) assay method (Viles Jr and Silverman, 1949). Absorbance of all samples were measured using a Perkin-Elmer spectrophotometer (Cecil 8000 series, Perkin-Elmer, USA).

**Soil chemical analyses**

At the time of litterbag distribution, soil samples (0-10 cm depth) from each subplot were collected in sealed plastic bags and transported to the laboratory for analysis (Lancaster, UK). Fresh soil samples were used to measure soil pH in water using a Hanna pH meter (soil: H\(_2\)O, 1:2.5 w: v, Hanna Instruments, USA). Dried and ground subsamples were analysed for total C and N content using a TruSpec CN elemental analyser (LECO). Further information regarding quality control for soil and plant material analyses is provided in Appendix A.

**Data analysis**

**Decomposition coefficient \((k)\)**

The decomposition coefficient was estimated from the rate of mass loss over time, assuming negative exponential decay (Olson, 1963, Wider and Lang, 1982). The decomposition constant (expressed as \( k \) in units of days\(^{-1}\)) was characterised by fitting a single exponential decay function (equation 3.1) using the initial mass at time 0 (\( M_0 \)), the mass remaining at the end of the experiment (\( M_t \)) and time since the start of the experiment (\( t \)) (Salinas et al., 2011). The decay function was fitted separately for each litterbag, resulting in 8 replicate values of \( k \) for each species of leaf and mesh size at each elevation site (total \( n = 639 \)). Higher \( k \) values represent faster mass loss (decomposition rate) compared to lower \( k \) values.

\[
M_t = M_0 e^{-kt}
\]

(Eq. 3.1)
Statistical modelling of decomposition along elevation gradient

Statistical analyses were performed using R version 3.2.1 (R Core Team, 2015). Main and interactive effects of ‘Leaf Species’ and ‘Mesh Size’ on decomposition \( k \) were assessed by two-way analysis of variance (ANOVA), separately for each site, with pairwise comparisons of significant effects conducted using Tukey’s HSD post hoc tests (significant differences identified where \( p < 0.05 \)). Prior to parametric analyses, the response variable \( (k) \) was tested for normality using the Shapiro-Wilk test and visual inspection of frequency distribution plots, and square-root transformed where necessary to meet the assumptions of the model.

Specific controls on decomposition \( (k) \) were examined further by using a statistical modelling approach. Linear mixed effects models (package lme4; Bates et al., 2015) were used to account for the nested experimental design (Bradford et al., 2016b), where subplot nested in site was the random effects structure of the model (random intercept model; Schielzeth and Nakagawa, 2013). A full model was first constructed followed by manual removal of non-significant terms in a systematic, stepwise process to achieve the best goodness of fit with the fewest factors (Table 3.3). Akaike Information Criterion (AIC) was used to guide model selection, where a lower AIC represented a better model fit to the data for the number of included parameters, with the significance of individual fixed effect terms assessed using likelihood ratio tests (Zuur et al., 2009). The resultant best-fit model was the simplest and also ecologically reasonable in terms of scientific understanding of the system, with validation of the final parsimonious model undertaken following guidance from Zuur et al. (2009). Further description of model construction and validation is provided in Supplementary Information (Method S3.1).

Mixed effect statistical analyses and model selection were also performed independently on the following subsets. Above (lowland forest sites) and below (montane forest and grassland sites) MAT of 20 °C, as used by Waring (2012) to determine the influence of different controls above and below a potential temperature threshold, and large (2.0 mm) and small (0.5 mm) mesh size to evaluate the distinct controls on decomposition in the presence/absence of macrofauna. This latter mesh size specific analysis was only performed for the lowland forest sites, since at higher
elevation sites (montane forests and grassland) mesh size had no significant effect on decomposition (see Results; Figure 3.2 and Table 3.4) suggesting that macrofauna made a negligible contribution to decomposition at these sites.

After model validation, the conditional (variance explained by fixed + random factors) and marginal $R^2$ (variance explained by fixed effects only) were calculated for the final best-fit models, according to the approach described by Nakagawa and Schielzeth (2013). To assess the relative contribution of each fixed effect to the model, null models (each excluding one fixed effect term in turn) were compared to the final model, to estimate the % variance explained by each fixed effect term separately (by subtraction of the marginal $R^2$ for final model - null model). This approach allowed identification of the fixed effects explaining most of the observed variance in the data, and therefore the relative importance of each parameter in describing decomposition.
Table 3.3: parameters included in the initial full model and final reduced best-fit model (- indicates non-significant term; p > 0.05, not present in the final model). Where independent variables were highly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6; Supplementary Tables S3.2 and S3.3), only one term was included in the initial full model to avoid co-linearity (chosen as the variable which gave the lowest AIC, highlighted in bold).

<table>
<thead>
<tr>
<th>Correlated Variables</th>
<th>Initial Full Model</th>
<th>Final Reduced Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Effects</td>
<td><strong>Temperature</strong>, <strong>Soil C</strong>, <strong>Soil C:N</strong> ratio, <strong>Soil N:P</strong> ratio, <strong>Microbial PLFA</strong>, <strong>Fungi:Bacteria</strong></td>
<td><strong>Temperature</strong></td>
</tr>
<tr>
<td><strong>Soil moisture</strong>, <strong>Soil pH</strong></td>
<td>Mean annual precipitation -</td>
<td></td>
</tr>
<tr>
<td><strong>Soil N</strong>, <strong>Soil P</strong></td>
<td>Soil moisture -</td>
<td>-</td>
</tr>
<tr>
<td><strong>Leaf N</strong>, <strong>Leaf P</strong>, <strong>Leaf C:N</strong> ratio, <strong>Leaf C:P</strong> ratio, <strong>Leaf N:P</strong> ratio, <strong>Leaf lignin:N</strong> ratio, <strong>Leaf lignin:P</strong> ratio</td>
<td><strong>Leaf N</strong></td>
<td><strong>Leaf N</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Leaf Cellulose</strong></td>
<td><strong>Leaf Cellulose</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Leaf Lignin</strong></td>
<td><strong>Leaf Lignin</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Mesh Size x Site_{1500m}</strong></td>
<td><strong>Mesh Size x Site_{1500m}</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Leaf Origin</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

Random Effect

| (1|Site/Subplot) |

*Mesh Size x Site_{1500m} interaction – where Site_{1500m} was a categorical variable defined as above or below 1500 m elevation, because macrofaunal abundance increases at lower elevations across the gradient (Palin et al., 2011), and mesh size (presence of macrofauna) had no significant effect on k at the sites above 1500 m, as identified by ANOVA a priori (see Results, Table 3.4).

Leaf Origin – binary term (0 or 1) where 1 represented leaves sourced from the same elevation as the site of decomposition, to evaluate whether plant-soil interactions had influenced microbial ability to decompose leaf-material of native-origin (Ayres et al., 2009).

*Additional soil properties determined by previous studies (see Table 3.2).
Apparent temperature sensitivity ($Q_{10}$) of decomposition

To examine the sensitivity of leaf decomposition to temperature, $Q_{10}$ coefficients, defined as the factor by which the decomposition constant ($k$) changes with a 10 ºC change in temperature (T), were calculated. $Q_{10}$ coefficients were first derived by fitting exponential curves to mean $k$-values (for each leaf species and mesh size at each site) against MAT, with regression-derived estimates of $k$ used to calculate the temperature sensitivity of decomposition across the full elevation gradient (according to equation 3.2) (Bothwell et al., 2014). $Q_{10}$ coefficients were also calculated separately across ecotones (lowland-lower montane forest: 194-1500 m; lower-upper montane forest: 1500-3025 m; and upper montane forest-grassland: 3025-3644 m), also using mean $k$-values (for each leaf species and mesh size) and MAT at each site (for $T_1$ and $T_2$), to assess if decomposition was more sensitive to temperature at higher elevations/lower temperatures (H1). These estimates of temperature sensitivity are observed-apparent, rather than intrinsic $Q_{10}$ coefficients (Scowcroft et al., 2000, Nottingham et al., 2015c). They reflect system-scale, apparent temperature sensitivities because they are a function of not only temperature but also other variables which differ among sites and co-vary with temperature (for example, plant communities, soil fertility, soil microbial community composition and macrofaunal abundance).

\[
Q_{10} = \left[ \frac{k_2}{k_1} \right]^{10} \left( \frac{T_2 - T_1}{T_2 - T_1} \right)
\]  
(Eq. 3.2)

3.3 Results

Leaf chemistry

Initial concentrations of foliar nutrients (C, N and P) and C chemistry (cellulose and lignin content) determined for the eight species of leaves used for the decomposition experiment are illustrated in Figure 3.1. Total C concentration varied between 45.1 - 54.3 % among the eight species. Leaf N concentration varied four-fold (0.61 - 2.47 %) and P concentration varied over two-fold (0.052 - 0.120 %). Concentrations of C, N
and P generally decreased with increasing elevation of leaf origin. Cellulose (13.4 – 26.5 %) and lignin (17.6 - 34.3 %) concentrations varied two-fold among species, exhibiting no apparent elevational trends.

**Rates of leaf decomposition**

Mean decomposition rate constants (k) varied three-fold among sites with exclusion of macrofauna and four-fold among sites with macrofaunal access permitted, with faster rates of decomposition observed at lower elevation sites (Figure 3.2). High inter- and intra-species variation in k within each site was also evident (Figure 3.3), especially at lower elevations where there was up to 16-fold variance in mean k among the eight different species of leaves (194 m site, 2.0 mm mesh; Figure 3.3e).

For the three higher elevation sites (1500 m, 3025 m, 3644 m), k varied significantly among ‘Leaf Species’, but there was no significant effect of ‘Mesh Size’ (Table 3.4). In contrast, for the lowland forest sites, significant main effects of ‘Leaf Species’ and ‘Mesh Size’ on k were identified for the 194 m site, with a significant ‘Leaf Species x Mesh Size’ interactive effect identified for the 210 m site (Table 3.4). At all sites, decomposition of *Alchornea latifolia* was consistently fast (high k) and decomposition of *Calophyllum brasiliense* was consistently slow (low k), with relative rates of decomposition among the other species varying among sites (Figure 3.3 and Supplementary Table S3.4). At the sites above 1500 m, macrofaunal access (determined by mesh size) had no significant effect on k, whereas at the lower elevation sites decomposition was significantly faster when macrofaunal access was permitted (Figure 3.2). Pairwise comparisons of the significant ‘Leaf Species x Mesh Size’ interactive effect for the 210 m site revealed that relative rates of decomposition among species differed depending on the presence/absence of macrofauna; for example, when macrofauna were excluded there was no significant difference in k among *Calophyllum brasiliense*, *Weinmannia crassifolia* and *Vismia*, whereas when macrofaunal access was permitted, decomposition of *Weinmannia crassifolia* and *Vismia* were significantly faster compared to *Calophyllum brasiliense* (Figure 3.3 and Supplementary Table S3.4).
Figure 3.1; initial foliar chemistry (total carbon (C), nitrogen (N), phosphorus (P), cellulose and lignin concentrations) for eight species of leaves, measured from homogenised sub-samples (homogenisation precluded estimates of variance). Raw data, including C:N, C:P, N:P, lignin:N, lignin:P ratios provided in Supplementary Table S3.1. Abbreviations of leaf species from Table 3.1, source elevation in parentheses.
Figure 3.2: mean rates of decomposition measured at each elevation site, with macrofauna permitted or excluded (determined by mesh size). Bars represent mean decomposition $k \times 10^{-3}$ (day$^{-1}$) ± 1SE measured for eight species of leaves over 10 months from September 2013-June 2014. Asterisks denote significant differences ($p < 0.05$) between $k$ with macrofauna permitted/excluded at each site, where ** $p < 0.01$, *** $p < 0.001$. 
Figure 3.3 a-e; decomposition $k \times 10^{-3}$ day$^{-1}$ for eight species of leaves at 5 elevation sites, measured over 10 months from September 2013-June 2014, with macrofauna excluded or permitted (determined by mesh size). Circles indicate individual $k$-values determined from each litterbag (8 replicates from 8 subplots at each elevation site) and bars represent mean $k \times 10^{-3}$ day$^{-1}$, $n = 8$. Abbreviations of leaf species from Table 3.1, source elevation of leaves in parentheses. Pairwise comparisons among species provided in Supplementary Table S3.4. Note different y-axis scales among panels a-c and d-e.
Table 3.4: main and interactive effects of leaf species (L) and mesh size (M) on decomposition k assessed by two-way ANOVA, separately by site. Significant effect identified where p < 0.05, not significant (ns) where p > 0.05.

<table>
<thead>
<tr>
<th>Site</th>
<th>Term</th>
<th>d.f.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tres Cruces (3644 m)</td>
<td>Leaf Species (L)</td>
<td>7</td>
<td>10.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mesh Size (M)</td>
<td>1</td>
<td>3.7</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L x M</td>
<td>7</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Wayqecha (3025 m)</td>
<td>L</td>
<td>7</td>
<td>57.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1</td>
<td>1.5</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L x M</td>
<td>7</td>
<td>0.7</td>
<td>ns</td>
</tr>
<tr>
<td>San Pedro (1500 m)</td>
<td>L</td>
<td>7</td>
<td>17.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1</td>
<td>3.8</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>L x M</td>
<td>7</td>
<td>1.5</td>
<td>ns</td>
</tr>
<tr>
<td>Tambopata-5 (210 m)</td>
<td>L</td>
<td>7</td>
<td>49.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1</td>
<td>41.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>L x M</td>
<td>7</td>
<td>4.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tambopata-6 (194 m)</td>
<td>L</td>
<td>7</td>
<td>17.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1</td>
<td>7.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>L x M</td>
<td>7</td>
<td>0.9</td>
<td>ns</td>
</tr>
</tbody>
</table>

Pairwise comparisons of significant effects provided in Supplementary Table S3.4.

Multiple drivers of decomposition

Across the elevation gradient, decomposition was explained foremost by temperature, leaf C and leaf N concentration, with an additional small but significant influence of leaf C chemistry (cellulose and lignin concentration) (Table 3.5). Greater temperatures, and concentrations of N and cellulose in leaves promoted k, whereas greater concentrations of C and lignin constrained k (as determined by model coefficients; Supplementary Table S3.5). Macrofaunal exclusion (determined by mesh size) retarded decomposition at lowland forest sites, but had a negligible effect at higher elevation sites, above 1500 m elevation.
At higher elevations, across the montane forest and grassland sites, temperature was the dominant control on decomposition (explaining 53.8 % variance in \( k \)) with secondary influences from leaf chemistry (23.5 % variance explained by foliar N, C, cellulose and lignin content together). In contrast, for the lowland forest sites, leaf chemical composition explained a greater proportion of the total variance in \( k \) (39.7 % variance explained by leaf N and C concentration), with an additional significant influence from macrofaunal activity (determined by mesh size, explaining 4.6 % variance in \( k \)). The proportion of observed variance in \( k \) explained by different foliar traits across the lowland sites also varied dependent on the composition of the decomposer community. With macrofaunal access permitted, leaf N concentration was the most important factor describing decomposition (explaining 34.3 % variance) with leaf C concentration also explaining 17.2 % variance, while C chemistry (cellulose and lignin) was not important (inclusion of terms did not significantly improve the model-fit). By comparison, decomposition at the lowland forest sites with exclusion of macrofauna was explained by the concentration of C and N in leaves, with additional small but significant effects of leaf cellulose and lignin (1.5 % and 2.3 % variance explained respectively).

In the case of the data-analysis subset for lowland forests, it was not possible to directly evaluate the effect of temperature on \( k \) due to the same MAT at the 210 m and 194 m sites (Table 3.1). Inclusion of soil temperature in the model instead, with a difference of 0.5 ºC between the two sites (Table 3.3) was also not significant (p > 0.05), but the difference may have been too small to drive a clear response. Nonetheless, despite a 9 ºC difference in MAT between the lower montane forest and lowland forest sites (17.4 ºC and 26.4 ºC respectively), when macrofauna were excluded there was no significant difference in mean \( k \) between the 1500 m, 210 m and 194 m sites (Figure 3.3) suggesting that greater temperatures at the lowland sites had not accelerated the rate of decomposition.

The mean apparent \( Q_{10} \) of decomposition across the elevation gradient was 1.6 ± 0.1 with macrofaunal exclusion (0.5 mm mesh), and 1.9 ± 0.2 with macrofaunal access permitted (2.0 mm mesh; Figure 3.4). Comparing mean \( Q_{10} \) coefficients among ecotones, the apparent \( Q_{10} \) derived by contrasting lowland with lower-montane forest sites (194-1500 m) was significantly lower than that derived by contrasting lower with
upper-montane forest (1500-3025 m) and upper-montane forest with montane grassland sites (3025-3644 m) (Figure 3.4), revealing that decomposition was more sensitive to temperature across higher elevations.
Table 3.5; estimated variance (var. %) explained by each term from best-fit linear mixed effects models, used to identify the relative importance of climatic, abiotic and biotic controls on leaf decomposition ($k$) across the elevation gradient (all sites), and with data subset by montane forests and grassland (1500 m, 3025 m and 3644 m) and lowland forests (194 m and 210 m). Additionally, for lowland forest sites, data subset by mesh size to assess controls on decomposition in presence/absence of macrofauna.

- denotes term not included in initial model, and ns denotes non-significant term (p > 0.05) not present in final parsimonious model.

<table>
<thead>
<tr>
<th>Estimated var. explained by each term (%)</th>
<th>All Sites ($n = 639$)</th>
<th>(MAT &lt; 20 °C) Montane Forest &amp; Grassland ($n = 384$)</th>
<th>(MAT &gt; 20 °C) Lowland Forest ($n = 255$)</th>
<th>Lowland Forest Including Macrofauna ($n = 127$)</th>
<th>Lowland Forest Excluding Macrofauna ($n = 128$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>17.7</td>
<td>53.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leaf Carbon (%)</td>
<td>9.5</td>
<td>9.2</td>
<td>15.9</td>
<td>17.2</td>
<td>14.6</td>
</tr>
<tr>
<td>Leaf Nitrogen (%)</td>
<td>12.6</td>
<td>11.1</td>
<td>23.8</td>
<td>34.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Leaf Cellulose (%)</td>
<td>1.1</td>
<td>2.4</td>
<td>ns</td>
<td>ns</td>
<td>1.5</td>
</tr>
<tr>
<td>Leaf Lignin (%)</td>
<td>0.8</td>
<td>0.8</td>
<td>ns</td>
<td>ns</td>
<td>2.3</td>
</tr>
<tr>
<td>Mesh Size x Site$_{1500m}$</td>
<td>6.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Mesh Size</td>
<td>-</td>
<td>ns</td>
<td>4.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Full model</strong></td>
<td><strong>54.6</strong></td>
<td><strong>71.0</strong></td>
<td><strong>41.8</strong></td>
<td><strong>45.0</strong></td>
<td><strong>35.1</strong></td>
</tr>
<tr>
<td><strong>Conditional R$^2$</strong> (% var. explained)</td>
<td><strong>49.6</strong></td>
<td><strong>66.8</strong></td>
<td><strong>34.1</strong></td>
<td><strong>38.9</strong></td>
<td><strong>26.1</strong></td>
</tr>
<tr>
<td><strong>Marginal R$^2$</strong> (% var. explained)</td>
<td><strong>5.0</strong></td>
<td><strong>4.2</strong></td>
<td><strong>7.7</strong></td>
<td><strong>6.1</strong></td>
<td><strong>9.0</strong></td>
</tr>
<tr>
<td><strong>Random Effects</strong> (% var. explained)</td>
<td><strong>5.0</strong></td>
<td><strong>4.2</strong></td>
<td><strong>7.7</strong></td>
<td><strong>6.1</strong></td>
<td><strong>9.0</strong></td>
</tr>
</tbody>
</table>

Estimated variance (var.) explained by each term in the model (%), calculated by comparing marginal R$^2$ from the final refined model and null models (with each term removed in turn). Conditional R$^2$ (variance explained by fixed + random effects) and marginal R$^2$ (variance explained by fixed effects only) were determined by the approach described by Nakagawa and Schielzeth (2013), and expressed as % variance explained. Model coefficients and SE for each fixed effect term provided in Supplementary Table S3.5. AIC and likelihood ratio test statistics for individual fixed effects following single term removal from best-fit models provided in Supplementary Table S3.6.
Figure 3.4: apparent temperature sensitivity ($Q_{10}$) of leaf decomposition derived across the elevation gradient and separately across ecotones (lowland-lower montane forest: 194-1500 m; lower-upper montane forest: 1500-3025 m; and upper montane forest-grassland: 3025-3644 m). Bars represent mean ± 1SE ($n = 8$). Different letters indicate significant differences (identified by Tukey’s HSD tests, $p < 0.05$).

3.4 Discussion

Decomposition across the gradient as a whole was explained principally by temperature and leaf C and leaf N concentration, while leaf C chemistry (cellulose and lignin content) had a smaller effect (Table 3.5). There were, however, differences in the relative importance of these controls at higher and lower elevations (Table 3.5), and differences in the apparent temperature sensitivity of decomposition across the gradient (Figure 3.4). Across the montane forest and grassland sites (MAT < 20 ºC), $k$ was strongly constrained by low temperatures, with secondary influences from foliar chemistry. By contrast, across the lowland forest sites (MAT > 20 ºC), high interspecies variation in $k$ (Figure 3.3 d-e) and significant differences in $k$ dependent on the presence/absence of invertebrate macrofauna (Figure 3.2) provided evidence for strong leaf trait and decomposer community controls on decomposition (Table 3.5). These findings were largely consistent with H1, however soil fertility (soil N and P concentration) did not explain a significant proportion of the observed variance in $k$. 

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Furthermore, leaf N concentration, rather than P, primarily explained decomposition rates in the lowland forest plots, contrary to the prevailing theory of P limitation to biological activity in lowland soils (H2).

Temperature was the dominant control on decomposition across the montane forest and grassland sites, suggesting that cool temperatures strongly constrain decomposition at higher elevations. This finding is supported by lower inter-species variation in k observed at these sites (Figure 3.3 a-c) such that the role of leaf traits was secondary to the constraint imposed by temperature (Table 3.5). Decomposition in lowland forests was less sensitive to temperature compared to decomposition at higher elevations (Figure 3.4). Indeed, when macrofauna were excluded, there was no significant difference in mean k among the 1500 m, 210 m and 194 m sites (Figure 3.3) with the corresponding apparent $Q_{10}$ of 1.0 ± 0.1 across the lower montane-lowland forest ecotone suggesting that k was independent of differences in temperature among these sites. The strong temperature dependence of decomposition at cooler higher-elevation sites was consistent with the relationship identified by Waring (2012), where temperature constrained decomposition in tropical montane forests (MAT < 20 °C) but did not influence k at warmer lowland sites. The higher apparent $Q_{10}$ for mid-to-high elevation sites (Figure 3.4) therefore indicates that these regions will be most sensitive to future climate warming, where temperature currently constrains decomposition, compared to lowland forests where temperature is already favourable for decomposition and leaf traits instead provide the dominant control. There was no significant effect of soil moisture nor MAP on k, consistent with previous studies of this gradient (Zimmermann et al., 2010a, Salinas et al., 2011). Soils along the Kosñipata transect are relatively moist throughout the seasonal cycle and hence water does not appear to limit biological activity (Prescott, 2010), despite marked variation in annual precipitation among sites.

Leaf chemical composition strongly mediated rates of decomposition, particularly in lowland forests (Table 3.5). Leaf N concentration consistently explained the greatest proportion of observed variance in k across models (Table 3.5), including for lowland forests, contrary to the hypothesis that foliar P content would more strongly drive decomposition (H2) due to the prevailing theory of P-limitation to biological activity in lowland soils (Nottingham et al., 2015a). As foliar N and P were
highly correlated, it is possible that high foliar nutrient concentration explains this response, rather than N content per se. However, leaf N concentration consistently explained more variation in $k$ than leaf P, across sites and for data subsets. Leaf N concentration, rather than P, was also a better predictor of leaf decomposition in a lowland forest in Bolivia (Bakker et al., 2011), and soil C:N ratio, rather than C:P ratio, influenced rates of decomposition across a lowland forest soil-fertility gradient in Panama (Dale et al., 2015). Together, these results challenge the generality of the theory of P limitation to microbial activity (and hence decomposition) in lowland tropical forests. One explanation for this is that fungi, which play an important role in the decomposition of leaves (de Boer et al., 2005, Strickland and Rousk, 2010), are most limited by N availability, as indicated by increased fungal abundance in response to N fertilisation in tropical lowland soils elsewhere (Liu et al., 2013, Fanin et al., 2015b). Alternatively, N from leaves may be used by microorganisms to support the production of extracellular phosphatase enzymes, to increase P acquisition (Allison and Vitousek, 2005, Nannipieri et al., 2011), with several tropical studies reporting fertilisation with N to increase phosphatase activity (Treseder and Vitousek, 2001, Wang et al., 2007). High phosphatase activity has been previously measured in lowland forest soils from the studied Peruvian gradient (Nottingham et al., 2015a), hence high apparent demand for N (from leaves) could be indicative of a fundamental constraint by P on the microbial community, if N was used for enzyme synthesis.

Soil fertility (soil N and P concentration) was not associated with observed rates of leaf decomposition ($p > 0.05$). Much support for the influence of soil fertility on decomposition in the tropics comes from fertilisation experiments where the availability of soil nutrients is artificially elevated (Tanner et al., 1998, Cleveland et al., 2002, Li et al., 2006, Kaspari et al., 2008), with relatively few studies using natural soil fertility gradients. Across a soil fertility gradient in Panama, although soil C:N ratio influenced the rate of leaf decomposition, a much greater proportion of variance was explained by the identity of the decomposing leaves (6.5% and 55% variance explained by soil C:N and leaf identity respectively; Dale et al. 2015). Moreover, while studies of leaf decomposition in Hawaii and Malaysia observed faster rates of decomposition on more fertile soils (Ostertag and Hobbie, 1999, Dent et al., 2006), soil fertility effects may have been more evident in these cases as climate was
relatively uniform across the study sites. In the study presented here, the constraint imposed by temperature at the more fertile montane forest sites may have prevailed over the influence of soil fertility. Irrespective of this, results demonstrate that climate (temperature) and leaf traits are dominant controls on leaf decomposition, while soil fertility may be hierarchically less important (Lavelle et al., 1993).

Macrofaunal activity was an important driver of decomposition in lowland forests but not in montane forests or grasslands (Figure 3.2). This was most likely due to low macrofaunal abundance at higher elevations, as a step-change in soil-feeding termite abundance has been previously reported at approximately 1000 m elevation along the studied gradient (Palin et al., 2011). It has been suggested that soil fauna accelerate decomposition in wet tropical forests, but have no effect where temperature or moisture are limiting (Wall et al., 2008). Results from this study are therefore consistent with this theory, whereby macrofauna made a small but significant contribution to decomposition in lowland forests, but at higher elevation sites, below a temperature-threshold which constrains macrofaunal abundance (Palin et al., 2011), decomposition was determined by microbial and microfaunal activity alone. Climatic warming may however move the thermal limit of invertebrate macrofauna up-slope (Colwell et al., 2008, Moret et al., 2016), with potential to accelerate future rates of leaf decomposition in montane forests if this occurs.

The presence of invertebrate macrofauna at lower elevations also influenced the relative importance of different foliar traits regulating rates of decomposition. When macrofaunal access was permitted, foliar N content was the most important determinant of $k$ (explaining over a third of the observed variance) while foliar C chemistry (lignin and cellulose content) had no significant influence. Given that termites are the most abundant macrofaunal decomposer in the tropics (Bignell and Eggleton, 2000) and dominate at the studied lowland forest sites (Dahlsjö et al., 2014), these findings are consistent with previous studies of termite-metabolism, with termites reported to preferentially feed on N-rich plant material (Prestwich et al., 1980) while being able to digest complex C compounds (Butler and Buckerfield, 1979, Brune, 2014). Foliar lignin content was however a significant predictor of $k$ in lowland forests when macrofauna were excluded, and in higher-elevation montane regions (where decomposition was microbially-mediated, no apparent influence of
macrofauna; Figure 3.2), indicating that decomposition by microorganisms and microfauna across the gradient was constrained by lignin-rich leaves. If microbial functional dissimilarity was prevalent then we may expect lignin content to more strongly constrain decomposition where fungal abundance is low (Strickland et al., 2009a, Strickland and Rousk, 2010). Fungal abundance is lower in lowland soils from the studied gradient (Whitaker et al., 2014b), and, although the signal was not strong, lignin content explained more variance in $k$ for lowland forests (with macrofaunal exclusion) than for the montane sites (2.3 % and 0.8 % variance explained respectively). This suggests that microbial community composition in soils may have, to some extent, influenced the decomposition of lignin-rich leaves, although this was not tested directly.

This study examined the relative importance of climatic, abiotic and biotic controls on decomposition using fresh leaves. The $k$-values from this study were therefore marginally greater compared to those determined for senescent leaves by a previous study along the same Peruvian elevation gradient (Salinas et al., 2011), likely due to higher foliar nutrients in fresh leaves compared to senescent litter (Aerts, 1996). Despite this, relative differences in $k$ among species were very consistent between both studies (Supplementary Figure S3.1), suggesting that the traits which determine decomposition are somewhat conserved or relative change is consistent among species during senescence. This indicates that it may be possible to predict the decomposability of leaf-litter from remotely sensed canopy leaf traits (Asner et al., 2014b), as found by a recent subtropical study where the spectral properties of green leaves accurately predicted the decomposability of senescent leaf-litter (Harguindeguy et al., 2015). Therefore, while fresh leaves were used here, it is reasonable to assume that the same traits also regulate the decomposition of leaf-litter, especially given that measured chemical traits for the eight plant species included in this study (Figure 3.1) were representative of leaf-litter chemistry reported for other tropical forests (Aerts, 1997, Hättenschwiler et al., 2008).

Given the importance of leaf traits in regulating rates of decomposition across the studied gradient, changes to the chemistry of leaf inputs have the potential to strongly influence future rates of C and nutrient cycling in the tropics. Increasing atmospheric N deposition (Hietz et al., 2011) reported even across remote Andean
ecosystems (Fabian et al., 2005, Boy et al., 2008) will likely increase foliar N concentration, and this could accelerate rates of decomposition. Climatic warming could also drive concomitant changes to leaf chemistry through changes to plant-species distributions (Feeley et al., 2011, Duque et al., 2015), influencing rates of decomposition independently of the direct response to temperature. Up-slope shifts in plant ranges may also increase the decomposability of leaves, as species from lower elevations on this transect have generally higher concentrations of foliar N (van de Weg et al., 2009). However, this premise depends on the extent to which foliar traits are maintained or are altered in response to warming and the new up-slope environment, which requires further investigation.

3.5 Conclusion

In this large-scale translocation experiment across a 3400 m tropical elevation gradient, the relative importance of multiple climatic, abiotic and biotic controls on decomposition were simultaneously evaluated, demonstrating the value of using natural environmental gradients for multi-factor experiments *in-situ*. Although temperature explained much of the variance associated with $k$ across the elevation gradient as a whole, temperature was a much stronger constraint across cooler mid-to-upper elevation sites, suggesting a greater sensitivity and vulnerability to accelerated C loss in this montane system under future climatic warming. The study also revealed a strong role for foliar chemistry (leaf C, N, cellulose and lignin content) in regulating rates of leaf decomposition across the gradient, and the role of invertebrate macrofauna in accelerating decomposition in lowland forests, warming-related changes to which have the potential to accelerate rates of decomposition above that predicted by the direct effect of temperature alone. Together these findings suggest that plant and soil biotic community composition will modulate the observed sensitivity of leaf decomposition to future climatic warming. A more integrated recognition of these factors is therefore required in order to more comprehensively predict future rates of soil C cycling across tropical lowland and montane systems in response to global change.
Supplementary Information

**Supplementary Table S3.1;** initial foliar traits (% dry mass basis) for eight species of leaves, measured from a homogenised sample for each species (homogenisation precluded estimate of variance)

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Source Elevation</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>Cellulose (%)</th>
<th>Non-cellulosic polysaccharides (%)</th>
<th>Lignin (%)</th>
<th>Lignin:N</th>
<th>Lignin:P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bixa arborea</em> (BX)</td>
<td>210</td>
<td>54.3</td>
<td>2.47</td>
<td>0.115</td>
<td>21.9</td>
<td>472</td>
<td>21.5</td>
<td>21.0</td>
<td>11.5</td>
<td>29.1</td>
<td>11.8</td>
<td>253</td>
</tr>
<tr>
<td><em>Caliphyllum brasiliense</em> (CB)</td>
<td>210</td>
<td>53.0</td>
<td>1.18</td>
<td>0.084</td>
<td>44.9</td>
<td>631</td>
<td>14.1</td>
<td>18.8</td>
<td>8.9</td>
<td>31.2</td>
<td>26.4</td>
<td>371</td>
</tr>
<tr>
<td><em>Alchornea latifolia</em> (AL)</td>
<td>1500</td>
<td>46.4</td>
<td>2.16</td>
<td>0.120</td>
<td>21.5</td>
<td>386</td>
<td>18.0</td>
<td>17.6</td>
<td>11.3</td>
<td>32.1</td>
<td>14.9</td>
<td>268</td>
</tr>
<tr>
<td><em>Vismia sp.</em> (VS)</td>
<td>1500</td>
<td>49.1</td>
<td>1.90</td>
<td>0.119</td>
<td>25.9</td>
<td>413</td>
<td>16.0</td>
<td>19.7</td>
<td>11.4</td>
<td>32.1</td>
<td>16.9</td>
<td>270</td>
</tr>
<tr>
<td><em>Clusia Alata</em> (CA)</td>
<td>3025</td>
<td>48.0</td>
<td>1.63</td>
<td>0.120</td>
<td>29.4</td>
<td>400</td>
<td>13.6</td>
<td>13.4</td>
<td>9.9</td>
<td>17.6</td>
<td>10.8</td>
<td>147</td>
</tr>
<tr>
<td><em>Weinmania crassifolia</em> (WC)</td>
<td>3025</td>
<td>49.5</td>
<td>1.03</td>
<td>0.107</td>
<td>47.9</td>
<td>463</td>
<td>9.7</td>
<td>19.6</td>
<td>11.9</td>
<td>34.3</td>
<td>33.2</td>
<td>321</td>
</tr>
<tr>
<td>Moss</td>
<td>3644</td>
<td>45.1</td>
<td>1.03</td>
<td>0.060</td>
<td>43.8</td>
<td>752</td>
<td>17.2</td>
<td>21.8</td>
<td>32.3</td>
<td>28.1</td>
<td>27.3</td>
<td>468</td>
</tr>
<tr>
<td><em>Calamagrostis Grass</em> (CTS)</td>
<td>3644</td>
<td>47.1</td>
<td>0.61</td>
<td>0.052</td>
<td>77.1</td>
<td>905</td>
<td>11.8</td>
<td>26.5</td>
<td>20.3</td>
<td>32.3</td>
<td>52.7</td>
<td>621</td>
</tr>
</tbody>
</table>
### Supplementary Table S3.2; matrix showing pairwise correlations between leaf chemical traits ($n = 8$). Data represent Spearman’s rank correlation coefficients for each pair of variables. Where leaf chemical traits were strongly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6; highlighted in bold) only one term included in the initial full model to avoid co-linearity.

<table>
<thead>
<tr>
<th>LEAF TRAITS</th>
<th>N (%)</th>
<th>P (%)</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>Lignin (%)</th>
<th>Cellulose (%)</th>
<th>Lignin:N (%)</th>
<th>Lignin:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>0.35</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.02</td>
<td>0.08</td>
<td>-0.12</td>
<td>-0.21</td>
<td>-0.31</td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.77*</td>
<td>-0.93*</td>
<td>-0.93*</td>
</tr>
<tr>
<td>P (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.78*</td>
<td>-0.97*</td>
<td>0.81*</td>
</tr>
<tr>
<td>C:N</td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
<td>-0.83*</td>
<td>0.38</td>
<td>0.38</td>
<td>0.81*</td>
<td>0.74*</td>
</tr>
<tr>
<td>C:P</td>
<td></td>
<td></td>
<td>-0.19</td>
<td>0.07</td>
<td></td>
<td>0.81*</td>
<td>0.69</td>
<td>0.81*</td>
<td></td>
</tr>
<tr>
<td>N:P</td>
<td></td>
<td></td>
<td>-0.45</td>
<td>0.05</td>
<td>-0.55</td>
<td>-0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin (%)</td>
<td></td>
<td></td>
<td>0.23</td>
<td>0.62</td>
<td>0.66</td>
<td>0.93*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lignin:N</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*denotes significant correlation $p < 0.05$
Supplementary Table S3.3: matrix showing pairwise correlations between site variables ($n = 5$ where measured at site-elevation scale, or $n = 40$ where measured at the site-subplot scale). Data represent Spearman’s rank correlation coefficients for each pair of variables. Where metrics were strongly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6; highlighted in bold), only one term included in the initial full model to avoid co-linearity.

<table>
<thead>
<tr>
<th>SITE VARIABLES</th>
<th>MAP (mm yr$^{-1}$)</th>
<th>Moisture (%)</th>
<th>Soil C (% dwt)</th>
<th>Soil N (% dwt)</th>
<th>Soil C:N</th>
<th>Soil P (mg kg$^{-1}$)</th>
<th>Soil N:P</th>
<th>Microbial PLFA</th>
<th>Fungi: Bacteria</th>
<th>Soil pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp. (°C)</td>
<td>0.56</td>
<td>-0.45</td>
<td>-0.68</td>
<td>-0.59</td>
<td>-0.81</td>
<td>-0.56</td>
<td>-0.56</td>
<td>-0.97</td>
<td>-0.97</td>
<td>-0.45</td>
</tr>
<tr>
<td>MAP (mm yr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Moisture (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Soil C (% dwt)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil N (% dwt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil C:N</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil P (mg g$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil N:P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Microbial PLFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil Fungi: Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*denotes significant correlation $p < 0.05$
**Supplementary Table S3.4**: pairwise comparisons of \( k \)-values among leaf species at each site, where different letters within each column indicate significant differences (\( p < 0.05 \)), determined by Tukey’s HSD post-hoc tests following ANOVA (Table 3.4).

Abbreviations of leaf species from Table 3.1 (source elevation in parentheses).

<table>
<thead>
<tr>
<th>Leaf Species</th>
<th>Tambopata-6 (194 m)</th>
<th>Tambopata-5 (210 m)</th>
<th>Tambopata-5 (210 m)</th>
<th>San Pedro (1500 m)</th>
<th>Wayqecha (3025 m)</th>
<th>Tres Cruces (3644 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL (1500 m)</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>CA (3025 m)</td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>b</td>
<td>ab</td>
</tr>
<tr>
<td>CTS (3644 m)</td>
<td>b</td>
<td>b</td>
<td>c</td>
<td>b</td>
<td>bc</td>
<td>ab</td>
</tr>
<tr>
<td>BX (210 m)</td>
<td>b</td>
<td>b</td>
<td>bc</td>
<td>b</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Moss (3644 m)</td>
<td>b</td>
<td>c</td>
<td>e</td>
<td>b</td>
<td>cd</td>
<td>ab</td>
</tr>
<tr>
<td>VS (1500 m)</td>
<td>b</td>
<td>bc</td>
<td>cd</td>
<td>b</td>
<td>d</td>
<td>bc</td>
</tr>
<tr>
<td>WC (3025 m)</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>b</td>
<td>d</td>
<td>bc</td>
</tr>
<tr>
<td>CB (210 m)</td>
<td>c</td>
<td>c</td>
<td>e</td>
<td>c</td>
<td>e</td>
<td>c</td>
</tr>
</tbody>
</table>

Post-hoc tests conducted separately for Tambopata-5 (210 m) with macrofauna excluded (0.5 mm mesh) and permitted (2.0 mm mesh) due to significant ‘Leaf Species x Mesh Size’ interactive effect identified by ANOVA (Table 3.4).
**Supplementary Table S3.5:** Coefficient (SE) for each fixed effect term, corresponding to best-fit models presented in Table 3.5.

- denotes term not present in the final best-fit model.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>(MAT &lt; 20 °C)</th>
<th>(MAT &gt; 20 °C)</th>
<th>Lowland Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Sites</td>
<td>Montane Forest &amp; Grassland</td>
<td>Lowland Forest Including Macrofauna Excluding Macrofauna</td>
</tr>
<tr>
<td>(n = 639)</td>
<td>(n = 384)</td>
<td>(n = 255)</td>
<td>(n = 127)</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>3.603 (0.35)</td>
<td>2.516 (0.28)</td>
<td>6.573 (0.68)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>0.077 (0.01)</td>
<td>0.077 (0.01)</td>
<td>-</td>
</tr>
<tr>
<td>Leaf Carbon (%)</td>
<td>-0.079 (0.01)</td>
<td>-0.054 (0.00)</td>
<td>-0.121 (0.01)</td>
</tr>
<tr>
<td>Leaf Nitrogen (%)</td>
<td>0.504 (0.04)</td>
<td>0.328 (0.03)</td>
<td>0.733 (0.07)</td>
</tr>
<tr>
<td>Leaf Cellulose (%)</td>
<td>0.030 (0.01)</td>
<td>0.031 (0.01)</td>
<td>-</td>
</tr>
<tr>
<td>Leaf Lignin (%)</td>
<td>-0.017 (0.00)</td>
<td>-0.012 (0.00)</td>
<td>-</td>
</tr>
<tr>
<td>Mesh Size</td>
<td>0.031 (0.03)</td>
<td>-</td>
<td>0.242 (0.05)</td>
</tr>
<tr>
<td>Site&lt;sub&gt;1500m&lt;/sub&gt;</td>
<td>-0.872 (0.16)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mesh Size x Site&lt;sub&gt;1500m&lt;/sub&gt;</td>
<td>0.211 (0.05)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Supplementary Table S3.6: Akaike information criterion (AIC) and likelihood ratio test statistic (LRT; in parentheses) following single term removal from best-fit models presented in Table 3.5. All LRT significant p < 0.05.

- denotes term not present in the final best-fit model.

<table>
<thead>
<tr>
<th></th>
<th>Lowland Forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Including Macrofauna</td>
</tr>
<tr>
<td></td>
<td>(n = 255)</td>
</tr>
<tr>
<td>All Sites</td>
<td>(n = 639)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>AIC for final best-fit model</th>
<th>Temperature (°C)</th>
<th>Leaf Carbon (%)</th>
<th>Leaf Nitrogen (%)</th>
<th>Leaf Cellulose (%)</th>
<th>Leaf Lignin (%)</th>
<th>Mesh Size</th>
<th>Site1500m</th>
<th>Mesh Size x Site1500m</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Sites</td>
<td>908</td>
<td>923 (17.8)</td>
<td>1028 (121)</td>
<td>1063 (157)</td>
<td>921 (15.6)</td>
<td>918 (11.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Montane Forest &amp; Grassland</td>
<td>91</td>
<td>101 (11.6)</td>
<td>195 (106)</td>
<td>213 (124)</td>
<td>121 (31.6)</td>
<td>100 (11.1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lowland Forest</td>
<td>526</td>
<td>586 (62.0)</td>
<td>521 (36.4)</td>
<td>612 (87.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>296 (6.4)</td>
<td>-</td>
</tr>
<tr>
<td>Including Macrofauna</td>
<td>288</td>
<td>321 (34.6)</td>
<td>321 (34.6)</td>
<td>347 (60.7)</td>
<td>-</td>
<td>-</td>
<td>302 (9.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Excluding Macrofauna</td>
<td>291</td>
<td>321 (32.5)</td>
<td>321 (32.5)</td>
<td>341 (52.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Supplementary Information Method S3.1;

Further description of model construction and validation

A full model was first constructed, including all possible fixed effects terms. To avoid co-linearity, where independent variables were highly correlated (identified by pairwise correlation analysis, where Spearman’s coefficient greater than 0.6 or less than -0.6, Supplementary Tables S3.2 and S3.3) only one term was included in the initial full model, chosen as the variable which gave the lowest AIC. In addition to a suite of climatic, soil and leaf metrics, ‘Leaf Origin’ and ‘Mesh Size’ were also included in the initial model. Leaf origin was defined as a binary term (0 or 1) where 1 represented that the leaf was sourced from the same elevation at which it was decomposing. An interaction term was also specified between mesh size and Site$_{1500m}$ (where Site$_{1500m}$ was a categorical variable defined as above or below 1500 m elevation), as macrofaunal abundance increases at lower elevations (Palin et al., 2011), and mesh size (permitting access by macrofauna) had no significant effect on $k$ at the sites above 1500 m (identified by ANOVA a priori; Table 3.4).

Full and reduced models (fitted by maximum likelihood), each excluding one fixed effect term in turn were compared by AIC, with a lower AIC representing a better model fit to the data for the given number of included parameters. The significance of individual fixed effects terms was determined using likelihood ratio tests, to assess the likelihood that a change in the AIC when a term was removed from the model occurred by chance (Zuur et al., 2009). Non-significant terms (those which did not improve the model-fit) were manually removed in a systematic, stepwise process to provide the best goodness of fit with the fewest factors. The resultant best-fit model (that with the lowest AIC) was the simplest and also ecologically reasonable in terms of scientific understanding of the system. The final model was fitted by restricted maximum likelihood and validated following guidance from Zuur et al. (2009), checking for normal distribution of model residuals and homogeneity of variance, assessed by visual inspection of residual plots.
Supplementary Figure S3.1; temperature adjusted $k$ at 15 °C ($k_{15}$), calculated according to approach described by Salinas et al. (2011), measured from senescent (Salinas et al. 2011) and fresh leaves (this study) for six species used in both studies (2.0 mm mesh, with macrofaunal access permitted by small holes/cuts dependent on study). Abbreviations of leaf species from Supplementary Table S3.1.
Chapter 4

Interactive non-climatic controls on leaf decomposition in tropical forests: leaf traits, soil fertility and the microbial community

Work presented in this chapter is based upon the manuscript intended for submission as; L. Hicks, J. Whitaker, A. T. Nottingham, D. S. Reay, P. Meir; Interactive non-climatic controls on leaf decomposition in tropical forests; (in preparation).

Contribution to work

LH led and implemented the study, with guidance from JW in experimental design. LH analysed the data and wrote the chapter, with all authors contributing to editing of the chapter.

Other acknowledgements

Professor S. C. Fry (University of Edinburgh) for analysis of plant material (carbon fractions), and the Analytical Chemistry Group, Centre for Ecology and Hydrology (Lancaster) for PLFA extractions.
Abstract

Decomposition is an important ecosystem process. Yet, while the effect of climate on decomposition is well understood, we lack knowledge of how fundamental non-climatic controls - including the chemical composition of plant material, soil fertility and microbial community composition - interact to determine rates of leaf decomposition, particularly across tropical ecosystems.

Temperature- and moisture-controlled microcosms were constructed to examine non-climatic drivers of decomposition, by comparing the rate of mass loss for four different species of leaves on contrasting soils from a 3400 m tropical elevation gradient. To assess the relationship between soil microbial community composition, leaf chemical traits, and resultant decomposition rates ($k$), microbial abundance and composition were determined by analysis of phospholipid fatty acids (PLFAs).

Leaf chemistry most strongly influenced rates of decomposition, with foliar cellulose and nitrogen (N) concentration together explaining 45 % of the observed variance in $k$. The chemical composition of leaves also modulated the effect of soil fertility on decomposition, with decomposition of leaves rich in cellulose faster on more fertile soils (those with greater concentrations of total N). However, despite marked differences in microbial abundance and community composition (relative abundance of different functional groups) among soils, these metrics were not associated with observed rates of decomposition.

Leaf chemical composition was the most important determinant of decomposition, in the absence of climatic variation, suggesting that modified foliar chemistry or altered leaf inputs as a consequence of global change could strongly influence future rates of decomposition across tropical ecosystems. The positive interaction between leaf cellulose and soil N concentration suggests that N enrichment, for example through atmospheric N deposition, may have a marked effect on decomposition in ecosystems characterised by leaves rich in accessible carbon, but a negligible influence where leaf-chemistry is unfavourable for decomposition and imposes an overriding constraint.
4.1 Introduction

Decomposition is a fundamental ecosystem process, determining rates of carbon (C) and nutrient cycling (Swift et al., 1979). Tropical soils make a globally important contribution to the terrestrial C cycle (Jobbágy and Jackson, 2000), therefore understanding the factors which regulate rates of decomposition in these ecosystems is of particular importance. Decomposition can be influenced by climate (temperature and precipitation) (Meentemeyer, 1978, Davidson and Janssens, 2006, Powers et al., 2009, Salinas et al., 2011), leaf chemical composition (Cornwell et al., 2008, Waring, 2012), soil fertility (Cleveland et al., 2011, Dale et al., 2015) and microbial community composition (Fanin et al., 2014, He et al., 2016). Yet the relative importance of these controls, and interactions among them remains poorly resolved. Although climate tends to exert a coarse-scale control on decomposition (Raich and Schlesinger, 1992, Lavelle et al., 1993, Liski et al., 2003), global-scale analyses have suggested a strong role for foliar traits (Cornwell et al., 2008, Zhang et al., 2008). Furthermore, a recent study across a latitudinal gradient of temperate forest demonstrated that our understanding of decomposition, typically based upon mean decomposition rates (local scale variance aggregated into mean values), may have masked and underestimated the importance of local-scale factors (such as soil fertility and biotic composition) in regulating rates of decomposition (Bradford et al., 2014). There is therefore a need to more closely examine how non-climatic factors (leaf chemical traits, soil fertility, and the microbial community) together regulate rates of decomposition, in order to more comprehensively predict responses to global change.

Leaf chemical composition can strongly influence the rate of decomposition (Aerts, 1997, Waring, 2012), with analysis of global datasets finding leaf traits to account for more variation compared to climate (Zhang et al., 2008), likely because of high inter-species based variation in foliar chemistry (Cornwell et al., 2008, Hättenschwiler et al., 2008). Decomposition is typically promoted by higher concentrations of foliar nutrients (Bakker et al., 2011, Waring, 2012) and labile C compounds, such as cellulose (Loranger et al., 2002, Hättenschwiler and Jørgensen, 2010), and constrained by high concentrations of more complex C compounds, such as lignin (Vaieretti et al., 2005, Freschet et al., 2012). However, the traits which best explain observed rates of decomposition are inconsistent among studies, limiting our
ability to predict how changes to the chemistry of plant inputs will influence future rates of decomposition in tropical systems.

Soil fertility can mediate rates of decomposition, as C and nutrient cycles are closely coupled by the nutrient demands of soil organisms which degrade organic matter (Finzi et al., 2011). As leaf stoichiometry tends to be wider and less tightly constrained compared to the stoichiometry of microbial biomass (Cleveland and Liptzin, 2007, Hättenschwiler et al., 2008), additional nutrients must often be acquired from soils to meet microbial metabolic demand. Low concentrations of essential soil nutrients (primarily nitrogen; N and phosphorus; P) can therefore constrain microbial activity and consequently rates of leaf turnover (Dent et al., 2006, Cleveland et al., 2011, Dale et al., 2015). However, few studies have investigated how soil fertility, varying widely across gradients of tropical lowland to montane forests and grasslands (Nottingham et al., 2015a) influences leaf-decomposition.

Microbial community composition may also regulate rates of decomposition, due to functional dissimilarity among different microbial groups (Strickland and Rousk, 2010). Fungi are typically able to degrade more chemically complex compounds compared to bacteria (Kjøller and Struwe, 2002, de Boer et al., 2005), with subtropical studies reporting faster rates of leaf mass-loss on soils with greater fungal abundance (García-Palacios et al., 2013b, He et al., 2016). The ratio of fungi:bacteria (F:B) was also an important determinant of C-substrate mineralisation in a study using soils from a tropical elevation gradient in Peru (Whitaker et al., 2014b). Further investigation is however required to assess the extent to which differences in microbial composition among tropical soils influences leaf breakdown.

While leaf traits, soil fertility and microbial composition can influence decomposition independently (consistent with the preceding discussion), the potential for interactions also exists. For example, a pan-tropical study using leaf-litter from two different plant species found that the type of leaf which decomposed fastest differed among sites (Powers et al., 2009), indicating that other factors, such as soil fertility and microbial composition, may have interacted with the decomposing leaves to determine the rate of decomposition. In another recent tropical study, fertilisation with N and P promoted decomposition of two species of leaves (which had higher
concentrations of soluble C) but had no effect on another species (with a lower concentration of soluble C) (Fanin et al., 2015c). In this case, the differential responses to fertilisation among different species of leaves was attributed to the relative availability of C, however further investigation is required to assess the generality of this interaction between leaf chemical composition and soil fertility.

Short and longer-term interactions between decomposing leaves and soil microorganisms have also been observed, with potential implications for resultant rates of decomposition. In one tropical study, leaf-mediated shifts in microbial composition were evident 98 days following amendment with different species of leaves (Fanin et al., 2014). These compositional shifts, in turn, influenced microbial mineralisation of a range of different C substrates, with similar responses observed by studies elsewhere in the tropics (Carney and Matson, 2005, Leff et al., 2012). Over longer time-scales, soil microorganisms may ‘adapt’ to more efficiently degrade plant material most frequently encountered, such that past resource history might constrain the ability of soil microorganisms to degrade new plant-inputs. The theory of home-field advantage (HFA) suggests that leaves decompose more rapidly on soil where the plant originates (home) relative to other soils (away) (Ayres et al., 2009). Whereas the theory of functional breadth (Van Der Heijden et al., 2008, Fanin et al., 2015a) states that where plant inputs are diverse and more recalcitrant, for example in forests, microorganisms will have a wide functional ability (functionally broad), but where plant inputs are more labile, for example in grasslands, microorganisms may be constrained in their ability to degrade more chemically complex leaves (functionally narrow). Evidence for these long-term leaf-microbe interactions in tropical ecosystems is very scarce; HFA has been supported by some tropical forest studies (de Toledo Castanho and de Oliveira, 2008, Vivanco and Austin, 2008), but not others (Gießelmann et al., 2011, Bachega et al., 2016), while the theory of microbial functional breadth, to date, has only been investigated comparing leaf decomposition among forests and grasslands in temperate regions (Keiser et al., 2011, Fanin et al., 2015a).

While improved understanding of interacting, non-climatic controls on decomposition is required in order to more comprehensively predict responses to global change, it remains difficult to ascertain the influence of soil fertility and
microbial composition across tropical elevation gradients *in-situ*, as many soil fertility and microbial metrics co-vary strongly with temperature (Sundqvist et al., 2013, Whitaker et al., 2014b, Nottingham et al., 2015a). Here, temperature- and moisture-controlled microcosms were constructed to examine non-climatic controls on leaf decomposition. Using soils collected from a 3400 m tropical elevation gradient in the Peruvian Andes, the influence of soil fertility and microbial community composition on the decomposition of four chemically contrasting species of leaves was assessed, to test the following hypotheses:

H1. Decomposition of more labile leaves (those with higher concentrations of foliar nutrients and cellulose, and lower concentrations of lignin) will be faster on more fertile soils (those with greater total N and P concentration), whereas for decomposition of nutrient-poor, lignin-rich leaves, inherent leaf recalcitrance will strongly constrain decomposition, irrespective of differences in soil fertility.

H2. Decomposition will be faster on soils with greater abundance of fungi relative to bacteria, due to the diverse capabilities of fungi in the degradation of simple to more chemically-complex substrates.

### 4.2 Materials and Methods

#### Study sites and field sampling

Soils were sampled in January 2014 from four sites along a 3400 m tropical elevation gradient located on the eastern flank of the Peruvian Andes (Table 4.1; Malhi et al., 2010), with soil fertility and microbial community composition varying among sites. The three highest elevation sites (3644 m, 3025 m, 1500 m) are located in or close to the Manu National Park, centred on the Kosñipata Valley, with the lowest elevation site (210 m) situated in the lowland Amazon basin, Tambopata Reserve, 240 km east of the main Kosñipata transect. Soil elevational trends are described by Whitaker et al. (2014b) and Nottingham et al. (2015a). Briefly, total C, N and P and their ratios (C:N, C:P, N:P) generally increase with elevation (Nottingham et al., 2015a). Soil microbial abundance and F:B ratio increase with elevation, while the ratio of gram positive:gram negative (GP:GN) bacteria decreases with elevation (Whitaker et al., 2014b).
Soil was collected to 10 cm depth, sampled from 5 subplots located at random around 1 ha permanent study plots at each elevation site, with soils from these 5 subplots used as 5 individual replicates. Surface soil (standardised 0-10 cm depth) was chosen to best represent the leaf-decomposition environment in the field. Sampled soils were stored in separate sealed plastic bags in a cool, dark room (Cusco, Peru) for a maximum of one week before transportation to the laboratory (Lancaster, UK). Upon arrival to the laboratory, soils were first gently homogenised by hand, removing large stones, woody debris, roots and un-decomposed leaf fragments, before being stored at 4 °C for a maximum of 4 weeks before further use. While cold storage can influence microbial function in tropical soils (Arnold et al., 2008), a previous study of forest and agricultural soils reported that total microbial abundance, microbial biomass C and enzyme activities were least affected by storage at 4 °C, compared to other storage methods (Lee et al., 2007). Moreover, in another study, although storage at 4 °C influenced microbial mineralisation of added C substrates, the change as a consequence of cold storage did not mask intrinsic differences in microbial community structure and function among different soils (Gonzales-Quinones et al., 2009). Here we compare relative treatment effects among soils. Therefore, while decomposition rates may not represent absolute in-situ values, the relative differences provide information on how different leaf tissue chemistries and variation in microbial community composition among different soils influence decomposition rates.

Leaves from four different tree species were selected, chosen as the two dominant species from two mid-elevation forest sites (Alchornea latifolia and Vismia sp. sourced from 1500 m, and Clusia alata and Weinmannia crassifolia sourced from 3025 m), with chemical traits varying among species (Table 4.3). Fresh leaves were collected directly from trees, to enable clear identification of species and to ensure that leaves were not partly decomposed prior to the start of the experiment, such that the large volume of leaves required for each species was as homogenous as possible. Leaves were air-dried and transported in paper bags, separately by species to the laboratory (Lancaster, UK).
Table 4.1; site characteristics (mean annual temperature; MAT, mean annual precipitation; MAP).

<table>
<thead>
<tr>
<th>Elevation (m asl)</th>
<th>MAT (°C)</th>
<th>MAP (mm yr⁻¹)</th>
<th>Soil Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata Lowland Rainforest</td>
<td>210</td>
<td>26.4</td>
<td>1900</td>
</tr>
<tr>
<td>San Pedro Lower Montane Forest</td>
<td>1500</td>
<td>17.4</td>
<td>5302</td>
</tr>
<tr>
<td>Wayqecha Upper Montane Forest</td>
<td>3025</td>
<td>11.1</td>
<td>1560</td>
</tr>
<tr>
<td>Tres Cruces Montane Grassland</td>
<td>3644</td>
<td>6.5</td>
<td>*760</td>
</tr>
</tbody>
</table>

*Measured at Acjanaco, 3450 m asl (Oliveras et al., 2014)

Soil analyses

Fresh soil from each subplot was used to measure pH in water, using a Hanna pH meter (soil: H₂O, 1:2.5 w: v, Hanna Instruments, USA), and gravimetric soil water content (soil dried at 105 °C for 24 hours to constant mass). Maximum water holding capacity (WHC) was measured using composite samples of soils from each elevation (composite of 5 subplots from each elevation) by saturating soils with deionised water before leaving to drain for 4 hours (with tops of funnels covered to prevent evaporation), and then drying at 105 °C to constant mass to calculate water content (Öhlinger and Kandeler, 1996). Dried and ground subsamples were analysed for total C and N content, using a TruSpec elemental analyser (LECO, USA), and total P content by sulphuric acid-hydrogen peroxide digestion followed by colorimetric analysis (Bran and Luebbe AutoAnalyser 3, Germany). Further information regarding quality control for soil analyses is provided in Appendix A.

Controlled microcosm experiment

To test the effect of both leaf traits and soil properties (fertility and microbial community composition) on decomposition, temperature- and moisture-controlled microcosms were constructed in petri dishes (Güsewell and Gessner, 2009). In total, 80 microcosms were constructed, each containing one of the four different species of
leaves, decomposing on soil from each elevation site, in a fully factorial design (4 leaf treatments x 4 soils x 5 replicates).

Leaves were cut into pieces no larger than 2 x 2 cm to homogenise surface area, and oven dried at 40 °C to constant mass, to standardise the initial mass whilst minimising potential changes to foliar chemistry. Soil moisture was adjusted to 80 % maximum WHC for each soil, by addition of sterile deionised water where necessary. Each microcosm contained 20.0 g fwt soil and 1.00 g dry leaf material, to measure mass loss over time. Exactly 1.00 g of leaf material was weighed, separately by species and placed directly onto the soil surface within each petri dish. Leaves were added in excess, relative to annual litterfall, so that sufficient material could be retrieved and measured at the end of the experiment and so that leaf-substrate supply would not constrain microbial activity over the duration of the study. 1.00 g of dry leaf material added to 20.0 g fwt soil was estimated as equivalent to approximately 30-35 times annual leaf litterfall at the forest sites across the gradient (Girardin et al., 2014), calculated on a leaf-soil mass basis (assuming decomposition occurs in the surface soil, 0-10 cm depth). Finally, leaves were moistened using sterile deionised water before microcosms were approximately 80% sealed with parafilm to limit moisture loss whilst allowing gas exchange, to prevent an anoxic environment developing due to production of carbon dioxide during decomposition.

Microcosms were incubated at 15.0 °C (representing the average MAT across the four sites) in the dark for 10 months. The total weight of each sealed microcosm at the start of incubation was recorded, and microcosms were weighed every 2-3 months over the course of the experiment to monitor moisture loss. Where mass loss exceeded 1.00 g of the starting weight, soil moisture was readjusted to the starting weight by addition of sterile deionised water, although this was rarely necessary. Microcosms were harvested in January 2015 (after 292 days incubation). Remaining leaf material was carefully cleaned of soil particles and was oven dried at 40 °C to constant mass before being weighed to calculate the rate of mass loss (see Data analysis).
Leaf analyses

Leaf mass per unit area (LMA; g cm\(^{-2}\)) was determined for a random subsample of 20 leaves for each species, using imageJ software (http://imagej.nih.gov/ij/) to measure the surface area of dried leaves. Oven dried (40 °C to constant mass) and ground homogenised subsamples for each species of leaf were analysed for total C and N content using a TruSpec elemental analyser (LECO, USA) and total P content by hydrogen peroxide-sulphuric acid digestion followed by colorimetric analysis (Bran Luebbe AutoAnalyser 3, Germany). Homogenous subsamples from each species were also analysed for cellulose, non-cellulosic polysaccharides and lignin content. Briefly, samples of ball-ground dry plant material were first washed in ethanol and then acetone to remove low molecular weight compounds and lipids, leaving insoluble polymers (polysaccharides, lignin, proteins, RNA, DNA). Lignin content was measured using the acetyl bromide method, described by Hatfield and Fukushima (2005). Non-cellulosic polysaccharides (TFA-hydrolysable, alcohol-insoluble polymers, including hemicelluloses, pectins, and any starch and fructans) were measured as monosaccharides obtained after hydrolysis in 2 M TFA at 120 °C for 1 hour by the p-hydroxybenzoic acid hydrazide (PAHBAH) assay for reducing sugars (Lever, 1972). Cellulose content was determined in the TFA residue by the anthrone/H\(_2\)SO\(_4\) assay method (Viles Jr and Silverman, 1949). Absorbance of all samples were measured using a Perkin-Elmer spectrophotometer (Cecil 8000 series). Further information regarding quality control for plant material analyses is provided in Appendix A.

Determining microbial community composition

At the end of the experiment, subsamples of soil were frozen at -80 °C and freeze-dried for analysis of phospholipid fatty acid (PLFA) biomarkers by a modified Bligh-Dyer extraction method (White et al., 1979). The number of soils extracted for PLFAs was rationalised due to the time and cost intensity of these measurements, such that only three selected at random out of five replicate samples were extracted (4 leaf treatments x 4 soils x 3 replicates). Phospholipids were extracted as part of the total lipid extract from 0.25-1.00 g freeze-dried soil (dependent on organic matter content). Gas
chromatography (GC) analysis of the fatty acid methyl esters (FAMEs) was carried out using an Agilent 6890 GC with fatty acids identified on an Agilent 5973 Mass Selective Detector. With every batch of 17 samples, one quality control standard and two blank solvent samples were also analysed. After normalisation to the internal standard (methyl nonadecanoate C19:0, Sigma Aldrich, UK), FAMEs in samples were quantified against a set of external standards containing all target compounds and the internal standard. Concentrations of all target compounds in samples were blank and recovery corrected and reported on a soil mass basis as PLFA nmol g\(^{-1}\) dwt soil. FAMEs were defined by standard nomenclature, as described by Frostegard et al. (1993).

To identify differences in microbial community composition among soils, and to test whether functional differences among different microbial groups affected rates of decomposition (H2), gram-positive bacteria (GP bacteria), gram-negative bacteria (GN bacteria) and fungi (F) were identified by specific biomarkers indicative of these different functional groups. Gram-positive bacteria were identified by the terminal and mid-chain branched fatty acids i-15:0, a-15:0, i-16:0, i-17:0, a-17:0 and GN bacteria by cyclopropyl saturated cy-17:0 and cy-19:0 and mono-saturated 16:1ω7 and 18:1ω7 fatty acids (Rinnan and Bååth, 2009). Saprotrophic fungi were identified by the 18:2ω6,9 marker (Wallander et al., 2013). Although 18:1ω9 is often also used as a fungal biomarker (Kaiser et al., 2010, De Deyn et al., 2011), this marker is also present in bacteria (Schoug et al., 2008), and while 18:1ω9 and 18:2ω6,9 tend to be closely correlated in forest soils, in agricultural soils the two markers were found to be poorly related (Frostegård et al., 2011). Here, although 18:2ω6,9 and 18:1ω9 were strongly correlated in forest soils, in the grassland soil the 18:1ω9 marker was high while the 18:2ω6,9 marker was low (Supplementary Figure S4.1). Hence only the 18:2ω6,9 marker was used as an indicator of fungal abundance in this study. The ratios of F: B and GP: GN bacteria were calculated to represent the relative abundance of these functional groups. Microbial abundance was calculated as the total concentration of all identified PLFAs (nmol g\(^{-1}\) dwt soil) in soils (14:0, 15:0, 16:0, 10Me16:0, 16:1ω5, 16:1ω9, br17:0, 10Me17:0, 17:1ω7, br18:0, 10Me18:0, 18:1ω9 and 18:1ω5 in addition to those previously listed as biomarkers for fungal and bacterial functional groups).
Data analysis

Decomposition coefficient ($k$)

Decomposition coefficients were estimated from the rate of mass loss over time, assuming negative exponential decay (Olson, 1963, Wider and Lang, 1982). The decomposition constant (expressed as $k$ in units of days$^{-1}$) was characterised by fitting a single exponential decay function (equation 4.1) using the initial leaf mass at time 0 ($M_0$), the leaf mass remaining at the end of the experiment ($M_t$) and the time since the start of the experiment (t) (Salinas et al., 2011). The decay function was fitted separately for each microcosm, resulting in 5 replicate values of $k$ for each soil/leaf treatment (total $n = 80$). Higher $k$-values represent faster mass loss (decomposition rate) compared to lower $k$-values.

$$M_t = M_0 e^{-kt}$$  \hspace{1cm} (Eq. 4.1)

Statistical analysis

Data were analysed using the R package 3.2.1. (R Core Team, 2015). To identify differences in microbial abundance and community composition in soils amended with different leaf treatments, the effect of ‘Leaf Treatment’ on total PLFA, F:B ratio and GP:GN bacteria ratio were tested by one-way analysis of variance (ANOVA), separately by soil. Main and interactive effects of ‘Soil’ and ‘Leaf Treatment’ on decomposition $k$ were also tested by two-way ANOVA. Prior to parametric analyses, response variables were tested for normality using the Shapiro-Wilk test and visual inspection of frequency distribution plots, and square-root transformed where necessary. Pairwise comparisons of significant effects were conducted by Tukey’s HSD post hoc tests, with significant differences identified where $p < 0.05$.

To identify which specific leaf traits and soil properties best explained the observed variation in $k$, a linear mixed effects (LME) modelling approach was employed (R package lme4; Bates et al., 2015). ‘Soil’ and ‘Leaf Species’ were included as random effects (random intercept model) and a suite of leaf trait, soil fertility and microbial composition metrics were specified as fixed effects (Table 4.2).
Two additional fixed-effect terms were also included in the initial model to evaluate the potential for HFA (Ayres et al., 2009) dependent on leaf origin, and microbial functional breadth (Fanin et al., 2015a), dependent on whether the soil originated from a forest or grassland (see Table 4.2 for further description). A full model (including all terms) was first constructed, followed by manual removal of non-significant terms in a systematic, stepwise process to achieve the best goodness of fit with the fewest factors. Akaike Information Criterion (AIC) was used to guide model selection, where a lower AIC represented a better model fit to the data for the given number of included parameters, with likelihood ratio tests used to evaluate the significance of individual fixed effects in improving the model-fit (Burnham and Anderson, 2002). The final best-fit model (that with the lowest AIC) was validated following guidance from Zuur et al. (2009), checking for normal distribution of model residuals and homogeneity of variance, by visual inspection of residual plots. After the final model was validated, the conditional (variance explained by fixed + random factors) and marginal $R^2$ (variance explained by fixed effects only) were calculated according to the approach described by Nakagawa and Schielzeth (2013). To evaluate the relative contribution of each fixed effect to the model, null models (each excluding one fixed effect term in turn) were compared to the final model, to estimate the % variance explained by each fixed effect term separately (by subtraction of marginal $R^2$ for full model - null model). This approach allowed identification of the fixed effects which explained most of the observed variance in the data, and therefore the relative importance of each parameter in describing $k$.

Finally, as the aim of this study was to also investigate the interactive effects of leaf traits and soil fertility on decomposition (H1), potential interactions among the main significant explanatory factors previously identified by the statistical model (leaf N, leaf cellulose, soil N), were tested. Each two-way interaction term was included in a more complex model, with comparison to the final simple-additive model to identify a significant interactive effect (by likelihood ratio tests). As such, a significant two-way interaction was identified for ‘Leaf Cellulose x Soil N’. In this case, the conditional and marginal $R^2$ was also calculated for this more complex model, with the variance explained by the interaction estimated by comparing the marginal $R^2$.
determined from the final simple model (without the interaction) to the marginal $R^2$
determined from the more complex model (with the interaction).

Table 4.2: Parameters included in the initial full model and final reduced best-fit model
(- denotes non-significant term $p > 0.05$, not present in the final model). Where independent
variables were strongly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6,
Supplementary Tables S4.1 and S4.2), only one term was included in the initial full model to
avoid co-linearity (chosen as the variable which gave the lowest AIC and explained the
greatest proportion of variance associated with $k$, highlighted in bold).

<table>
<thead>
<tr>
<th>Correlated Variables</th>
<th>Initial Full Model</th>
<th>Final Reduced Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed Effects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf N, Leaf P, Leaf C:N, Leaf N:P</td>
<td>Leaf N</td>
<td>Leaf N</td>
</tr>
<tr>
<td>Leaf Cellulose, Leaf C, Leaf Lignin, Leaf Lignin:N, LMA</td>
<td>Leaf Cellulose</td>
<td>Leaf Cellulose</td>
</tr>
<tr>
<td>Soil Total PLFA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soil F:B ratio</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soil GP:GN bacteria ratio</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Random Effects</strong></td>
<td>(1</td>
<td>Soil) + (1</td>
</tr>
</tbody>
</table>

*Leaf origin was included as a binary term (0 or 1), to evaluate the potential for HFA (Ayres et al., 2009), where 0 denoted leaves sourced from a different elevation to the soil, and 1 denoted leaves sourced from the same elevation as the soil.

*Forest-Grassland were included as categorical terms to evaluate the potential for differences in microbial functional breadth among forest and grassland soils (Fanin et al., 2015a).
4.3 Results

Leaf traits

Initial leaf traits are summarised in Table 4.3. The concentration of C in leaves varied between 45.7-49.1 % among the four species. *Weinmannia crassifolia* had the lowest concentration of both N and P (0.90 % and 0.070 % respectively) whilst *Alchornea latifolia* had the greatest concentration of both N and P (1.94 % and 0.118 % respectively). The concentration of cellulose varied between 12.7 % (*Vismia*) and 28.8 % (*Alchornea latifolia*) and lignin concentration varied between 20.4 % (*Clusia alata*) and 40.5 % (*Weinmannia crassifolia*). Leaf mass per area varied between 0.0110 g cm\(^{-2}\) (*Vismia*) and 0.0249 g cm\(^{-2}\) (*Clusia alata*).

Soil biotic and abiotic properties

Soil properties are summarised in Table 4.4. The concentration of total C in soils (0-10 cm depth) varied between 2.0 % (soil from lowland forest; Tambopata, 210 m) and 48.8 % (soil from upper montane forest; Wayqecha, 3025 m). Soil from Tambopata (210 m) had the lowest concentration of total N (0.2 %) and P (0.042 %), while soil from Wayqecha (3025 m) had the greatest concentrations of total N (2.3 %) and P (0.111 %). Soils from San Pedro (1500 m) and Tres Cruces (3644 m) both had the same concentration of total N (1.2 %), but the concentration of total P was greater in soil from San Pedro (0.104 %) and lower in soil from Tres Cruces (0.056 %). Soil pH did not vary markedly amongst soils from the three forest sites (3.8-4.0), but was higher (pH 4.8) for the grassland soil from 3644 m.

Microbial abundance and community composition, measured at the end of the experiment, varied distinctly among the four soils. Total PLFA varied almost 9-fold among soils, with the highest concentration of PLFAs (nmol g\(^{-1}\) dwt) in the soil from Wayqecha (3025 m) and the lowest in the soil from Tambopata (210 m). The ratio of F:B was greatest in the soil from Wayqecha (3025 m) and was considerably lower in the other three soils, whereas the ratio of GP:GN bacteria was greatest in the soil from Tambopata (210 m) and decreased with increasing elevation (Figure 4.1). Generally, microbial abundance (total PLFA) and community composition (F:B ratio and GP:GN
bacterial ratio) did not vary significantly among leaf treatments (p > 0.05; data not shown). However, in the soils from Wayqecha (3025 m) and San Pedro (1500 m) there were significant differences in the ratio of F:B and GP:GN bacteria respectively, dependent on amendment with different species of leaves (Figure 4.1). In the soil from Wayqecha, the abundance of fungi relative to bacteria was significantly greater in soils which had been amended with *Clusia alata* compared to those which had been amended with *Alchornea latifolia*. In the soil from San Pedro, the ratio of GP:GN bacteria was greater in soils amended with *Vismia* and *Weinmannia crassifolia* compared to those amended with *Alchornea latifolia* and *Clusia alata* (significant differences shown in Figure 4.1). A similar trend was also evident in the Tambopata soil (210 m), where the relative abundance of GP:GN bacteria was marginally higher in soils amended with *Weinmannia crassifolia* and lower in soils amended with *Clusia alata* (marginal difference, p = 0.09, identified by Tukey’s post-hoc test).
Table 4.3; initial foliar properties (% dry mass basis) for four species of leaves, measured from a homogenised sample for each species (homogenisation precluded estimate of variance for chemical traits). Leaf mass per area (LMA) measured from random sample of 20 leaves for each species, where data indicates mean (1SE).

<table>
<thead>
<tr>
<th>Leaf Species</th>
<th>Source</th>
<th>Elevation (m asl)</th>
<th>C</th>
<th>N</th>
<th>P</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>Cellulose</th>
<th>Non-cellulosic polysaccharides</th>
<th>Lignin</th>
<th>Lignin: N</th>
<th>LMA (g cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alchornea latifolia</em> (AL)</td>
<td>1500</td>
<td>45.9</td>
<td>1.94</td>
<td>0.118</td>
<td>23.7</td>
<td>389</td>
<td>16.4</td>
<td>28.8</td>
<td>12.0</td>
<td>31.6</td>
<td>16.3</td>
<td>0.0145 (0.0006)</td>
<td></td>
</tr>
<tr>
<td><em>Vismia</em> (VS)</td>
<td>1500</td>
<td>49.1</td>
<td>1.77</td>
<td>0.117</td>
<td>27.7</td>
<td>420</td>
<td>15.1</td>
<td>12.7</td>
<td>10.6</td>
<td>34.1</td>
<td>19.3</td>
<td>0.0110 (0.0007)</td>
<td></td>
</tr>
<tr>
<td><em>Clusia alata</em> (CA)</td>
<td>3025</td>
<td>47.2</td>
<td>1.37</td>
<td>0.117</td>
<td>34.5</td>
<td>403</td>
<td>11.7</td>
<td>22.3</td>
<td>10.2</td>
<td>20.4</td>
<td>14.9</td>
<td>0.0249 (0.0012)</td>
<td></td>
</tr>
<tr>
<td><em>Weinmannia crassifolia</em> (WC)</td>
<td>3025</td>
<td>46.2</td>
<td>0.90</td>
<td>0.070</td>
<td>51.7</td>
<td>660</td>
<td>12.8</td>
<td>18.1</td>
<td>12.2</td>
<td>40.5</td>
<td>45.0</td>
<td>0.0205 (0.0017)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4; initial soil characteristics (0-10 cm depth, % dwt). Data represent mean (1SE), n = 5 from 5 individual replicates from each elevation site.

<table>
<thead>
<tr>
<th>Elevation (m asl)</th>
<th>Total C (% dwt)</th>
<th>Total N (% dwt)</th>
<th>Total P (% dwt)</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>pH</th>
<th>80% WHC (g H₂O g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata</td>
<td>210</td>
<td>2.0 (0.5)</td>
<td>0.2 (0.02)</td>
<td>0.042 (0.006)</td>
<td>10.1 (0.7)</td>
<td>51 (8)</td>
<td>4.9 (0.4)</td>
<td>3.8 (0.1)</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>15.8 (2.4)</td>
<td>1.2 (0.12)</td>
<td>0.104 (0.006)</td>
<td>13.1 (0.8)</td>
<td>151 (17)</td>
<td>11.3 (0.7)</td>
<td>4.0 (0.1)</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>48.8 (0.8)</td>
<td>2.3 (0.05)</td>
<td>0.111 (0.003)</td>
<td>21.7 (0.5)</td>
<td>441 (15)</td>
<td>20.3 (0.4)</td>
<td>4.0 (0.1)</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>14.6 (1.6)</td>
<td>1.2 (0.17)</td>
<td>0.056 (0.005)</td>
<td>12.0 (1.1)</td>
<td>266 (31)</td>
<td>22.9 (3.6)</td>
<td>4.8 (0.1)</td>
</tr>
</tbody>
</table>
Figure 4.1; microbial abundance (total PLFA; nmol g⁻¹ dwt) and community composition (fungi:bacteria ratio; F:B and gram positive:gram negative bacteria ratio; GP:GN) measured in four soils c. 300 days after amendment with four different species of leaves (abbreviations for leaf species from Table 4.3, * denotes leaves sourced from same elevation as soil). Data represent mean ± 1SE (n = 3); significant differences (p < 0.05) indicated by different letters, marginal differences (p < 0.10) indicated by different letters in parentheses, both identified by Tukey’s HSD tests.
Effect of leaf identity and soil origin on rates of decomposition

Mean decomposition rate constants ($k$) varied more than 2-fold among leaf treatments and 1.5-fold among soils (Table 4.5), with 60.4% of the total observed variance in $k$ explained by leaf treatments and 12.4% of variance explained by soil (Table 4.6). A significant ‘Soil x Leaf Treatment’ interactive effect on $k$ was also identified by ANOVA (Table 4.6), whereby differences in $k$ among the four species of leaves depended on the soil on which it was decomposing (Figure 4.2). On soils from the three higher-elevation montane sites (1500 m, 3025 m and 3644 m), relative rates of decomposition among the four species of leaves were consistent and varied in the order *Alchornea latifolia* > *Clusia alata* > *Weinmannia crassifolia* > *Vismia*, whereas on soil from the lowland forest site (210 m) decomposition varied in the order *Alchornea latifolia* > *Vismia* > *Weinmannia crassifolia* > *Clusia alata* (although not all differences among species were significant; see Figure 4.2). Decomposition of the two faster decomposing species, *Alchornea latifolia* and *Clusia alata*, were significantly faster on soils from the mid-elevation montane forests (1500 m and 3025 m) compared to $k$ measured on soils from the lowland forest (Tambopata; 210 m) and montane grassland (Tres Cruces; 3644 m). Decomposition of *Vismia* was significantly faster on the soil from the lowland forest (Tambopata; 210 m), with no significant difference in $k$ among the three montane soils. Decomposition of *Weinmannia crassifolia* was consistently slow, and did not vary significantly amongst the four soils.

**Table 4.5;** decomposition rate constants ($k \times 10^{-3}$ day$^{-1}$) where data represents mean (1SE), determined from leaf mass loss after c. 300 days under controlled temperature (15 °C) and moisture (80% maximum water holding capacity) conditions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tambopata (210 m)</th>
<th>San Pedro (1500 m)</th>
<th>Wayqecha (3025 m)</th>
<th>Tres Cruces (3644 m)</th>
<th>Mean (across soils)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Weinmannia crassifolia</em></td>
<td>1.29 (0.10)</td>
<td>1.30 (0.11)</td>
<td>1.26 (0.08)</td>
<td>0.97 (0.09)</td>
<td>1.21 (0.08)</td>
</tr>
<tr>
<td><em>Clusia alata</em></td>
<td>1.17 (0.13)</td>
<td>1.62 (0.09)</td>
<td>1.77 (0.05)</td>
<td>1.10 (0.08)</td>
<td>1.42 (0.17)</td>
</tr>
<tr>
<td><em>Vismia</em></td>
<td>1.47 (0.16)</td>
<td>1.12 (0.03)</td>
<td>1.13 (0.11)</td>
<td>0.86 (0.04)</td>
<td>1.14 (0.12)</td>
</tr>
<tr>
<td><em>Alchornea latifolia</em></td>
<td>1.62 (0.05)</td>
<td>3.19 (0.17)</td>
<td>3.64 (0.22)</td>
<td>2.25 (0.29)</td>
<td>2.67 (0.46)</td>
</tr>
<tr>
<td>Mean (across leaf treatments)</td>
<td><strong>1.39 (0.04)</strong></td>
<td><strong>1.81 (0.21)</strong></td>
<td><strong>1.95 (0.26)</strong></td>
<td><strong>1.29 (0.45)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.6: main and interactive effects of ‘Soil’ and ‘Leaf Treatment’ on decomposition $k$, assessed by two-way ANOVA. Significant effect identified where $p < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>Sum of Squares (SS)</th>
<th>% SS</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil (S)</td>
<td>3</td>
<td>0.80</td>
<td>12.4</td>
<td>22.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leaf Treatment (L)</td>
<td>3</td>
<td>3.90</td>
<td>60.4</td>
<td>112.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x L</td>
<td>9</td>
<td>1.02</td>
<td>15.8</td>
<td>9.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>64</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.2: decomposition $k$ ($\times 10^{-3} \text{ d}^{-1}$) for four different species of leaves decomposing on four different soils (Tambopata; 210 m, San Pedro; 1500 m, Wayqecha; 3025 m, Tres Cruces; 3644 m). Decomposition $k$ determined after c. 300 days incubation under controlled climatic conditions (15 °C and 80 % maximum water holding capacity). Bars represent mean decomposition rate constant, $k \pm 1SE (n = 5)$. Different letters indicate significant differences ($p < 0.05$) assessed by Tukey’s HSD post hoc tests, following ANOVA (presented in Table 4.6).
Effect of leaf traits, soil fertility and microbial composition

Statistical modelling revealed that decomposition $k$ was explained foremost by leaf chemical traits. Greater concentrations of cellulose and N in leaves enhanced the rate of decomposition, with these two traits together explaining 45.3% of the observed variance in $k$ (Table 4.7a). Soil fertility (soil N concentration) had a smaller, secondary influence on $k$, the effect of which was dependent on the initial concentration of cellulose in the decomposing leaves (significant ‘Leaf Cellulose x Soil N’ interaction; Table 4.7b). As the concentration of cellulose in leaves increased, the degree to which soil N concentration influenced $k$ increased (steeper slope-response gradient; Figure 4.3). Where cellulose concentration in leaves was lower, soil N concentration had a lesser effect (more shallow slope-response gradient) tending towards a small negative effect of soil N on $k$ for decomposition of leaves with very low (< 15%) concentrations of cellulose. Microbial abundance (total PLFA) and community composition (GP:GN bacteria, F:B ratio) did not improve the model fit ($p > 0.05$) and hence these terms were not present in the final model (Table 4.2). However, the ‘Forest-Grassland’ term was significant and explained 8.1% variance in $k$ (Table 4.7a), indicating that decomposition of leaves (sourced from trees) on the grassland soil was slower relative to decomposition of leaves on the forest soils.

![Figure 4.3](image-url)

**Figure 4.3:** Interactive ‘Leaf cellulose x Soil N’ effect on decomposition $k$ (x 10$^{-3}$ day$^{-1}$), whereby the relationship between $k$ and soil N concentration (% dwt) was dependent on the initial concentration of cellulose (% dwt) in decomposing leaves. Relationship determined from model coefficients provided in Table 4.7b.
Table 4.7; estimated variance (var. %) explained by each term from best-fit linear mixed effects models (a) simple additive model, and (b) more complex model (including leaf x soil interaction) to identify specific controls on leaf decomposition (square root transformed k). Fixed effect parameter estimates (model coefficient), standard errors (SE), and random effect variance and standard deviation (SD).

Microbial abundance (total PLFA), F:B ratio, GP:GN bacterial ratio and leaf origin removed from model as non-significant terms (p > 0.05; Table 4.2).

<table>
<thead>
<tr>
<th>a) Simple Model</th>
<th>Model Coefficient</th>
<th>SE</th>
<th>Estimated var. explained by each term (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Intercept)</td>
<td>0.259</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Leaf Nitrogen (%)</td>
<td>0.200</td>
<td>0.13</td>
<td>6.9</td>
</tr>
<tr>
<td>Leaf Cellulose (%)</td>
<td>0.030</td>
<td>0.01</td>
<td>38.4</td>
</tr>
<tr>
<td>Soil Nitrogen (%)</td>
<td>0.087</td>
<td>0.03</td>
<td>6.9</td>
</tr>
<tr>
<td>Grassland</td>
<td>-0.171</td>
<td>0.05</td>
<td>8.1</td>
</tr>
<tr>
<td>Random Effects</td>
<td>Variance</td>
<td>SD</td>
<td>Full Model</td>
</tr>
<tr>
<td>Soil</td>
<td>0.0005</td>
<td>0.022</td>
<td>Conditional R² (% var. exp.) 73.5</td>
</tr>
<tr>
<td>Leaf Species</td>
<td>0.0095</td>
<td>0.098</td>
<td>Marginal R² (% var. exp.) 62.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Random Effects (% var. exp.) 11.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b) Complex Model</th>
<th>Model Coefficient</th>
<th>SE</th>
<th>Estimated var. explained by each term (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Intercept)</td>
<td>0.850</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Leaf Nitrogen (%)</td>
<td>0.200</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Leaf Cellulose (%)</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Soil Nitrogen (%)</td>
<td>-0.399</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Grassland</td>
<td>-0.171</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Estimated var. explained by interaction term (%)</td>
<td>12.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf Cellulose x Soil N</td>
<td>0.024</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Random Effects | Variance | SD  | Full Model |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.0010</td>
<td>0.031</td>
<td>Conditional R² (% var. exp.) 86.9</td>
</tr>
<tr>
<td>Leaf Species</td>
<td>0.0101</td>
<td>0.101</td>
<td>Marginal R² (% var. exp.) 74.6</td>
</tr>
<tr>
<td>Random Effects</td>
<td>0.0010</td>
<td>0.031</td>
<td>Random Effects (% var. exp.) 12.3</td>
</tr>
</tbody>
</table>

Estimated variance (var.) explained (exp.) by each term in the model (%), calculated by comparing marginal R² from the full refined model and null models (with each term removed). Conditional R² (variance explained by fixed + random effects) and marginal R² (variance explained by fixed effects only) were determined by the approach described by Nakagawa and Schielzeth (2013), and expressed as % variance explained.

AIC, likelihood ratio test statistic and associated p-values for individual fixed effects following single term removal provided in Supplementary Table S4.3.
4.4 Discussion

Under controlled climatic conditions, decomposition \((k)\) was strongly regulated by leaf chemical traits, with foliar cellulose and N concentration together explaining nearly half of the total observed variance in \(k\) (Table 4.7). Soil fertility (soil N concentration; Table 4.7) had a smaller, secondary influence on \(k\), the effect of which was dependent on the initial concentration of cellulose in the decomposing leaves (Figure 4.3), in support of H1. However, despite marked differences in microbial community composition among soils (Figure 4.1), the relative abundance of different functional groups (F:B and GP:GN bacteria ratio) were not associated with the observed rates of decomposition, contrary to H2. Instead, leaves decomposed more slowly on the grassland soil, relative to the forest soils, with differences in microbial functional breadth providing one potential explanation for this response.

Leaf trait-soil fertility interaction

Leaf chemical traits most strongly influenced decomposition rates under controlled temperature and moisture conditions, with greater concentrations of foliar cellulose and N associated with faster rates of decomposition (Table 4.7a). These trends were consistent with findings from an in-situ decomposition study along the same elevation gradient in Peru (Chapter 3), as well other tropical studies where faster rates of decomposition were associated with greater concentrations of cellulose (Loranger et al., 2002, Bachega et al., 2016) and foliar nutrients (Bakker et al., 2011, Waring, 2012). The chemical composition of leaves also appeared to modulate the influence of soil fertility on \(k\), as the extent to which soil N concentration regulated decomposition was dependent on the initial concentration of cellulose in the decomposing leaves (Figure 4.3). This relationship was akin to leaf-soil interactions identified by previous tropical decomposition studies. In one lowland forest study, the availability of labile C (dissolved organic C; DOC) in leaves modulated the effect of NP- fertilisation on decomposition, where fertilisation increased the rate of mass loss for leaves rich in DOC but did not influence mass loss of another DOC-poor species (Fanin et al., 2015c). In this case, C-limitation (low DOC) was thought to have strongly constrained decomposition, irrespective of differences in nutrient availability. Likewise, in a
tropical montane forest study, N-fertilisation promoted mass loss of lignin-poor leaves, whereas decomposition of lignin-rich leaves was most strongly constrained by inherent leaf-recalcitrance (Hobbie, 2000). Cellulose is a readily degradable source of C (energy) for decomposer microorganisms (Kalbitz et al., 2006). Given this, in the study presented here, when the concentration of cellulose in decomposing leaves was lower, microorganisms may have become constrained by the lower concentration of accessible-C (Hättenschwiler et al., 2011), irrespective of differences in availability of other essential soil nutrients (N and P). In contrast, when the availability of cellulose was greater and provided microorganisms with sufficient C to meet their energy demand, the availability of N in soils appeared to further modulate rates of decomposition. This study therefore provides further support that the influence of soil fertility on decomposition is dependent on the C-chemistry of decomposing leaves (Hobbie, 2000, Fanin et al., 2015c), whereby low availability of accessible-C in decomposing leaves can lead to energy starvation for decomposer microorganisms which imposes an overriding constraint, irrespective of differences in soil fertility.

**No clear effect of microbial community composition on decomposition**

Despite predicting that decomposition would be faster in soils with greater fungal abundance (H2), as observed by previous subtropical studies (García-Palacios et al., 2013b, He et al., 2016), metrics of microbial community composition did not improve the model-fit here (p > 0.05, Table 4.2). It is often assumed that fungi are more efficient decomposer organisms compared to bacteria, due to their diverse capabilities in degrading a range of substrates, including more chemically complex compounds such as lignin (Kjøller and Struwe, 2002, de Boer et al., 2005). However, several studies have challenged the generality of this theory. In one study using temperate soils, rates of decomposition in bacterially-dominated soils were equal or faster compared to those dominated by fungi (Güsewell and Gessner, 2009). Moreover, higher resolution analyses of microbial functional diversity have demonstrated significant differences in leaf-degrading ability among fungal taxa (Osono and Takeda, 2002, Strickland et al., 2009b), with some fungi targeting more labile sources of C (Hanson et al., 2008, van der Wal et al., 2013). As such, while positive relationships between soil F:B ratio and
rates of leaf decomposition have been previously reported (García-Palacios et al., 2013b, He et al., 2016), the relative abundance of these functional groups may be too coarse an indicator (not accounting for functional differences among fungal taxa) which may explain why the ratio of F:B was not a significant predictor of \( k \) here. Higher resolution analyses are therefore required, to more comprehensively assess the role of the microbial community in regulating rates of decomposition.

**Short and longer term leaf-microbe interactions**

Significant differences in microbial community composition were evident in some soils which had been amended with chemically-contrasting leaves (Figure 4.1), suggesting that short term (< 300 days) leaf-mediated shifts in microbial composition had occurred. Whilst it is possible that new microorganisms were introduced to microcosms with the leaves, there was no systematic change to microbial composition (based on the relative abundance of microbial groups) evident across all soils. This indicates that the small changes observed in some cases were a result of compositional shifts in the original soil microbial community rather than as a result of new microorganisms introduced to the microcosms. In the case of the soils from San Pedro (1500 m) and Tambopata (210 m), the ratio of GP:GN bacteria were lower in soils which had been amended with cellulose-rich leaves (*Alchornea latifolia* and *Clusia alata*) relative to soils which had been amended with cellulose-poor leaves (*Vismia* and *Weinmannia crassifolia*; Table 4.3 and Figure 4.1). This trend is consistent with our understanding of GP and GN bacteria, whereby GN bacteria are associated with high growth rates under resource-rich conditions (Kramer and Gleixner, 2008, Fanin et al., 2014) whereas GP bacteria dominate when the quality of available C is poorer (Fierer et al., 2007). Although microbial community composition had no significant influence on observed rates of leaf-decomposition here, a previous study of the same Peruvian gradient found the ratio of GP:GN bacteria to be an important determinant of soil-respiration responses following the amendment of soils with a range of different C substrates (Whitaker et al., 2014b), indicating that leaf-mediated community shifts could influence future soil C dynamics.
From the statistical model, decomposition of leaves (sourced from trees) on the grassland soil was slower, relative to decomposition on the forest soils (Table 4.7a). This finding was comparable to that reported from temperate studies (Keiser et al., 2011, Fanin et al., 2015a), whereby slower rates of decomposition on grassland soils compared to forest soils was attributed to differences in microbial functional breadth, influenced by long-term resource history. The functional breadth hypothesis states that microorganisms in soils where the supply of plant material is relatively labile, for example grasslands, are less able to degrade more chemically complex plant residues, for example from forests (Van Der Heijden et al., 2008). Along the studied gradient, dominant species of vegetation at the grassland site (Calamagrostis grass and moss) had generally higher concentrations of cellulose and lower concentrations of lignin, relative to concentrations measured in dominant species of leaves from lowland and montane forest sites (Chapter 3). Long-term resource history therefore provides one potential explanation for slow decomposition on the grassland soil, as microbial adaptation to energy-rich and less chemically complex plant material in the grassland may have resulted in a functionally narrow microbial community (relative to the forest soils), constraining degradation of the added leaf treatments here. Alternatively, other differences among the grassland and forest soils may have contributed to this effect.

Soil pH was considerably higher in the grassland soil (pH 4.8) compared to the forest soils (pH 3.8-4.0; Table 4.4). However, decomposition is typically constrained on more acidic soils (Motavalli et al., 1995), opposite to the trend observed here. Low availability of inorganic N and P in these grassland soils (A. Nottingham, personal communication), despite moderate concentrations of total N and P (Table 4.4) may have also constrained the rate of decomposition. However, soil microbes are able to access organic nutrients by the synthesis of extracellular enzymes (Allison and Vitousek, 2005, Caldwell, 2005), such that total N and P is a good indicator of the total pool of nutrients available to microorganisms. While differences in microbial functional breadth among forest and grassland soil-microorganisms provides one plausible explanation for slower rate of decomposition on the grassland soil relative to forest soils, this requires further investigation.
Global change and future rates of decomposition

In this temperature- and soil moisture-controlled study, leaf chemical composition (cellulose and N concentration) was the dominant control on decomposition, with a secondary influence of soil fertility (Table 4.7). The influence of leaf composition also prevailed over soil fertility (55 % and 6.5 % of variance explained by leaf-species and soil C:N ratio respectively) in a decomposition study across a soil fertility gradient in lowland Panama (Dale et al., 2015). These results therefore suggest that modified leaf inputs, as a consequence of global change, have the potential to strongly influence future rates of decomposition across the tropics. Increasing atmospheric nutrient deposition (Okin et al., 2004, Hietz et al., 2011), observed even in remote Andean forests (Fabian et al., 2005, Boy et al., 2008), will increase foliar nutrient concentrations (Homeier et al., 2012) and consequently increase leaf decomposability. Warming-driven changes to tree species distributions (Feeley et al., 2011, Duque et al., 2015) could also markedly alter the chemical composition of leaf inputs to soil, potentially increasing the decomposability of leaves as species from lower elevations along the studied Peruvian gradient generally have higher concentrations of foliar N (van de Weg et al., 2009). While the potential influence of global change on foliar cellulose content is less evident, one study observed that tropical grasses grown under warmer conditions had lower concentrations of cellulose (Ford et al., 1979), which could constrain rates of decomposition. However, this requires further investigation. The apparent interplay between leaf cellulose concentration and soil fertility suggests that future N deposition will accelerate decomposition in ecosystems characterised by leaves rich in accessible C, but will have a relatively minor influence in ecosystems characterised by leaves low in accessible C, for which inherent leaf recalcitrance will continue to impose the overriding control.

Finally, if long-term resource history constrained the ability of grassland soil microorganisms to degrade leaves from contrasting forest environments, then this could have important implications for future rates of decomposition in these high-elevation tropical systems. Although the forest-grassland ecotone transition in the Peruvian Andes has remained relatively stable over the last four decades, there is some evidence for warming-driven advancement of the treeline upslope into the grassland, especially in areas protected from grazing (Harsch et al., 2009, Lutz et al., 2013).
Upslope migration of the treeline would result in markedly altered leaf inputs to the soil, slow decomposition of which, as indicated by findings presented here, would result in reduced rates of C and nutrient cycling in these soils. Moreover, no change in microbial abundance nor community composition was evident in these grassland soil microcosms c. 300 days after amendment with chemically-contrasting species of leaves (Figure 4.1), suggesting that microbial compositional shifts in response to new plant material did not readily occur. One temperate-region study found that initial functional differences between grassland and forest soil microbial communities were sustained with time (Keiser et al., 2011), however, longer-term evaluation of how grassland microorganisms respond to changing leaf inputs merits further investigation, to directly test this theory and to better assess future rates of decomposition in montane grassland systems.

4.5 Conclusion

Leaf chemical composition most strongly regulated rates of decomposition under controlled temperature and soil moisture conditions. The chemistry of leaves also modulated the effect of soil fertility on decomposition, with the decomposition of leaves rich in cellulose faster on more fertile soils (greater concentration of N). Microbial community composition was not however associated with observed rates of decomposition. Instead, differences in microbial functional breadth among forest and grassland soils may have contributed to the slower rate of decomposition on the grassland soil, relative to forest soils, although this requires further investigation. Overall, findings from this controlled study show that the chemical composition of leaves is important in regulating rates of decomposition, independently of climate. Therefore, while temperature may exert a coarse-scale control on decomposition (Lavelle et al., 1993, Bradford et al., 2016b), and climatic warming will likely directly accelerate rates of decomposition across the tropics (Salinas et al., 2011, Bothwell et al., 2014), we must also consider how parallel changes to the chemistry of leaf inputs to soil, whether in response to elevated temperature or through climate-related shifts in plant community composition, will also influence future rates of C cycling across tropical ecosystems.
Supplementary Figure S4.1; abundance of 18:1ω9 and 18:2ω6,9 markers measured in four soils following c. 300 days incubation with four different species of leaves.
**Supplementary Table S4.1;** matrix showing pairwise correlations between leaf chemical traits \((n = 4)\). Data represent Spearman’s rank correlation coefficients for each pair of variables. Where chemical traits are highly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6, highlighted in bold) only one term included in the initial full model to avoid co-linearity, chosen as the term which gave the lowest AIC.

*denotes significant relationship \(p < 0.05\)

<table>
<thead>
<tr>
<th>LEAF TRAITS</th>
<th>N (%)</th>
<th>P (%)</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>Cellulose (%)</th>
<th>Lignin (%)</th>
<th>Lignin:N (%)</th>
<th>LMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (%)</td>
<td>-0.20</td>
<td>-0.32</td>
<td>0.20</td>
<td>0.40</td>
<td>-0.40</td>
<td>-0.80</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.32</td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
<td>0.94</td>
<td>-1.00*</td>
<td>-0.80</td>
<td>0.80</td>
<td>0.40</td>
<td>-0.40</td>
<td>-0.40</td>
</tr>
<tr>
<td>P (%)</td>
<td></td>
<td></td>
<td>-0.94</td>
<td>-0.94</td>
<td>0.63</td>
<td>0.63</td>
<td>-0.63</td>
<td>-0.63</td>
<td>0.00</td>
</tr>
<tr>
<td>C:N</td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
<td>-0.80</td>
<td>-0.40</td>
<td>0.40</td>
<td>0.40</td>
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</tr>
<tr>
<td>C:P</td>
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<td></td>
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<td>-0.40</td>
<td>0.80</td>
<td>0.80</td>
<td>-0.32</td>
</tr>
<tr>
<td>N:P</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cellulose (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Lignin (%)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin:N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00*</td>
<td>-0.63</td>
</tr>
</tbody>
</table>

*denotes significant relationship \(p < 0.05\)
**Supplementary Table S4.2:** matrix showing pairwise correlations between soil fertility (n = 20) and microbial community (n = 48) metrics. Data represent Spearman’s rank correlation coefficients for each pair of variables. Where soil properties are highly correlated (Spearman’s coefficient greater than 0.6 or less than -0.6, highlighted in bold) only one term included in the initial full model to avoid co-linearity, chosen as the term which gave the lowest AIC.

*denotes significant relationship p < 0.05
**Supplementary Table S4.3;** Akaike information Criterion (AIC; AIC for full best-fit model in parentheses), likelihood ratio test statistic (LRT) and associated p value, following single term removal from best fit models of leaf-decomposition ($k$); corresponding to (a) simple additive model, and (b) more complex model (including leaf x soil interaction) presented in Table 4.7.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>AIC</th>
<th>LRT</th>
<th>p value</th>
<th>Fixed Effects</th>
<th>AIC</th>
<th>LRT</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Simple Model</td>
<td></td>
<td></td>
<td></td>
<td>b) Complex Model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf Nitrogen (%)</td>
<td>-51.4</td>
<td>4.6</td>
<td>0.030</td>
<td>Leaf Nitrogen (%)</td>
<td>-54.0</td>
<td>52.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leaf Cellulose (%)</td>
<td>-46.2</td>
<td>9.8</td>
<td>0.002</td>
<td>Leaf Cellulose (%)</td>
<td>-48.0</td>
<td>8.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Soil Nitrogen (%)</td>
<td>-48.0</td>
<td>8.0</td>
<td>0.005</td>
<td>Soil Nitrogen (%)</td>
<td>-47.8</td>
<td>8.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Grassland</td>
<td>-47.8</td>
<td>8.3</td>
<td>0.004</td>
<td>Grassland</td>
<td>-54.0</td>
<td>52.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Leaf Cellulose x Soil N</td>
<td>-54.0</td>
<td>52.6</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5

Root-soil interactions stimulate soil carbon loss and increase the apparent sensitivity of soil respiration to climatic warming

Work presented in this chapter is based upon the manuscript intended for submission as; L. Hicks, A. T. Nottingham, J. Whitaker, D. S. Reay, A. J. Q. Ccahuana, P. Meir; Root-soil interactions stimulate soil carbon loss and increase the apparent sensitivity of soil respiration to climatic warming; Global Change Biology (in preparation).

Contribution to work
LH led and implemented the study, with assistance from AN and PM regarding the experimental design, and AC in the field. LH analysed the data and wrote the chapter, with all authors contributing to editing of the chapter.

Other acknowledgements
Rosa Castro and Jenny Michel for their many hours of help extracting and scanning roots.
Abstract

Tropical soils are a globally important store of terrestrial carbon (C), with soil respiration a major source of atmospheric carbon dioxide (CO₂). Roots play a potentially important role in the soil C cycle, yet our understanding of how root activity and root-soil interactions influence soil C dynamics in tropical systems remains limited. Improved understanding of the role of roots in modulating soil C dynamics will be particularly important in predicting responses to global change, as climatic warming has the potential to increase root productivity and alter the species composition of root communities across elevation and latitudinal gradients.

Here we investigate how warming-related changes in the composition of root communities and root productivity will affect soil C dynamics. To do so, we conducted a soil translocation experiment, whereby intact soil cores were reciprocally translocated among four sites along a 3400 m elevation gradient, spanning 20 °C (6.5-26.4 °C) in mean annual temperature. Soil cores were divided into two treatments: cores where root growth was permitted (ingrowth cores; root community-soil interaction), and cores where root growth was excluded (controls). These treatments enabled partitioning of soil CO₂ effluxes into heterotrophic (Rh) and root-rhizosphere (Rr) derived components, to examine how root-soil interactions influenced soil C dynamics (CO₂ effluxes, total soil C content) and whether the presence of roots influenced the apparent temperature sensitivity ($Q_{10}$) of soil respiration to climatic warming.

Roots made a substantial contribution to the total soil CO₂ efflux across the elevation gradient, with greater fine root biomass (roots < 2 mm diameter, within ingrowth cores) strongly associated with greater CO₂ effluxes derived from roots and the rhizosphere (Rr) at all sites. Following translocation of soil cores downslope to the lowland forest site (210 m; warming treatment), Rr-CO₂ effluxes from lower montane forest and montane grassland soils were elevated, compared to Rr-CO₂ effluxes measured from the native lowland forest soil. This difference remained significant when Rr-CO₂ was normalised per g fine root biomass, indicating that the high Rr-CO₂ effluxes were not simply a result of greater root proliferation within these lower montane forest and montane grassland soils. Indeed, following 24 months at the
lowland forest site, there was no significant difference in fine root biomass among the
different soils. Instead, the high Rr-CO$_2$ effluxes corresponded to substantial depletion
of total soil C and N from these ingrowth cores, suggesting that root-soil interactions
had stimulated this loss. The observed, apparent temperature sensitivity of soil
respiration varied among soils, but was consistently greater when roots were permitted
(Rh + Rr; $Q_{10}$ = 1.8-4.6) compared to the apparent temperature sensitivity of
heterotrophic soil respiration alone (Rh; $Q_{10}$ = 1.5-2.4), further highlighting the
potential importance of root activity in determining responses to future climatic
warming. Overall, findings from this study suggest that through root-soil interactions,
climate-related changes to plant distributions and root productivity may have large
impacts on soil C cycling and storage across tropical elevation gradients. Further
understanding of the mechanisms underlying these root-soil interactions will be
critical for predicting responses to global change.
5.1 Introduction

Tropical soils are a globally important store of terrestrial carbon (C) (Jobbágy and Jackson, 2000), yet the fate of these large soil C stocks in response to global change remains uncertain (Cox et al., 2013, Cusack et al., 2016). Climatic warming of up to 5 °C is projected to occur over the course of this century (Urrutia and Vuille, 2009, IPCC, 2013), which will likely directly stimulate microbial activity and accelerate the rate of C return to the atmosphere (Melillo et al., 2002, Davidson and Janssens, 2006, Lu et al., 2013, Wieder et al., 2013). However, increased temperature (together with elevated concentrations of atmospheric carbon dioxide; CO$_2$) may also enhance plant productivity, and in turn increase the quantity of plant-derived C inputs to soils (Wood et al., 2012, Cernusak et al., 2013, Giardina et al., 2014). Furthermore, climate-related shifts in the distribution of plant communities, occurring across gradients of tropical lowland to montane forests (Chen et al., 2011, Feeley et al., 2011) and grasslands (Harsch et al., 2009, Lutz et al., 2013), as well as latitudinal gradients (Frenne et al., 2013), will also result in parallel changes to belowground root communities and root-derived C inputs to soils. These indirect consequences of climatic warming could strongly influence soil C dynamics and modulate the direct response to temperature, because root-derived C inputs have been shown to strongly influence the total CO$_2$ efflux from soils (Högberg et al., 2001, Metcalfe et al., 2007a) and root-soil interactions may stimulate loss of pre-existing soil C (Bird et al., 2011, Cheng et al., 2014). A more integrated understanding of the combined effects of temperature and root-soil interactions on soil C cycling across tropical lowland and montane systems is therefore required to more comprehensively predict responses to global change.

The efflux of CO$_2$ from soil (Rs) can be partitioned into that derived from free-living soil microorganisms oxidising soil organic matter (SOM) (heterotrophic respiration; Rh) and that derived from autotrophic root respiration and microbial mineralisation of root-derived C in the rhizosphere (root-rhizosphere respiration; Rr) (Kuzyakov, 2006, Sayer and Tanner, 2010). Roots therefore play a potentially important role in the soil C cycle, by directly respiring CO$_2$ to the atmosphere, and by providing a source of labile C belowground which is readily mineralised by soil microorganisms (Grayston et al., 1997, Jones et al., 2009). The supply of labile C-substrates from roots is crucial for regulating soil respiration (Högberg et al., 2001,
as, without these inputs, the pool of labile soil C may become depleted, constraining microbial activity, reducing effluxes of CO₂ and dampening responses to increasing temperature (Curiel Yuste et al., 2010, Melillo et al., 2011, Billings and Ballantyne, 2013). Moreover, as well as being directly mineralised by microorganisms, root-derived C inputs may stimulate microbial activity and enhance the mineralisation of pre-existing SOM (Cheng et al., 2014, Finzi et al., 2015), with studies reporting roots to increase soil C turnover, resulting in net C loss compared with unplanted soils (Dijkstra and Cheng, 2007, Bird et al., 2011). This mechanism (whereby root exudation stimulates microbial mineralisation of SOM) may also increase the availability of soil nutrients for uptake by plants (Dijkstra et al., 2013, Murphy et al., 2015, Nie and Pendall, 2016), such that root-soil interactions could strongly influence future responses to global change. For example, an experimental study of temperate forest under elevated atmospheric CO₂ reported an increase in the flux of plant-derived C belowground which stimulated microbial activity and resulted in increased microbial decomposition of SOM (Drake et al., 2011). The associated release of N from organic matter was, in turn, taken up by plants and used to support increased primary productivity, contributing to a positive feedback which sustained the elevated soil CO₂ efflux over time. However, despite the potential importance of root-soil interactions in regulating the efflux of CO₂ from soils, our understanding of these interactions in tropical systems remains limited.

While it is evident that roots make an important contribution to the total CO₂ efflux from soils, there remains considerable uncertainty regarding how root activity and root-soil interactions will influence the temperature sensitivity (Q₁₀) of soil respiration to climatic warming. Some temperate-region (Boone et al., 1998), boreal (Gaumont-Guay et al., 2008) and arctic (Grogan and Jonasson, 2005) studies have found soil respiration including roots (Rh + Rr) to be more sensitive to temperature, compared to root-free soil (Rh). Yet other temperate and boreal studies have reported the reverse relationship to be true (Bhupingderpal-Singh et al., 2003, Hartley et al., 2007), with Rr instead most dependent on the allocation of photosynthates to roots (coupled to productivity; Janssens et al, 2001). It has been suggested that the apparent temperature sensitivity of soil respiration (Rh + Rr) may be greatest when increased plant productivity (and associated root-exudation) occurs in parallel with increasing
temperature (Curiel Yuste et al., 2004, Schindlbacher et al., 2009). As such, climate-related changes to root productivity have the potential to strongly influence the observed temperature sensitivity of soil respiration. However, these ideas are yet to be tested in the tropics.

While changes to root communities and root-derived C inputs may affect soil C dynamics, differences in soil properties may also mediate the growth of these roots. Root productivity has been shown to increase in patches of greater soil fertility (Fitter, 1994, Hodge, 2004), with a lowland forest study observing increased root proliferation into soil characterised by more readily obtainable nutrients (Sayer et al., 2006). As such, differences in soil organic matter content and fertility, varying widely across tropical elevation gradients (Nottingham et al., 2015a), may be important in modulating the growth of roots from different plant communities, in turn potentially influencing root-soil interactions and soil C dynamics. It is therefore important to evaluate how soil properties influence the growth of new root communities, as well as examining how changes in root communities will affect soil C processes.

Elevation gradients offer a valuable opportunity to investigate the effect of temperature on soil C dynamics, with experimental warming imposed by translocation of soils downslope (Zimmermann et al., 2009a, Luan et al., 2014). This is a particularly useful approach in the tropics, where in-situ warming experiments are yet to be implemented (Cavaleri et al., 2015, Nottingham et al., 2015c). In a previous study, intact soil cores were translocated across a 3000 m elevation gradient (spanning 14 ºC range in mean annual temperature; MAT) in the Peruvian Andes, to evaluate the temperature dependence of heterotrophic soil respiration, finding that the $Q_{10}$ of Rh was greater for soils originating from higher elevations (Zimmermann et al., 2009a). This study, however, did not evaluate the role of roots and root-soil interactions, which can make an important contribution to soil C processes (Högberg et al., 2001, Drake et al., 2011, Cheng et al., 2014), and may be strongly influenced by future climatic warming (Chen et al., 2011, Feeley et al., 2011, Wood et al., 2012). Further consideration of how warming-related changes to root productivity and root communities may modulate the observed, apparent temperature sensitivity of soil CO$_2$ effluxes is therefore required to more comprehensively predict responses to global change.
Here we investigate the combined direct (temperature) and indirect (change to root communities and productivity) effects of climatic warming on soil C dynamics across a tropical elevation gradient in the Peruvian Andes. Intact soil cores were reciprocally translocated among four elevation sites spanning a 20 °C range in MAT (6.5–25.4 °C), to evaluate the role of root-soil interactions in regulating soil C dynamics, and how these interactions may influence the apparent temperature sensitivity of soil respiration to future climatic warming.

The experiment tests the following hypotheses:

H1. The apparent temperature sensitivity ($Q_{10}$) of soil respiration will be lower when roots are excluded. Firstly, because roots are an important source of organic C substrate, meaning that in the absence of roots (and root-derived C inputs) soil microorganisms will become constrained by restricted substrate supply, reducing the $Q_{10}$ of Rh. Secondly, because root productivity is greater at warmer-lower elevation sites, which will increase the Rr component of soil respiration, leading to a greater apparent $Q_{10}$ for Rh + Rr.

H2. Greater root productivity at the warmer-lower elevation site, coupled with greater root proliferation into fertile montane soils rich in organic matter (translocated downslope) will contribute to a higher apparent temperature sensitivity (for Rh + Rr) for the montane forest and grassland soils compared to the lowland forest soil.

### 5.2 Materials and Methods

**Study sites**

Soil cores were reciprocally translocated among four sites along a tropical elevation gradient situated on the eastern flank of the Peruvian Andes, spanning a MAT range of 20 °C (6.5-26.4 °C; site characteristics summarised in Table 5.1). The three highest elevation sites are located in or close to the Manu National Park, centred on the Kosñipata Valley, with the lowest elevation site located in the Amazon basin, Tambopata Reserve, south-eastern Peru. All sites from the lowland to upper montane forest have continuous closed canopy forest cover, with montane grassland above the treeline, which is situated at approximately 3500 m above sea level. Mean annual
temperature decreases with increasing elevation, exhibiting little seasonal variation. Mean annual precipitation (MAP) peaks at the mid-elevation site (5302 mm year\(^{-1}\) at 1500 m) and is distinctly seasonal across the gradient, with a dry season from May-September and a wet season from November-March (Rapp and Silman, 2012). Despite distinct wet and dry seasons, there is little seasonal variation in soil moisture (Girardin et al., 2013), and evidence to date indicates that plants and soils at all sites are rarely moisture limited over the seasonal cycle (Zimmermann et al., 2010a, van de Weg et al., 2014).

The composition of vegetation changes across the elevation gradient, with dominant tree families Leythidaceae, Bixacea, Moraceae and Fabaceae in the lowland rainforest (210 m), shifting to Euphorbiaceae, Fabaceae and Lauraceae in the lower montane forest (1500 m) and Clusiaceae, Cunoniaceae and Rosaceae in the upper montane forest (3025 m) (Girardin et al., 2010, Salinas et al., 2011). Above the treeline, tussock forming grasses dominate, from the genera *Calamagrostis*, *Scirpus* and *Festuca* (Oliveras et al., 2014). Standing stocks of fine root biomass and root residence time increase with elevation, whereas fine root net primary productivity (NPP) decreases with increasing elevation, such that fine root NPP in lowland forest soils is approximately six-times greater than in upper montane forest sites (6.80 ± 1.00 and 1.23 ± 0.40 Mg C ha\(^{-1}\) yr\(^{-1}\) at 210 m and 3025 m elevation sites respectively) (Girardin et al., 2010). High seasonality in root growth has also been observed, with greatest root production during the dry season at the lower montane forest site (Girardin et al., 2013), and during the wet season at the montane grassland (Oliveras et al., 2014), upper montane forest (Girardin et al., 2013) and lowland forest (Malhi et al., 2014, Girardin et al., 2016) sites.

Soil fertility generally increases with elevation, with higher concentrations of total C, nitrogen (N) and phosphorus (P) in montane forest soils compared to lowland soils (Nottingham et al., 2015a). Extractable PO\(_4\)-P also increases with elevation from lowland to montane forest soils, while mineralised N (NH\(_4\) + NO\(_3\)) decreases with increasing elevation (Nottingham et al., 2015a). Soils across the gradient are acidic, with little variation in soil pH among the forest sites (Whitaker et al., 2014b).
Table 5.1: summary characteristics of the four study sites (mean annual temperature; MAT, mean annual precipitation; MAP).

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Elevation (m asl)</th>
<th>Vegetation Type</th>
<th>MAT (°C)</th>
<th>MAP (mm yr(^{-1}))</th>
<th>Underlying Geology</th>
<th>Soil Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata</td>
<td>210</td>
<td>Lowland rain forest</td>
<td>26.4</td>
<td>1900</td>
<td>Pleistocene alluvial terrace</td>
<td>Haplic Cambisol</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>Lower montane forest</td>
<td>17.4</td>
<td>5302</td>
<td>Plutonic Intrusion (granite)</td>
<td>Cambisol</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>Upper montane forest</td>
<td>11.1</td>
<td>1560</td>
<td>Palaeozoic shales-slates</td>
<td>Umbrisol</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>Montane Grassland</td>
<td>6.5</td>
<td>*760</td>
<td>Palaeozoic shales-slates</td>
<td>Umbrisol</td>
</tr>
</tbody>
</table>

*measured at Ajanaco, 3450 m asl (Oliveras et al., 2014)
Soil classification from Quesada et al. (2010)
Translocation of soil cores

Intact soil cores were reciprocally translocated among four sites, spanning a MAT range of 20 °C (6.5 – 26.4 °C; Table 5.1) in October 2013. At each site, twenty-four intact cores of mineral soil (10 cm diameter, 30 cm depth) were excavated, and divided into two treatments. Twelve of these cores were contained in plastic tubes with fine mesh (35 µm) at the base of the core, to prevent root ingrowth whilst allowing drainage of water (hereafter referred to as control cores) and twelve cores were contained in plastic tubes with a series of 15 holes (2 cm diameter) around the sides and coarse (2.0 mm) mesh at the bottom of the core (with total area of 125 cm² open to root access, equivalent to c. 13 % surface area of the core), permitting root-soil interactions (hereafter referred to as ingrowth cores). Mineral soil was used for this experiment, removing the organic rich topsoil due to large differences in the depth of the organic horizon among sites, which would have confounded comparisons among soils.

Soil cores were re-installed at each site, in three separate subplots located outside the perimeter of 1 ha permanent study plots of the Andes Biodiversity and Ecosystem Research Group (ABERG; http://www.andesconservation.org), with the exception of the 3644 m site where translocated cores were situated in three subplots within a smaller fenced area (approximately 8 m x 8 m) to protect from disturbance. These three subplots acted as individual spatial replicates, such that 24 cores were re-installed at each elevation site (4 soils x 2 core treatments (control/ingrowth) x 3 replicates; Figure 5.1). Cores were carefully inserted into holes cut into the soil using a hand auger, with remaining gaps around cores refilled with local soil. In order to focus on the role of belowground root inputs, coarse 2.0 mm mesh covers were secured to the tops of all cores with plastic bands to prevent inputs of plant material from aboveground over the duration of the study.

Additional shallow soil collars (10 cm diameter, to 3 cm depth) were installed at each subplot, adjacent to translocated cores. Care was taken not to cut coarse roots when installing these collars, hereafter referred to as undisturbed controls. At each site, three of these soil collars were installed to measure total soil respiration (including the organic soil horizon) and three soil collars to measure mineral soil respiration (organic horizon removed), with one of each soil collar in each subplot (3 spatial
replicates; Figure 5.1). The collars with organic soil removed (mineral soil respiration only) were used as an undisturbed control, for comparison with disturbed soil ingrowth cores which had been excavated and reinstalled at the same elevation. To enable comparison with CO₂ effluxes measured from disturbed ingrowth cores, and to focus on the role of belowground root inputs, these soil collars were also covered with coarse mesh, to prevent plant-inputs from aboveground entering the soil over the duration of the study.

The different soil cores and collars were used to partition soil respiration (Rs) fluxes and evaluate the disturbance effect caused by the translocation process. Heterotrophic respiration (Rh) was defined as the CO₂ efflux from respiration of free-living soil microorganisms (mineralising SOM), while root-rhizosphere respiration (Rr) was defined as the CO₂ efflux from autotrophic root respiration in addition to microbial respired CO₂ from rhizosphere microorganisms and mycorrhizae metabolising labile root exudates and dead root material (Sayer and Tanner, 2010). Hereafter, these definitions of Rh and Rr will be used to describe and refer to these different sources of CO₂.

i. Control core (R_{control}); Rh (mineral soil)

ii. Ingrowth core (R_{ingrowth}); Rh + Rr (mineral soil)

iii. Undisturbed mineral soil; Rh + Rr (from mineral soil only, organic horizon removed), used to identify disturbance effects by comparison with CO₂ efflux from ingrowth cores excavated and reinstalled at the same elevation

iv. Undisturbed organic + mineral soil; Rh + Rr (total soil respiration, including organic soil horizon, leaf-litter layer removed)

Henceforth, the following notation is used to refer to soils translocated across the gradient, dependent on the original source elevation and final elevation after translocation;

Soil_{source elevation – final elevation}
For example, soil originating from Wayqecha (3025 m) which was translocated downslope to Tambopata (210 m) is described by Wayqechaa_{3025\text{m}-210\text{m}}, and soil originating from San Pedro (1500 m) translocated upslope to Tres Cruces (3644 m) is described by San Pedro_{1500\text{m}-3644\text{m}}. Soil excavated and re-installed at the same elevation site, for example Wayqecha (3025 m) soil excavated and re-installed at 3025 m is described by Wayqechaa_{3025\text{m}-3025\text{m}}.

Figure 5.1; example subplot illustrating distribution of soil cores and collars in the field (undisturbed soil collar including organic soil not shown), and schematic diagram of control and ingrowth cores.
Soil chemical analysis – pre translocation

Samples of mineral soil from each subplot were retained in sealed plastic bags and transported to the laboratory in the UK (Lancaster). Fresh soil was used to measure soil pH in water using a Hanna pH meter (1:2.5 soil: H₂O, Hanna Instruments, USA). Total C and N content was measured from dried (60 °C to constant mass) and ground subsamples using an elemental analyser (LECO, USA). Total P content was determined by sulphuric acid digestion followed by phosphate detection by automated molybdate colorimetric analysis (Bran Luebbe AA3 Autoanalyser, Germany). While soil pH and total C and N content were measured using soil sub-samples from the individual subplots from each elevation site (n = 3), total P content was measured from a single bulked sample of soil from each elevation (by even mixing of the three individual spatial replicates to produce a homogenous sample), due to insufficient soil remaining for individual analyses.

Soil CO₂ efflux measurements

The CO₂ efflux from soil cores and collars were measured using a closed chamber system with a 10 cm diameter survey chamber and portable Li-Cor 8100-IRGA (LI-COR Biosciences, USA). Briefly, in a closed chamber system, air is circulated in a closed loop between the chamber and infra-red-gas analyser (IRGA) which measures the increase in concentration of CO₂ over a specified period of time, from which the CO₂ efflux is calculated (Nottingham et al., 2012a). For all measurements, the increase in CO₂ concentration was measured over 120 seconds, with the efflux internally calculated and reported as µmol CO₂ m⁻² second⁻¹. Mesh covers on the tops of soil cores, used to prevent above-ground plant inputs to soil, were temporarily removed during CO₂ measurements. Soil temperature and soil moisture (0-10 cm depth) was measured within each soil core and collar immediately after each CO₂ measurement, using a thermometer and soil moisture probe (ThetaProbe, DeltaT Devices, UK).
Intra-annual CO₂ measurements

Intra-annual measurements of CO₂ efflux from each soil core and collar occurred over 24 months following translocation (October 2013-2015), at intervals of approximately 3 months. The first set of measurements were taken in December 2013, two months after cores were first translocated to allow soils to equilibrate after the initial disturbance from excavation and translocation (Zimmermann et al., 2009a). Measurements were always taken in the morning, between 07:00 - 10:00 am, to minimise potential effects of diurnal variation on respiration rates (although a separate study revealed diurnal variation to be minimal; Appendix C). At each time point, the CO₂ efflux from each soil core and collar was measured twice, with the mean of these two measurements used for further data analysis. Where inconsistency between the two measurements arose, with an elevated first measurement compared to the second, a third measurement was taken. In this occurrence, the first measured flux was assumed to be an artefact of soil disturbance from placing the survey chamber onto the core and therefore the first artificially elevated measurement excluded, using the mean of the second two measurements for further data analysis.

After each set of intra-annual measurements, control cores were gently rotated by 90° to sever very fine root and mycorrhizal in-growth through the 35 µm mesh at the base of the core (Johnson et al., 2001). Ingrowth cores were not disturbed for the duration of the study. In total, a series of 9 measurements were taken at each site over 24 months, with one fewer measurement at the lowest elevation site (210 m) as site access was restricted in September 2014.

Harvesting soil cores

Soil cores were harvested at the end of the dry season in October 2015, approximately 24 months after they were first translocated across the gradient. Cores were removed by carefully digging around the plastic tubes, taking care not to disturb roots which were inside the cores. The intact cores were wrapped separately in plastic bags to prevent contamination among cores, and to prevent loss of soil and roots from the cores during transportation. All cores were transported back to the laboratory (Cusco, Peru) and stored in a cool, dark room for a maximum of 5 days before further processing
occurred. Each core was carefully deconstructed by pushing the soil out from the plastic tubing, aiming to keep the core intact before roots were extracted and soil samples collected.

**Quantification of fine root biomass and length**

Fine root biomass and root length in ingrowth cores were quantified using the method described by Metcalfe et al. (2007b), which corrects for underestimation of fine roots. The soil in each core was briefly and gently homogenised before root extraction commenced, taking care not to break roots. Roots were manually extracted during 4 x 10 minute intervals so that total root extraction (root mass and length) could be estimated by extrapolation from cumulative root extraction-time plots. Roots extracted during each 10-minute interval were first rinsed in water to remove soil particles, before roots were scanned as greyscale images at 600 DPI resolution. Roots were then oven dried at 60 °C until constant mass and weighed. Scanned images were analysed using Image-J software (http://imagej.nih.gov/ij/) by manually tracing roots, to measure total root length (m) extracted during each 10-minute time interval. Subsamples of the remaining, homogenised soil from each ingrowth core after roots had been extracted, as well as homogenised samples of soil from each control core, were retained in separate sealed plastic bags and transported to the UK for further chemical analysis.

Roots were classified as very fine (< 1 mm diameter), fine (1-2 mm diameter) and coarse (> 2 mm diameter) (Cavelier, 1992, Metcalfe et al., 2008). Extracted roots were predominantly very fine and fine, with coarse roots only present in some cores, consistent with previous observations from the gradient (Girardin et al., 2010, Girardin et al., 2013). Root biomass and root length were quantified for very fine and fine roots only (hereafter referred to as fine roots), as fine roots account for the majority of belowground root production and the greatest proportion of root-litter inputs (Jackson et al., 1996). Additionally, as coarse roots are much longer lived (Röderstein et al., 2005) they are more likely to have originated in the soil cores prior to translocation. Due to relatively fast turnover of fine roots, especially at warmer temperatures/lower elevations, it was assumed that all fine roots measured in cores had grown after
translocation, although this will have resulted in a small overestimation at higher elevation sites where the residence time of roots is greater than 24 months (Girardin et al., 2010). Finally, owing to difficulty in differentiating between live and dead root matter, all extracted fine roots were used to estimate total root biomass and length. This approach may have also resulted in overestimation of root biomass and length, but assuming the same proportion of live/dead roots at each elevation, it provided a relative measure among soil cores (after 24 months at the same elevation).

To estimate the total fine root mass (g) and total fine root length (m) in each core, extracted fine root dry mass and fine root length were plotted cumulatively against time (10, 20, 30 and 40 minutes extraction) separately for each soil core, with logarithmic curves fitted to these data. The total fine root mass and length in each core was determined where the logarithmic curve plateaued such that the increase in root mass or length predicted between two consecutive time points was no greater than 1% of the total cumulative root mass or length predicted by the curve, consistent with the approach described by Metcalfe et al. (2007b). From these estimates of total fine root mass in each core, total fine root biomass (roots < 2 mm diameter, mineral soil to 30 cm depth) was calculated and reported as Mg C ha⁻¹, assuming root biomass was 50% C (Jackson et al., 1997). Specific fine root length (SFRL; m g⁻¹) was calculated from total estimated fine root biomass and length data (root length/root mass), separately for each core.

**Soil chemical analysis- post translocation**

Total C and N content was measured from dried (60 °C to constant mass) and ground soil subsamples from each core (n = 96), using an elemental analyser (LECO, USA). To measure soil pH in water (1:2.5 w:v soil:H₂O, Hanna pH meter, Hanna Instruments, USA), fresh soil subsamples were bulked across replicates by evenly mixing to produce a homogenous sample for each soil/elevation/core treatment (n = 32).
Data analysis

Partitioning CO$_2$ effluxes

Soil CO$_2$ effluxes were partitioned into those arising from free-living soil microorganisms (Rh) and those associated with roots and microbial respiration in the rhizosphere (Rr) by a mass balance approach. Rh was determined directly as the CO$_2$ efflux measured from control cores (Figure 5.1), while Rr was estimated as the difference in CO$_2$ efflux from ingrowth and control cores (R$_{\text{ingrowth}}$ – R$_{\text{control}}$), for corresponding pairs of cores (same elevation/soil/subplot/time point). Where the partitioning of Rr resulted in a negative flux (when R$_{\text{control}}$ > R$_{\text{ingrowth}}$), a negligible CO$_2$ efflux from roots was assumed, and the Rr component was re-defined as a zero flux. This was only necessary for a limited number of cases (c. 10 % of measurements), most notably for soils translocated upslope to 3644 m where measured CO$_2$ effluxes were relatively low across all soils/core treatments (Supplementary Figure S5.1a), likely a function of cool temperatures constraining root productivity. The % contribution of roots (Rr) to the total soil CO$_2$ efflux (Rh + Rr) at each elevation site was estimated using CO$_2$ effluxes measured from native soils (excavated and re-installed at the same elevation site), with all Rr data used for further statistical analysis to assess how root-soil interactions in cores translocated across the gradient influenced the soil CO$_2$ efflux.

Assessing root-soil interactions

Data were analysed using the R package 3.2.1. (R Core Team, 2015). Repeated measures analysis of variance (ANOVA) was used to assess variation in Rr-CO$_2$ efflux with time (separately by elevation), in which ‘Soil’, ‘Time’ and their interaction were fixed effects and core identity was included as a random factor, to account for the repeated measurements of CO$_2$ effluxes over time following translocation. The response variable (Rr-CO$_2$ efflux, for each elevation) was first tested for normality using the Shapiro-Wilk test, and square-root transformed where necessary to meet the assumptions of the model. Two-way ANOVA was also used to test the main and interactive effects of ‘Elevation’ and ‘Soil’ on fine root biomass (Mg C ha$^{-1}$), and SFRL (m g$^{-1}$) measured from ingrowth cores. Pairwise comparisons of significant
effects identified by ANOVA were conducted by Tukey’s HSD post hoc tests, with significant differences identified where p < 0.05. To evaluate whether there was a significant relationship between the amount of fine root biomass within ingrowth cores (measured following destructive harvesting in October 2015), and CO₂ effluxes derived from roots and the rhizosphere (also measured in October 2015 immediately prior to harvesting), Pearson’s correlation coefficients (r) were determined separately for each elevation. Root-rhizosphere CO₂ effluxes were also calculated on a fine root mass basis (CO₂ g⁻¹ fine root mass), to normalise for differences in root biomass among soil cores. Finally, to assess whether root-soil interactions influenced total C and N content in soils, pairwise comparisons among control and ingrowth cores (after 24 months at the same elevation site) were conducted using Tukey’s HSD tests.

**Apparent temperature sensitivity (Q₁₀) of soil respiration**

To determine the temperature dependence of soil CO₂ effluxes (with root growth permitted; Rh + Rr, and excluded; Rh alone), first-order exponential curves of the form displayed in equation 5.1 (Paul and Clark, 1996, Boone et al., 1998) were fitted; where $R$ is the soil CO₂ efflux, $T$ is soil temperature (0-10 cm depth), and $a$ and $b$ are fitted constants. These exponential curves were fitted to soil temperature-CO₂ efflux data collected over 24 months (at intervals of approximately 3 months) following translocation of soils across the elevation gradient (responses driven by among-site temperature variation), in each case separately by soil and core type (control/ingrowth core).

$$R = ae^{bT} \quad \text{(Eq. 5.1)}$$

Regression-derived estimates of CO₂ effluxes were used to calculate the temperature sensitivity ($Q_{10}$) of soil respiration with root growth permitted (Rh + Rr) and excluded (Rh only), separately for each soil (according to equation 5.2), where the $Q_{10}$ of respiration was defined as the factor by which respired CO₂ ($R$) changed with a 10 °C
These $Q_{10}$ coefficients, derived using between-site temperature variation across the gradient, are estimates of the observed, apparent temperature sensitivity rather than intrinsic temperature sensitivity of soil respiration (Nottingham et al., 2015c). They reflect a system-scale temperature sensitivity of soil CO$_2$ efflux, because they are a function of not only temperature, but also of other variables which differ among sites and co-vary with temperature, for example different tree (root) communities, rates of root productivity and root metabolism. Furthermore, although reasonable to assume the same microbial community and enzyme assemblages originated in soil cores sourced from the same elevation (prior to translocation), over the 24 months following translocation across the gradient, temperature-related community shifts may have occurred, with potential to influence the observed, long-term temperature sensitivity of soil respiration (Bradford et al., 2008, Karhu et al., 2014).

$$Q_{10} = \left[ \frac{R_2}{R_1} \right]^{10(T_2-T_1)}$$

(Eq. 5.2)

5.3 Results

Initial soil properties

Soil properties (mineral soil) measured prior to translocation are summarised in Table 5.2. The concentration of total C and N in mineral soil increased approximately eleven-fold and eight-fold with elevation respectively, whereby total C ranged from 1.3 % (at 210 m) to 14.9 % (at 3644 m) and total N ranged from 0.15 % (at 210 m) to 1.14 % (at 3644 m). Total phosphorus (P) concentration varied five-fold from 0.027 % (at 210 m) to 0.111 % (at 3025 m). The ratio of C: N was lowest in soil from the lowest elevation site (8.7 at 210 m) and greatest in soil from the upper montane forest site (17.6 at 3025 m), whereas the ratio of C:P was also lowest in soil from the lowest elevation site (48.1 at 210 m) but greatest in soil from the montane grassland site.
(144.7 at 3644 m). Across the gradient, concentrations of resin extractable PO$_4$ (mineral soil) vary three-fold, from 2.5 mg kg$^{-1}$ (at 210 m) to 7.4 mg kg$^{-1}$ (at 3025 m) (A. Nottingham, unpublished data). The availability of inorganic N (NH$_4$ + NO$_3$) in soils across the gradient has only been measured to 10 cm depth, whereby N mineralisation is greater in soils from the two lower elevations relative to soils from the two higher elevations (Nottingham et al., 2015a). Soil pH did not vary considerably among soils from the three lower elevation forest sites (pH 4.0- 4.4), but was higher in the grassland soil from 3644 m (pH 4.9).

**Estimation of disturbance effect**

Firstly, to evaluate the effect of disturbance, as a result of excavating and re-installing soil cores, CO$_2$ effluxes measured from ingrowth cores (disturbed soil cores; Rh + Rr) and undisturbed soil collars (Rh + Rr) at each site were compared (Supplementary Figure S5.2). Although CO$_2$ effluxes from disturbed soil cores were typically elevated compared to undisturbed soil collars, fluxes measured from the disturbed cores tended to closely track seasonal variation in CO$_2$ measured from the undisturbed soil collars. This was with the exception of the lower montane forest site (1500 m), where there was a negligible disturbance effect evident during the wet season (no difference between disturbed and undisturbed CO$_2$ efflux), whereas during the dry season CO$_2$ from disturbed soil cores were markedly elevated relative to the undisturbed soil collars (Supplementary Figure S5.2c). Nonetheless, irrespective of these small disturbance effects, here CO$_2$ effluxes measured from disturbed-control and disturbed-ingrowth cores are compared, using the relative difference among these cores to estimate the Rr component. As such, while these values may be artificially elevated, they provide a good indication of the CO$_2$ efflux associated with roots and the rhizosphere relative to that associated with microbial-heterotrophic respiration, and enable comparison of the relative responses to translocation among the different soils.
Table 5.2: soil properties (mineral soil, pre-translocation) at each elevation site. Data represent mean (SE) where \( n = 3 \) from 3 subplots at each elevation site. Total C, N and P reported on a soil dry mass basis. Total P measured from a homogenised sample bulked across 3 subplot-samples at each elevation site, which precluded estimate of variance.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Elevation (m asl)</th>
<th>pH</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>Total P (%)</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>PO(_4)-P (mg P kg(^{-1}))</th>
<th>NH(_4) + NO(_3) (µg N g(^{-1}) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata</td>
<td>210</td>
<td>4.0 (0.05)</td>
<td>1.3 (0.1)</td>
<td>0.15 (0.02)</td>
<td>0.027</td>
<td>8.7 (0.7)</td>
<td>48.1</td>
<td>5.6</td>
<td>2.5</td>
<td>27.6</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>4.3 (0.06)</td>
<td>8.6 (1.7)</td>
<td>0.58 (0.09)</td>
<td>0.089</td>
<td>14.9 (1.0)</td>
<td>96.6</td>
<td>6.5</td>
<td>4.5</td>
<td>27.2</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>4.4 (0.21)</td>
<td>14.6 (0.8)</td>
<td>0.83 (0.04)</td>
<td>0.111</td>
<td>17.6 (0.2)</td>
<td>131.5</td>
<td>7.5</td>
<td>7.4</td>
<td>12.3</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>4.9 (0.03)</td>
<td>14.9 (2.2)</td>
<td>1.14 (0.12)</td>
<td>0.103</td>
<td>13.1 (1.2)</td>
<td>144.7</td>
<td>11.1</td>
<td>3.0</td>
<td>10.7</td>
</tr>
</tbody>
</table>

\(^a\)Extractable P (mineral soil) determined by A. Nottingham (unpublished data)

\(^b\)Mineralised N (0-10 cm depth) determined by Nottingham et al. (2015a and unpublished data for Tres Cruces)
Root-rhizosphere respiration and root-soil interactions

Effluxes of CO₂ measured from control (Rh) and ingrowth (Rh + Rr) cores (Supplementary Figure S5.1) were used to partition the root-rhizosphere component (Rr) of respired CO₂ from four soils reciprocally translocated among four elevation sites (Figure 5.2). At the three higher elevation sites (1500 m, 3025 m and 3644 m), Rr varied significantly with time, but there was no significant difference in Rr among soils (Table 5.3). At the lowest elevation site (210 m) a significant ‘Soil x Time’ interactive effect was identified by repeated measures ANOVA (Table 5.3). Pairwise comparisons of this significant interactive effect revealed that Rr from soils originating from Tres Cruces₃₆₄₄₉₂₁₀₉, Wayqecha₃₀₂₅₉₂₁₀₉ and San Pedro₁₅₀₀₉₂₁₀₉ translocated downslope to 210 m were typically greater during the wet season compared to the dry season, whereas Rr from the Tambopata₂₁₀₉₂₁₀₉ soil at its native elevation showed little seasonal variation (Figure 5.2d). At this lowland forest site there were also marked differences in the magnitude of Rr effluxes among soils, with Rr from the Tres Cruces₃₆₄₄₉₂₁₀₉ and San Pedro₁₅₀₀₉₂₁₀₉ soils significantly greater than Rr measured from native Tambopata₂₁₀₉₂₁₀₉ soil at the same elevation. Root-rhizosphere respired CO₂ fluxes from the Wayqecha₃₀₂₅₉₂₁₀₉ soil were also generally higher than those measured from the native Tambopata₂₁₀₉₂₁₀₉ soil, but were lower than those measured from the Tres Cruces₃₆₄₄₉₂₁₀₉ and San Pedro₁₅₀₀₉₂₁₀₉ soils at the same elevation (Figure 5.2d). Although there were no significant differences in Rr among soils at the three higher elevation sites, examination of the significant ‘Time’ effect by post-hoc tests highlighted some seasonal trends. At the 1500 m site, discernible seasonal fluctuations in Rr were evident, with Rr greatest during the dry season and lowest during the wet season (Figure 5.2c). At the 3644 m and 3025 m sites, seasonal trends were less clear, however Rr typically increased over the course of the dry season, tending to peak during the mid-wet season before declining towards the end of the wet season (Figures 5.2 a-b).

Over the 24-month duration of the study, the % contribution of Rr to the total soil CO₂ efflux (mineral soil) was greatest at the upper montane forest and lowland forest sites, where Rr accounted for 28-71 % of total soil CO₂ at 3025 m (Supplementary Figure S5.1b) and 37-75 % of total soil CO₂ at 210 m (Supplementary Figure S5.1d). In the montane grassland and lower montane forest sites, Rr made a
negligible contribution to the total soil CO$_2$ efflux during the wet season (November - March), where there tended to be no difference among the CO$_2$ efflux measured from control (Rh) and ingrowth (Rh + Rr) cores (Supplementary Figure S5.1a and S5.1c). However, during the dry season (May-September) root-rhizosphere derived CO$_2$ accounted for up to 38 % of the total soil CO$_2$ efflux at 3644 m (Supplementary Figure S5.1a) and up to 56 % of the total soil CO$_2$ efflux at 1500 m (Supplementary Figure S5.1c).

Table 5.3: main and interactive effects of ‘Soil’ and ‘Time’ on CO$_2$ resired from root-rhizosphere (Rr), tested by repeated measures ANOVA, separately by elevation. Core identity was included as a random factor to account for repeated measurements from cores over 24 months following translocation.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3644 m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil (S)</td>
<td>3</td>
<td>0.5</td>
<td>ns</td>
</tr>
<tr>
<td>Time (T)</td>
<td>8</td>
<td>8.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x T</td>
<td>24</td>
<td>1.2</td>
<td>ns</td>
</tr>
<tr>
<td><strong>3025 m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>1.1</td>
<td>ns</td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>4.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x T</td>
<td>24</td>
<td>1.5</td>
<td>ns</td>
</tr>
<tr>
<td><strong>1500 m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>1.8</td>
<td>ns</td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>8.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x T</td>
<td>24</td>
<td>0.9</td>
<td>ns</td>
</tr>
<tr>
<td><strong>210 m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>16.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>T</td>
<td>7</td>
<td>6.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x T</td>
<td>21</td>
<td>2.4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Significant effect identified where p < 0.05, not significant (ns) where p > 0.05.
Figure 5.2; root-rhizosphere respired CO$_2$ (Rr) at (a) 3644 m, (b) 3025 m, (c) 1500 m, (d) 210 m measured from four soils; Tres Cruces (TC; originating from 3644 m), Wayqecha (WAY; originating from 3025 m), San Pedro (SP; originating from 1500 m) and Tambopata (TAM; originating from 210 m) over 24 months following translocation. Data represent mean ± 1SE (n = 3). Blue/white shading represents wet/dry seasons respectively. Raw data (Rh and Rh + Rr) used to determine Rr illustrated in Supplementary Figure S5.1. Note different y-axis scale for panel d.
Fine root biomass measured from ingrowth cores varied significantly among elevations, but not among soils (Table 5.4). Examination of the significant ‘Elevation’ effect revealed that root biomass in ingrowth cores which had been translocated to the upper montane forest site (3025 m) was significantly greater than fine root biomass in ingrowth cores at the 1500 m and 3644 m sites (Figure 5.3a). Root biomass measured from ingrowth cores at the 210 m site was also significantly greater than that measured from ingrowth cores at the 3644 m site. The effect of ‘Elevation’ on specific fine root length was marginal (p = 0.07; Table 5.4), whereby SFRL tended to be greater in ingrowth cores which had been translocated to the 3644 m site compared to that measured from ingrowth cores following 24 months at the 210 m site (Figure 5.3b).

Greater fine root biomass within ingrowth cores was significantly correlated with greater CO₂ effluxes derived from roots and the rhizosphere at all sites (p < 0.05; Figure 5.4). Normalisation of measured Rr-CO₂ g⁻¹ of root biomass in each core revealed that at the 210 m elevation site, CO₂ g⁻¹ root biomass was significantly greater from Tres Cruces 3644m-210m and San Pedro 1500m-210m soils, compared to CO₂ efflux g⁻¹ root biomass measured from Wayqecha 3025m-210m and Tambopata 210m-210m soils at the same elevation (Figure 5.4d). There were no significant differences in root biomass-normalised CO₂ efflux among soils at the other elevation sites (Figure 5.4 a-c; p > 0.05, data not shown).

**Table 5.4:** main and interactive effects of ‘Soil’ and ‘Elevation’ on fine root biomass and specific fine root length (SFRL), tested by ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>d.f.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fine root biomass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mg C ha⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevation (E)</td>
<td>3</td>
<td>9.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Soil (S)</td>
<td>3</td>
<td>0.5</td>
<td>ns</td>
</tr>
<tr>
<td>E x S</td>
<td>9</td>
<td>0.6</td>
<td>ns</td>
</tr>
<tr>
<td><strong>SFRL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(m g⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>2.6</td>
<td>0.07</td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>0.2</td>
<td>ns</td>
</tr>
<tr>
<td>E x S</td>
<td>9</td>
<td>0.4</td>
<td>ns</td>
</tr>
</tbody>
</table>

Significant effect identified where p < 0.05, marginal effect where p < 0.10, and not significant (ns) where p > 0.10.
Figure 5.3: (a) fine root biomass (Mg C ha\(^{-1}\)) and (b) specific fine root length (SFRL; m g\(^{-1}\)) measured from ingrowth cores containing soils originating from Tambopata (TAM; 210 m), San Pedro (SP; 1500 m), Wayqecha (WAY; 3025 m) and Tres Cruces (TC; 3644 m), following 24 months at 210 m, 1500 m, 3025 m, 3644 m elevation sites. Bars represent mean + SE (\(n = 3\)). Two-way ANOVA of main and interactive effects of ‘Elevation’ and ‘Soil’ on fine root biomass and SFRL presented in Table 5.5.
Figure 5.4 a–d; correlation (Pearson’s r coefficient) between root-rhizosphere CO₂ efflux (Rr µmol CO₂ m⁻² s⁻¹) and fine root biomass (g), measured from ingrowth soil cores in October 2015 following 24 months at 3644 m, 3025 m, 1500 m and 210 m, where different symbols indicate soils originally sourced from different elevations (Tres Cruces; 3644 m, Wayqecha; 3025 m, San Pedro; 1500 m, Tambopata; 210 m). Root-rhizosphere (Rr) CO₂ efflux normalised g⁻¹ root biomass (µmol CO₂ m⁻² s⁻¹ g⁻¹ root), where bars indicate mean ± 1SE (n = 3); different letters indicate significant differences, p < 0.05 identified by Tukey’s HSD tests. Note different axis-scales.
Post-translocation soil C and N content

Total C and N content in soils 24 months following translocation among four sites across the 3400 m elevation gradient are illustrated in Figure 5.5. Total C and N measured from ingrowth soil cores tended to be lower than that measured from root-free control soils, however these differences were only significant in a number of cases. Most notably, significant depletion of total C and N was evident from the Tres Cruces3644m-210m and San Pedro1500m-210m ingrowth cores which had been translocated downslope to the 210 m site, relative to the control soil cores also translocated to 210 m but without root ingrowth. In these cases, total soil C content in the ingrowth cores was over 40 % lower than total C content in equivalent control cores (without root growth). This substantial depletion of total C corresponded to high Rr-CO$_2$ effluxes (Figure 5.2d) and Rr-CO$_2$ normalised g$^{-1}$ root biomass (Figure 5.4d) measured from these soils following translocation to 210 m. Total C content in the Wayqecha3025m-210m soil following translocation downslope to 210 m was also significantly lower in the ingrowth core compared to the control core (c. 20 % lower), however there was no significant difference in total N content among the control and ingrowth soils in this case. Total C was also significantly depleted in the Wayqecha3025m-3644m ingrowth soil following translocation upslope to 3644 m, while both total C and N were significantly depleted in the San Pedro1500m-3025m ingrowth soil following translocation upslope to 3025 m. Soil pH did not vary substantially among equivalent control and ingrowth cores (< 0.5 difference in pH among control and ingrowth cores; Supplementary Table S5.1) following 24 months translocation among sites.
Figure 5.5: total soil C (% dwt) and N (% dwt) content in control and ingrowth soil cores for soils originating from (a) Tres Cruces 3644 m, (b) Wayqecha 3025 m, (c) San Pedro 1500 m and (d) Tambopata 210 m, 24 months following translocation across elevation gradient. Bars represent mean ± 1SE (n = 3). Asterisks denote significant differences among control/ingrowth treatments, where * p < 0.05, ** p < 0.01. Note different y-axis scales for panel d.
Apparent temperature sensitivity of soil respiration

Soil temperature explained 19-59% of variance in CO₂ efflux measured from soils following translocation across the elevation gradient (Figure 5.6), with temperature explaining a greater proportion of the variance in CO₂ efflux for the montane forest and grassland soils (34-59%) compared to the lowland forest soils (only 19-23% variance in CO₂ efflux explained by temperature). \(Q_{10}\) coefficients, determined from among sites temperature variation, were consistently greater for Rh + Rr compared to \(Q_{10}\) values for Rh alone (Table 5.5). The difference in the apparent \(Q_{10}\) of soil respiration with exclusion (Rh) and inclusion of roots (Rh + Rr) was most marked for the Tres Cruces \(_{3644m}\) (Rh = 1.9 ± 0.1, Rh + Rr = 4.4 ± 0.2) and San Pedro \(_{1500m}\) soils (Rh = 2.5 ± 0.1, Rh + Rr = 4.6 ± 0.6).

It was reasonable to assume that temperature was the primary environmental variable driving among site differences in soil CO₂ efflux as, although soil moisture content was not directly manipulated, and irrespective of large differences in MAP among sites across the gradient (Table 5.1), soils tended to maintain moisture content characteristic of their native elevation over the duration of the study period (Supplementary Figure S5.3). Furthermore, the relationship between soil moisture content and CO₂ efflux is typically parabolic (Sotta et al., 2004), whereas here there was a weak negative-linear relationship, which was likely a function of soils tending to be wetter at the coolest-highest elevation site and drier at the warmer-lowest elevation site, rather than a direct response to small differences in soil moisture.
Table 5.5; temperature dependence ($R^2$) and sensitivity ($Q_{10}$; mean ± SE, $n = 3$) of heterotrophic soil respiration (Rh; from control soil cores) and heterotrophic plus root-rhizosphere respiration (Rh + Rr; from ingrowth soil cores), for four soils (Tambopata, San Pedro, Wayqecha, Tres Cruces; source elevation in parentheses) derived from among site temperature variation following translocation across 20 °C temperature gradient.

<table>
<thead>
<tr>
<th>Derived from among site temperature variation</th>
<th>Tambopata (210 m)</th>
<th>San Pedro (1500 m)</th>
<th>Wayqecha (3025 m)</th>
<th>Tres Cruces (3644 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature Dependence ($R^2$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh</td>
<td>0.22**</td>
<td>0.62***</td>
<td>0.49***</td>
<td>0.33***</td>
</tr>
<tr>
<td>Rh + Rr</td>
<td>0.19**</td>
<td>0.48***</td>
<td>0.36***</td>
<td>0.51***</td>
</tr>
<tr>
<td>Apparent Temperature Sensitivity ($Q_{10}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh</td>
<td>1.5 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>Rh + Rr</td>
<td>1.8 ± 0.1</td>
<td>4.6 ± 0.9</td>
<td>2.9 ± 0.4</td>
<td>4.4 ± 0.2</td>
</tr>
</tbody>
</table>

** denotes $p < 0.01$, *** denotes $p < 0.001$ ($n = 35$)
Figure 5.6: temperature dependence ($R^2$) of soil respiration for soils originating from (a) Tres Cruces$_{3644m}$, (b) Wayqecha$_{3025m}$, (c) San Pedro$_{1500m}$, (d) Tambopata$_{210m}$, measured over 24 months following reciprocal translocation across a 20 °C mean annual temperature gradient. All relationships significant $p < 0.01$ ($n = 35$; see Table 5.5). Closed symbols indicate soil respiration with exclusion of roots (control cores; Rh) and open symbols indicates soil respiration including the contribution from roots and the rhizosphere (ingrowth cores; Rh + Rr). Data represent mean ± 1SE ($n = 3$). Note different y-axis scales among panels.
5.4 Discussion

Roots made a substantial contribution to total soil CO$_2$ effluxes across the gradient, with strong seasonality in Rr evident at some sites (Figure 5.2) corresponding to seasonal peaks in root-productivity reported by previous studies (Girardin et al., 2013, Malhi et al., 2014). The direct effect of root ingrowth on soil CO$_2$ efflux was demonstrated by the strong positive relationship between fine root biomass within soil cores and the efflux of CO$_2$ derived from roots and the rhizosphere (Rr; Figure 5.4), in agreement with other tropical and temperate studies (Metcalfe et al., 2007a, Pregitzer et al., 2008). The apparent temperature sensitivity ($Q_{10}$) of soil respiration was consistently greater when roots were permitted (Rh + Rr), compared to the apparent $Q_{10}$ of Rh alone, in support of H1. However, while greater root proliferation into the organic-rich mineral montane soils following translocation downslope was predicted (hypothesised to in turn contribute to a higher apparent $Q_{10}$ for these soils; H2), no significant difference in fine root biomass among lowland and montane soils was found after 24 months at the lowest elevation site (Figure 5.3). Instead, normalisation of Rr-CO$_2$ effluxes g$^{-1}$ root biomass revealed significantly greater Rr-CO$_2$ g$^{-1}$ root for the grassland and lower montane forest soils at 210 m, compared to the native lowland forest soil (Figure 5.4). This corresponded to substantial depletion of total C and N from these ingrowth soil cores, compared to equivalent root-free translocated-control soil cores (Figure 5.5), suggesting that root-soil interactions had stimulated this loss (Dijkstra and Cheng, 2007, Bird et al., 2011).

Root-soil interactions stimulate substantial soil carbon loss

Following translocation of grassland (Tres Cruces$_{3644m-210m}$) and lower montane forest (San Pedro$_{1500m-210m}$) soils downslope to the lowland forest site, effluxes of CO$_2$ from roots and the rhizosphere (Rr; Figure 5.2d) and Rr-CO$_2$ normalised g$^{-1}$ root biomass (Figure 5.4d) were significantly greater compared to CO$_2$ effluxes from the native Tambopata$_{210m-210m}$ soil. These high root-rhizosphere derived CO$_2$ effluxes corresponded to significant depletion of total C and N in these ingrowth soil cores, compared to the root-free control cores (Figure 5.5a and 5.5c), suggesting that the high measured CO$_2$ effluxes were real, and root-soil interactions had stimulated this loss.
Furthermore, while the signal was not as strong (Figure 5.2d), there was also evidence that root-soil interactions stimulated C loss from the Wayqecha 3025m-210m soil following translocation downslope to 210 m (Figure 5.5b). Previous temperate and subtropical studies have also reported roots to increase the turnover and loss of soil C, compared to unplanted root-free soils (Bader and Cheng, 2007, Dijkstra and Cheng, 2007, Bird et al., 2011). Root exudation of labile C substrates can stimulate microbial growth, activity and the production of extracellular enzymes (De Nobili et al., 2001, Carney et al., 2007, Zhu et al., 2014), leading to increased decomposition of SOM and net loss of soil C; a phenomenon known as rhizosphere priming (Cheng et al., 2014). Indeed, a laboratory-based study using organic-soils from the same Peruvian gradient reported that inputs of labile C enhanced the mineralisation of pre-existing SOM (Whitaker et al., 2014a), indicating that labile C exudates from roots could induce a similar response. Mineral soils from higher elevations along the studied gradient are less chemically and physically protected against microbial decomposition compared to soils from the lowland forest (Zimmermann et al., 2012), suggesting that the montane soils would be most amenable to root-mediated decomposition of SOM, as observed (Figure 5.5). Further investigation is however required to substantiate these findings, including measurements of microbial abundance, community composition and enzymatic activity in the rhizosphere soils (samples were retained for future analysis) to probe the mechanism underlying this response.

While total C content measured from ingrowth soil cores was typically lower than that measured from root-free control cores across elevations, several factors may explain why root activity at the lowland forest site, in particular, most strongly (and significantly) stimulated soil C loss (Figure 5.5). Firstly, some studies have reported the magnitude of root-mediated soil C loss to vary among different plant species, attributed to differences in the quantity and lability of the exudates from different root communities (Cheng et al., 2003, Wang et al., 2016). Plant species distributions across the gradient may have therefore influenced the response. Alternatively, the intensity of photosynthesis can determine the degree of root-mediated soil C loss, by directly influencing the flow of photosynthates to roots (Högberg et al., 2001, Kuzyakov, 2002), with one study reporting a positive relationship between plant productivity and soil C loss (Dijkstra et al., 2006). Frequent cloud immersion at higher elevations across
the gradient, known to reduce photosynthetically active radiation (Letts and Mulligan, 2005, van de Weg et al., 2012) may have therefore diminished the quantity of root exudation by plants at the montane sites, relative to the lowland forest, lessening the potential for strong root-soil interactions. Indeed, process-based modelling of gross primary productivity (GPP) along the studied gradient revealed that across the montane forest sites, differences in photosynthetically active radiation strongly influenced GPP, whilst temperature was important in explaining differences in GPP among lowland and montane regions (van de Weg et al., 2014). Cloud immersion is likely to reduce with future climatic warming (Foster, 2001), suggesting that if these factors do explain the substantial net C loss from ingrowth soil cores at the lowland site, then increased root-exudation (as a consequence of reduced cloud immersion and increasing temperature), together with climate-related changes to the species composition of root communities (Feeley et al., 2011, Duque et al., 2015) could induce future C loss from soils across higher-elevation montane regions.

Significant depletion of N was also evident from the Tres Cruces 3644m-210m and San Pedro 1500m-210m ingrowth soils following 24 months at the 210 m site (Figure 5.5). It is postulated that root-soil interactions may be an important mechanism for enhancing N supply to plants in systems constrained by low N-availability (Drake et al., 2011, Cheng et al., 2014, Murphy et al., 2015, Nie and Pendall, 2016), but may be less prevalent in systems constrained by P, because microbial acquisition of P is decoupled from decomposition of SOM (Dijkstra et al., 2013). The prevailing theory states that strongly weathered and leached tropical lowland soils will be most deficient in rock-derived P (Walker and Syers, 1976, Reed et al., 2011a) whilst N remains relatively abundant (Hedin et al., 2009). Consistent with this, high microbial-demand for P (relative to N) has been observed in the lowland soils from this study gradient (Nottingham et al., 2015a). However, another study at this lowland site observed that the relative growth rate of trees was enhanced by fertilisation with N and P together, compared to tree growth in control plots and those fertilised with N and P separately (Fisher et al., 2013), suggesting that plants are co-limited by both N and P. Root-soil interactions therefore, may be an important mechanism for increasing the availability of N for uptake by plants in this lowland forest. Further consideration of nutrient dynamics in soils with and without root growth is required to more fully interpret the
response (particularly of mineralised NO₃, NH₄ and PO₄). Nonetheless, irrespective of the underlying mechanism, root-soil interactions resulted in net C loss from montane soils under an experimental warming treatment, indicating that root-soil interactions could represent a quantitatively important mode of future C loss across montane systems.

**Roots increase the apparent temperature sensitivity of soil respiration**

The apparent temperature sensitivity of soil respiration, including the contribution from roots (Rh + Rr), was consistently greater compared to the temperature sensitivity of heterotrophic soil respiration alone (Rh; Figure 5.6 and Table 5.5). This was in agreement with temperate (Boone et al., 1998), boreal (Gaumont-Guay et al., 2008) and arctic (Grogan and Jonasson, 2005) studies (where $Q_{10}$ coefficients were derived from seasonal temperature variation), as well as previous findings from the elevation gradient in Peru where the $Q_{10}$ of soil respiration (Rh + Rr) was typically higher and more variable (Zimmermann et al., 2010a) compared to $Q_{10}$ values determined for Rh (with exclusion of roots) (Zimmermann et al., 2009a). Several different factors may have contributed to this response.

Firstly, root metabolic processes may have an intrinsically higher $Q_{10}$ compared to microbial metabolism. However, other studies examining the temperature sensitivity of soil respiration have reported root-rhizosphere respiration to be decoupled from temperature and instead dependent on the supply of photosynthates to roots (Bhupinderpal-Singh et al., 2003, Tang et al., 2005, Hartley et al., 2007). As such, the higher apparent temperature sensitivities observed for Rh + Rr in this study were likely strongly influenced by differences in root productivity across the gradient, assuming that greater root productivity results in greater soil CO₂ efflux. This would only confound findings if the differences were unrelated to temperature, yet a previous study across the Peruvian gradient found that root productivity was strongly related to temperature, and unrelated to differences in soil moisture and precipitation (Girardin et al., 2010). Thus, greater root productivity (and associated root exudation) at the warmest, lowest elevation site (Girardin et al., 2013) likely contributed to a higher gross Rr-CO₂ efflux (Figure 5.2d), rather than intrinsically greater CO₂ per g of root
with increasing temperature. This is further supported by the measurements of Rr-CO₂ normalised per g root biomass (Figure 5.4) as, despite marked temperature variation across the gradient, and irrespective of differences in plant species composition, Rr-CO₂ g⁻¹ root was largely consistent (c.1-2 µmol CO₂ m⁻² s⁻¹ g⁻¹) at all elevations (with the exception of Rr from soils translocated downslope to 210 m where root-soil interactions appear to have stimulated additional soil C loss). This indicates that while climatic warming may not directly enhance the efflux of CO₂ from roots (low intrinsic temperature sensitivity), temperature-related increases in root productivity could increase the Rr-component of soil respiration, in turn increasing the observed, apparent temperature sensitivity of soil respiration to warming.

While the apparent Q₁₀ of Rh + Rr was consistently greater than the apparent Q₁₀ of Rh for all soils, considerable variation among soils was also evident (Table 5.5). A previous study of the Peruvian gradient reported the Q₁₀ of total soil respiration (Rh + Rr; derived from seasonal temperature variation in-situ) to be positively related to the proportion of soil C contained within particulate organic matter (SOM unprotected from microbial decomposition) (Zimmermann et al., 2012). In the study presented here, the Tres Cruces 3644m (Figure 5.6a) and San Pedro 1500m (Figure 5.6c) soils exhibited the greatest apparent Q₁₀ of Rh + Rr. These were also the soils for which root-soil interactions at the warmest-210 m site stimulated high Rr-CO₂ effluxes (Figure 5.2d) and associated soil C loss (Figure 5.5), indicating that the organic matter in these soils was amenable to microbial and root-mediated decomposition. We may therefore expect to observe the greatest temperature sensitivity of soil respiration to future climatic warming where soils are less physically and chemically protected (Zimmermann et al., 2012), and where parallel warming-related increases in root-productivity (and exudation) stimulates the greatest soil C loss.

Depletion of the labile pool of soil C in control cores over the duration of the study, because fresh C-inputs from above and belowground were excluded, may have also contributed to the lower Q₁₀ derived for Rh compared to Rh + Rr. In a long-term (10 year) soil warming experiment, microbial activity became constrained by depletion of the labile soil C pool, such that soil respiration became insensitive to differences in temperature among control and warmed plots (Melillo et al., 2002, Kirschbaum, 2004). Likewise, in a controlled laboratory study, the Q₁₀ of soil
respiration decreased over time with the reduction of labile-C substrates (Curiel Yuste et al., 2010), indicating that the availability of labile C can strongly influence the temperature sensitivity of heterotrophic respiration (Fissore et al., 2013). As such, microbial C starvation in the control cores over the 24 months following translocation may have inhibited microbial activity and dampened the observed response to temperature, contributing to the lower apparent $Q_{10}$ coefficients for Rh. Differences in the pool of (labile) soil C available to microorganisms may also explain differences in the apparent $Q_{10}$ of Rh among soils (Table 5.5). From the previous translocation study across the Kosñipata transect, soil organic C content was strongly positively correlated with the $Q_{10}$ of Rh (Zimmermann et al., 2009a), a trend broadly reflected by the results presented here whereby estimated $Q_{10}$ coefficients for Rh were lower for the C-poor lowland soil compared to those estimated for the C-rich montane soils (Table 5.2 and Table 5.5).

Microbial-community level responses to long-term warming/cooling imposed by translocation across the gradient may have also contributed to differences in the observed temperature sensitivity of Rh among soils. While reasonable to assume that the same microbial community and enzyme assemblages originated in soil cores sourced from the same elevation (prior to translocation), over the 24 months following translocation, temperature-related community shifts may have occurred. Some studies have reported that microbes appear to acclimate to temperature overtime, dampening the long-term temperature sensitivity of Rh (Bradford et al., 2008, Crowther and Bradford, 2013). However, an experimental study using soils from the Arctic to the Amazon (including soils from the studied Peruvian gradient) found that microbial responses to prolonged change in temperature more often enhanced the temperature sensitivity of Rh (Karhu et al., 2014). Moreover, these enhancing responses were most pronounced for soils originating from cooler systems and for soils with greater ratios of C:N. Given this, it may be expected that microbial-community responses in the montane soils (lower MAT, greater C:N ratio; Table 5.2) more strongly enhanced the $Q_{10}$ of Rh, compared to microbial-responses in the lowland soil, although this requires further investigation.

The lower apparent $Q_{10}$ estimated for lowland soils, compared to montane soils, may however be an artefact of the experimental design, because soil originating
from the lowest elevation site was only translocated upslope and therefore only exposed to experimental cooling. A soil translocation study across a temperate forest gradient reported soils to respond more strongly to warming than to cooling (Luan et al., 2014), raising the possibility that the temperature sensitivity for the lowland soil, derived from the response to cooling, may have underestimated the potential response to warming. Therefore, while translocation experiments are a useful approach for examining the temperature dependence and sensitivity of soil C dynamics, \textit{in-situ} warming experiments are urgently required to more directly examine responses to elevated temperature (Cavaleri et al., 2015, Nottingham et al., 2015c), particularly in lowland forests where translocation cannot be used to directly test responses to warming. Further analysis of different soil C fractions (i.e. particulate and mineral-associated soil organic C) is necessary in order to identify the pool of C available to microorganisms, to aid interpretation of differences in the $Q_{10}$ of respiration among soils (Zimmermann et al., 2012, Doetterl et al., 2015). Additional data is also required to verify the apparent rhizosphere priming effect, and to probe the underlying mechanism. However, despite the need for further inquiry, this study has revealed that root-soil interactions are important in determining soil C dynamics, and could strongly influence the observed temperature sensitivity of soil respiration under future climatic warming, paving the way for further, more focused, investigations.

\section*{5.5 Conclusion}

This large-scale soil translocation experiment across a 3400 m tropical elevation gradient in the Peruvian Andes has demonstrated that roots are likely to play a crucial role in determining future soil C dynamics in response to climatic warming. Root-soil interactions stimulated substantial net C loss from soils following translocation downslope, indicating that warming-driven changes to root productivity, exudation and/or root-species composition could stimulate considerable loss of C from soils. Root-mediated soil C loss could therefore represent a quantitatively important mode of future C loss from montane soils, potentially acting as a positive feedback to climate change, depending on the extent to which increased assimilation of C by plants balance these soil C losses. The presence of roots also increased the observed, apparent
temperature sensitivity of total soil respiration (Rh + Rr), compared to the apparent temperature sensitivity of soil respiration with exclusion of roots (Rh). Together these results suggest that predictions of how tropical soils will respond to climatic warming determined from studies of the temperature sensitivity of heterotrophic respiration alone, without consideration for the root-rhizosphere component, have likely underestimated the magnitude of their future response to changes in temperature.
Supplementary Information

Supplementary Table S5.1: soil pH measured from control and ingrowth soil-cores 24 months after translocation among four elevation sites (210 m, 1500 m, 3025 m, 3644 m). Measured from homogenised sampled bulked across three replicates, which precluded estimate of variance.

<table>
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<tr>
<th>Destination</th>
<th>Tres Cruces 3644 m Soil</th>
<th>Wayqecha 3025 m Soil</th>
<th>San Pedro 1500 m Soil</th>
<th>Tambopata 210 m Soil</th>
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<tbody>
<tr>
<td></td>
<td>Control Core</td>
<td>Ingrowth Core</td>
<td>Control Core</td>
<td>Ingrowth Core</td>
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<tr>
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</tr>
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</tbody>
</table>
Supplementary Figure S5.1 a-b: respired CO$_2$ (µmol m$^{-2}$ s$^{-1}$) from soils (Tres Cruces$_{3644}$m, Wayqecha$_{3025}$m, San Pedro$_{1500}$m, Tambopata$_{210}$m) over 24 months following translocation to (a) 3644 m and (b) 3025 m. Solid circles represent control soil cores (Rh) and open circles represent ingrowth soil cores (Rh + Rh). Data represent mean ± 1SE ($n = 3$). Red dashed line represents soil temperature (0-10 cm depth, internal soil cores, secondary axis), and blue/white shading represents wet/dry seasons. Note difference in y-axis scales.
Supplementary Figure S5.1 c-d: respired CO$_2$ (µmol m$^{-2}$ s$^{-1}$) from soils (Tres Cruces$_{3644}$m, Wayqecha$_{3025}$m, San Pedro$_{1500}$m, Tambopata$_{210}$m) over 24 months following translocation to (c) 1500 m and (d) 210 m. Solid circles represent control soil cores (Rh) and open circles represent ingrowth soil cores (Rh + Rh). Data represent mean ± 1SE ($n = 3$). Red dashed line represents soil temperature (0-10 cm depth, internal soil cores, secondary axis), and blue/white shading represents wet/dry seasons. Note difference in y-axis scales.
**Supplementary Figure S5.2**; estimation of disturbance effect on soil CO₂ efflux (Rh + Rr) at (a) 3644 m, (b) 3025 m, (c) 1500 m, (d) 210 m due to excavation and re-installation of soil cores. Closed squares indicate CO₂ efflux from undisturbed controls (measured from undisturbed soil collars, adjacent to translocated cores at each elevation site), and open squares indicate CO₂ efflux from disturbed soil cores (ingrowth cores, excavated and re-installed at the same elevation site). Data represent mean ± 1SE (n = 3). Red dashed line represents soil temperature (°C, 0-10 cm depth, secondary axis), and blue/white shading represents wet/dry seasons respectively.
Supplementary Figure S5.3; variation in moisture content measured from four soils (a) Tres Cruces\textsubscript{3644m}, (b) Wayqecha\textsubscript{3025m}, (c) San Pedro\textsubscript{1500m}, (d) Tambopata\textsubscript{210m} over 24 months following translocation among four elevation sites (3644 m, 3025 m, 1500 m, 210 m). Data represent mean ± 1SE (n = 3). Soil moisture content typically explained a low proportion (3-12%) of variance in respired CO\textsubscript{2} measured from these soils, compared to temperature (see Figure 5.6).
Chapter 6

Nitrogen availability as a determinant of organic matter cycling in tropical upper montane forest and grassland soils

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Contribution to work
LH led and implemented the study, with advice from JW, AN, and PM regarding the experimental design. AS analysed trace gas and PLFA samples for $\delta^{13}$C values, with help from LH. LH analysed the data and wrote the chapter, with all authors contributing to editing of the chapter.

Other Acknowledgements
Grant-in-kind from the NERC Stable Isotope Facility, and Andy Gray (University of Edinburgh, Crew Labs) for chemical analysis of soil samples (total phosphorus and loss on ignition).
Abstract

Tropical soils are a globally important store of terrestrial carbon (C), regulated by soil microorganisms through the mineralisation of plant residues and soil organic matter (SOM). Microbial decomposition of SOM can be accelerated or retarded by the combined effects of plant-derived C and nutrient inputs through a phenomenon known as ‘priming’. However, the extent to which priming responses are dependent on the availability of nutrients in tropical soils remains poorly resolved, especially across gradients of lowland to montane forest and grassland, where soil fertility can vary widely.

To test whether nutrient availability influences the mineralisation of added C inputs and pre-existing SOM, four soils from a 3400 m tropical elevation (and soil fertility) gradient were amended with simple and complex 13C-labelled substrates (as surrogates of plant-derived C) in combination with inorganic nitrogen (N) and phosphorus (P) treatments. Isotopic partitioning was used to identify sources (substrate- or SOM-derived) of respired C.

Nutrient treatments did not influence the amount of substrate-respired C for any of the soils, but did affect the direction and magnitude of priming responses in some cases. For the upper montane forest and grassland soils, +N and +NP induced strong negative priming of SOM, indicating that mineralisation of organic matter in these soils was primarily regulated by microbial demand for N. By contrast, in the lower montane and lowland forest soils, C addition stimulated positive priming of SOM, which was unaffected by additional nutrient treatments, indicating that microorganisms in these soils were primarily constrained by the availability of labile C. The assimilation of 13C substrates into functionally active microorganisms was also traced, revealing that while nutrient treatments did not influence microbial incorporation of added C substrates, C substrate complexity strongly shaped the pathway for microbial C use.

Overall, these results have potentially contrasting implications for the response of C cycling in montane and lowland systems, under future global change. Increased N availability has the potential to reduce SOM mineralisation and increase net C sequestration in higher elevation soils, while increase C inputs may stimulate C loss from lower elevation soils.
6.1 Introduction

Tropical soils are a globally important store of terrestrial carbon (C) (Jobbágy and Jackson, 2000), with soil microorganisms playing a decisive role in regulating the soil C cycle through the mineralisation of plant residues and soil organic matter (SOM). However, because C and nutrient cycles are tightly coupled according to microbial metabolic demands (Cleveland and Liptzin, 2007, Finzi et al., 2011) the availability of essential nutrients can constrain microbial activity (Cleveland et al., 2002, Fisher et al., 2013) and hence the rate of C turnover in soils. In the tropics, atmospheric nutrient deposition (Okin et al., 2004, Boy et al., 2008, Hietz et al., 2011) is likely to increase soil nutrient availability, while climatic warming together with elevated concentrations of atmospheric carbon dioxide (CO₂) may also increase inputs of labile plant-derived C (Wood et al., 2012, Cernusak et al., 2013). Yet our ability to predict how soil-C turnover will be affected by changes to plant-derived C and nutrient supply is limited by poor understanding of the mechanistic interactions between C inputs, nutrient availability and soil microorganisms, which operate to determine soil C dynamics in tropical ecosystems.

Microbial decomposition of SOM can be accelerated or retarded by the combined effects of plant-derived C and nutrient inputs through a phenomenon known as ‘priming’. Priming effects are defined as a change to the mineralisation of SOM in response to labile inputs of C or nutrients (Kuzyakov et al., 2000, Qiao et al., 2016), resulting in enhanced (positive priming) or reduced (negative priming) mineralisation of SOM. The direction of priming is often mediated by soil nutrient availability and microbial nutrient demand (Dijkstra et al., 2013). For example, in soils where nutrient availability is low, inputs of labile C may be used as a source of energy to support microbial activity, with microorganisms co-mineralising SOM (positive priming) to liberate and acquire ‘limiting’ nutrients from soil (nutrient-mining; Craine et al., 2007, Chen et al., 2014). Positive priming may also occur when C and nutrient inputs correspond to microbial stoichiometric requirements (Cleveland and Liptzin, 2007), which optimises microbial activity and decomposition of SOM (Chen et al., 2014). Whereas, in contrast, negative priming may occur in soils of high nutrient availability if inputs of labile C are used in preference to more recalcitrant SOM (Blagodatskaya et al., 2007). Negative priming may also occur in response to labile nutrient inputs,
particularly in nutrient-poor soils, due to reduced mining of SOM for nutrient acquisition (Janssens et al., 2010, Bird et al., 2011). However, despite the potential opposing impacts on soil C stocks, dependent on the direction of priming, the extent to which nutrient availability determines priming responses in tropical soils remains uncertain. Amendment of tropical lowland soils with inorganic nitrogen (N) and phosphorus (P) treatments in combination with labile C reduced priming of SOM relative to the intensity of priming in response to C alone (Nottingham et al., 2015b), whereas in a different tropical-region study, amendment of lowland and montane forest soils with a nitrogenous C substrate stimulated strong positive priming of SOM (Whitaker et al., 2014a). Improved understanding of the mechanisms which determine the direction of priming responses in tropical soils is therefore required to better predict responses to global change.

Nitrogen and P are essential nutrients required by microorganisms to build and maintain cellular structures, and as such the availability of N and P is critical for microbial growth and activity (Sterner and Elser, 2002, Hartman and Richardson, 2013). As the stoichiometry (C:N:P) of organic matter (Hättenschwiler et al., 2008) is typically wider than that of microbes (Cleveland and Liptzin, 2007), additional N and P must often be acquired from the soil to meet microbial metabolic demands. However, the different sources from which N and P are derived in terrestrial ecosystems means that either element may constrain microbial activity in tropical soils. Strongly weathered and leached lowland soils may be more deficient in rock-derived P (Walker and Syers, 1976, Reed et al., 2011a). Whereas tropical montane forest and grassland soils may be more deficient in N because of slow accumulation via biological N-fixation (due to cooler temperatures; Tanner et al., 1998) while P remains abundant due to landslide events and near-surface weathering (Porder et al., 2007). Fertilisation with N and P, in montane and lowland forests respectively, has been shown to increase microbial respiration (Cleveland et al., 2002, Fisher et al., 2013) and growth of microbial biomass (Cusack et al., 2011, Turner and Wright, 2014), consistent with the prevailing theory. However, simultaneous addition of N and P in two tropical forest studies stimulated greater microbial respiration (Reed et al., 2011b) and leaf-litter mass loss (Barantal et al., 2012) compared to responses induced by N or P alone, demonstrating the potential for N-P co-limitation. While a change in nutrient
acquisition investment by soil microorganisms along a Peruvian elevation gradient suggests a shift with elevation in the relative demand for P and N by microbes (Nottingham et al., 2015a), there have been few studies utilising the contrasting soils from tropical elevation gradients to discern whether and how microbial demand for nutrients influences C mineralisation, and hence how alleviation of potential nutrient constraints to microorganisms will impact future soil C dynamics.

While the availability of soil nutrients may constrain microbial activity in tropical soils (Cleveland et al., 2002, Fisher et al., 2013), nutrient availability may also influence the relative abundance of different microbial functional groups which exhibit contrasted nutrient requirements (Keiblinger et al., 2010, Strickland and Rousk, 2010). Experimental evidence from tropical and temperate studies suggests that fungi are more limited by N-availability (Liu et al., 2013, Fanin et al., 2015b), whereas bacteria may be more constrained by P-availability (Güsewell and Gessner, 2009, Fanin et al., 2015c). As such, microbial community composition, mediated by the availability of N and P (Fanin et al., 2015b), may have further implications for C turnover in tropical soils, given that different microbial functional groups have been attributed to the mineralisation of labile and more recalcitrant sources of C (Waldrop and Firestone, 2004, Fontaine and Barot, 2005, Strickland et al., 2009a). For example, increased bacterial abundance in tropical soils facilitated mineralisation of labile C compounds, whereas fungal growth was associated with the mineralisation of more recalcitrant soil C fractions (Cusack et al., 2011). Taken together, these findings suggest that mineralisation of more complex C substrates and recalcitrant SOM, primarily by fungi, may be dependent on N-availability whereas mineralisation of more labile C substrates, by bacteria, could be more dependent on P-availability. However, further testing is required to assess the extent to which fungi and bacteria are differentially constrained by N and P in contrasting tropical soils, and how these differences, in turn, influence mineralisation of simple and more complex C substrates.

It is evident that complex interactions between nutrient availability and soil microorganisms strongly influence the dynamics of soil C cycling across tropical ecosystems. Yet further mechanistic understanding is required to more comprehensively predict how these interactions will determine future soil C dynamics, particularly in response to increased C inputs (Wood et al., 2012, Cernusak et al., 2013).
and nutrient availability (Okin et al., 2004, Hietz et al., 2011), as consequences of global change. To examine how soil microorganisms use simple and more complex C substrates, and whether the use of these substrates and fate of pre-existing SOM is dependent on availability of N and P, four soils from a 3400 m tropical elevation and soil fertility gradient were amended with two chemically contrasting $^{13}$C-labelled substrates (simple and complex) in combination with inorganic nutrient treatments (+N, +P, +NP) to test the following hypotheses:

H1. Amendment of soils with ‘limiting’ nutrients (predicted as N for montane forest and grassland soils, and P for lowland forest soils), will stimulate microbial activity and enhance mineralisation of added C substrates, with greater mineralisation of simple C compared to more complex C.

H2. Addition of C substrates alone will stimulate positive priming of SOM, as microorganisms will co-metabolise existing SOM to meet nutrient demands, whereas C supplied in combination with a limiting nutrient will induce reduced or negative priming (due to reduced nutrient-mining).

H3. Fungal assimilation of more complex C will be enhanced by N addition (particularly in montane forest and grassland soils), whereas bacterial assimilation of simple C will be enhanced by P addition (particularly in lowland forest soils).

### 6.2 Materials and Method

#### Study sites and field sampling

Soils were sampled in January 2015 from four sites along a 3400 m tropical elevation gradient located on the eastern flank of the Peruvian Andes (Table 6.1; Malhi et al., 2010). The three highest elevation sites are located in or close to the Manu National Park, centred on the Kosñipata Valley. The lowest elevation site is in the Tambopata Reserve in the lowland Amazon basin, 240 km east of the main Kosñipata transect. All sites from lowland Amazonian rainforest to upper montane cloud forest have continuous forest cover. Montane grassland occurs above the treeline, which is situated at approximately 3500 m above sea level.
Soil fertility and microbial community composition change distinctly with elevation along the gradient. Detailed description of these elevational trends is provided by Whitaker et al. (2014b) and Nottingham et al. (2015a). Briefly, total C, N and P in soils and their ratios (C:N, C:P, N:P) generally increase with elevation. Extractable PO$_4$-P also typically increases with elevation, while mineralised N (NH$_4$ + NO$_3$) decreases with elevation (Nottingham et al., 2015a). These elevational trends do not wholly extend to the grassland soils where mineralised N (NH$_4$ + NO$_3$) and extractable PO$_4$ are both very low (A. Nottingham, personal communication), suggesting low N and P availability despite moderate concentrations of total N and P in these soils (Table 6.2). The ratio of fungi to bacteria in soils increases with elevation, while the ratio of gram positive to gram negative bacteria decreases with elevation (Whitaker et al., 2014b).

Organic soil was collected from four subplots outside the perimeter of the 1 ha permanent study plots at each elevation, with soil from these subplots used as four individual replicates. Soil from the organic layer was used for this experiment to enable comparison among soils from different elevations which vary markedly in the depth of the organic horizon (Table 6.1). Soils were transported in sealed plastic bags to the laboratory in the UK and refrigerated at 4 °C for 4 weeks between collection and the start of the experiment.
Table 6.1: summary of site characteristics and organic soil properties (mean annual temperature; MAT, mean annual precipitation; MAP, water holding capacity; WHC). Data represent mean (1SE), where \( n = 4 \) from 4 subplots at each elevation site.

<table>
<thead>
<tr>
<th>Elevation (m asl)</th>
<th>MAT (°C)</th>
<th>MAP (mm yr(^{-1}))</th>
<th>Soil Classification</th>
<th>Organic Layer (cm)</th>
<th>Soil LOI(^\circ) (%)</th>
<th>80% Max. WHC (g H(_2)O g(^{-1}) fwt soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata</td>
<td>210</td>
<td>26.4</td>
<td>Haplic Cambisol</td>
<td>1.5 (0.3)</td>
<td>28.7 (3.2)</td>
<td>0.55</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>17.4</td>
<td>Cambisol</td>
<td>6.0 (0.4)</td>
<td>69.0 (6.1)</td>
<td>0.69</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>11.1</td>
<td>Umbrisol</td>
<td>13.3 (1.2)</td>
<td>91.2 (1.4)</td>
<td>0.74</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>6.5</td>
<td>Umbrisol</td>
<td>2.7 (0.2)</td>
<td>51.4 (1.6)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

\(^\circ\) Loss on ignition (LOI) as estimation of organic matter content

* Measured at Acjanaco, 3450 m asl (Oliveras et al., 2014)
Soil analyses

Soil from each subplot was gently homogenised by hand, removing stones, woody debris, coarse roots and un-decomposed leaf fragments. Fresh subsamples of soils were used to measure gravimetric water content (soil oven dried to constant mass at 105 °C) and pH (soil: H₂O, 1:2.5 w: v, Hanna Instruments, USA). Organic matter content was estimated from mass loss on ignition by heating dried and ground subsamples at 500 °C for 8 hours in a muffle furnace. Dried (60 °C until constant mass) and ground subsamples were used to measure total C and N content, using a TruSpec CN elemental analyser (LECO, USA). Total P content was measured using a modified dry ash procedure (Enders and Lehmann, 2012) with sulphuric acid-hydrogen peroxide digestion followed by phosphate detection by automated molybdate colorimetric analysis (Bran Luebbe AA3 Autoanalyser, Germany).

Maximum water holding capacity (WHC) was measured using composite samples of soils from each elevation (homogenised composite of soil from four subplots from each elevation). Soils were saturated with deionised water and left to drain for 6 hours in a fully humid airspace, with top of funnels covered with plastic film to prevent evaporation, before drying at 105 °C to constant mass to calculate water content (Öhlinger and Kandeler, 1996). Soil moisture content was standardised across soils to 80 % maximum WHC (by addition of sterile deionised water where necessary, allowing for later addition of C and nutrient treatments in 400 μl + 400 μl solution per assay), chosen so that microbial respiration was not constrained by very low or high soil water content (Meir et al., 1996, Davidson et al., 2000).

Amendment of soils with 13C-labelled substrates and labile nutrients

To determine if soil nutrient availability influenced microbial mineralisation of C substrates and SOM, soils were amended with 13C-labelled xylose or hemicellulose in combination with three inorganic nutrient treatments, plus controls, in a fully factorial design. For controls, sterile deionised water was added in place of C and/or nutrient treatments. Xylose (Sigma-Aldrich, UK) and hemicellulose (IsoLife, The Netherlands) were selected as two ecologically relevant C substrates, where xylose represented a simple C substrate (monosaccharide, building block for hemicellulose) and
hemicellulose a more complex molecule (polysaccharide, constituent of plant cell wall; Figure 6.1). $^{13}$C-labelled xylose and hemicellulose of 10.8 atom % enrichment were created by mixing uniformly $^{13}$C-labelled substrates (whereby $^{13}$C was evenly distributed across the C molecule) with equivalent non-labelled substrates. Carbon substrates were added in concentration of 0.2 mg C g$^{-1}$ fwt soil, equivalent to 0.16 -0.33 % of total C in the soils (Table 6.2). This dose rate was chosen based upon experimental findings by Whitaker et al. (2014b) whereby this concentration of added C equated to 53-100 % of initial microbial biomass C, such that microbial activity and respiration would be stimulated by the added substrate without inducing a significant increase in microbial growth (Blagodatskaya and Kuzyakov, 2008). Xylose and hemicellulose treatments were prepared by dilution in sterile deionised water so that each substrate was added in 400 µl solution per assay. As hemicellulose is insoluble, it was diluted into suspension by sonication and vortexed for 5 seconds prior to addition to soils.

Figure 6.1; chemical structure of xylose ($C_5H_{10}O_5$), simple C substrate (left) and hemicellulose ($C_6H_{10}O_5$), more complex C substrate (right).

Three inorganic nutrient treatments (N, P and N+P) were included in the experiment. Nitrogen was added as ammonium nitrate (NH$_4$-NO$_3$), phosphorus as monosodium phosphate (NaH$_2$PO$_4$) and a combined N+P treatment where ammonium nitrate and monosodium phosphate were added in solution together. Concentrations of these nutrient treatments were determined to correspond to mean C:N:P stoichiometry of
soil microbial biomass (60:7:1) which was reported to be tightly constrained at a global scale (Cleveland and Liptzin, 2007). Hence, soils were amended with N and P treatments in a fixed ratio with the added C substrate (0.2 mg C g\(^{-1}\) fwt soil), with C:N ratio of 60:7 (equivalent to 0.02 mg N g\(^{-1}\) fwt soil) and C: P ratio of 60:2 (equivalent to 0.007 mg P g\(^{-1}\) fwt soil). Phosphorus was added in excess of C: P ratio 60:1 in case of immobilisation of phosphate by soil aluminium and iron compounds, which may have been most notable in soils from lowland forest which have a high P-sorption capacity (Quesada et al., 2010). Nutrient treatments were prepared by dissolving into sterile deionised water so that each treatment was added in 400 \(\mu\)l solution per assay.

Prior to the start of the experiment, soils were pre-incubated in the dark at 16.0 °C (chosen as the average MAT of the four soils used in experiment; Table 6.1) for 24 hours to allow equilibration to the experimental incubation temperature. Aliquots of 8.0 g fwt soil were placed into 160 ml glass Wheaton bottles (Wheaton Science Products, USA) before the soils were amended with one of the \(^{13}\)C-labelled substrates (xylose, hemicellulose or control, sterile de-ionised water) in combination with one of the nutrient treatments (+N, +P, +NP, or control, sterile deionised water). The headspace of each bottle was flushed with compressed air for 1 minute to achieve a standard starting atmosphere before bottles were sealed with butyl rubber stoppers and aluminium crimp caps. Bottles were over-pressurised by injecting 20 ml compressed air, to partly compensate for subsequent headspace gas sampling, and were incubated at 16.0 °C in the dark for 7 days. In total, 192 soil assays were incubated. Samples of compressed air were taken to measure starting gas concentrations, at time 0. Two headspace gas samples were taken from each bottle at 3 time points (24 hours, 48 hours and 168 hours after C substrate-nutrient addition) by taking 5.0 ml gas samples with an air-tight syringe and injecting into 3 ml evacuated extainer vials (Labco, UK), to determine the evolution of CO\(_2\). After 7 days, at the end of the experiment, soils were frozen at -80°C and freeze dried for analysis of phospholipid fatty acid (PLFA) biomarkers.
CO₂ and δ¹³C-CO₂ analyses

The concentration of CO₂ in gas samples were determined using a PerkinElmer Autosystem Gas Chromatograph (GC; PerkinElmer, USA) fitted with a flame ionization detector containing a methaniser (Case et al., 2012), with results calibrated against certified gas standards of 500, 1000, 4000 and 8000 parts per million CO₂ (BOC Ltd. Guildford, UK). Carbon dioxide fluxes were calculated using the linear accumulation of CO₂ concentrations in headspace gas samples from 0, 24, 48 and 168 hours, using the approach described by Holland et al. (1999). Linear fluxes best described the data, whereby the Pearson coefficient for accumulation of CO₂ with time was greater than 0.95 for each bottle. The total CO₂ flux was calculated on a soil dry mass basis (CO₂-C μg g⁻¹ soil dwt day⁻¹) and also on a soil C mass basis (CO₂-C μg g⁻¹ soil C day⁻¹), to normalise for differences in C content among soils.

δ¹³C values of CO₂ were measured by isotope ratio mass spectrometry (IR-MS) using a trace-gas preconcentrator coupled to an isotope ratio mass spectrometer (IRMS, Isoprime Ltd., UK). Between 5 and 60 μl headspace gas sample (dependent on the concentration of CO₂ in the sample, previously determined by GC) was manually introduced into the injection port of the pre-concentrator using a gas-tight syringe. Water was eliminated via a perchlorate chemical trap and the CO₂ cryogenically preconcentrated by liquid nitrogen prior to gas chromatography column separation and introduction to the Micromass Isoprime IRMS via an open split. The resultant ¹³C:¹²C isotope ratio was compared to pulses of known reference CO₂ (PDB standard) to determine δ¹³C values of CO₂. The instrument was calibrated on each day of analysis, with blanks run prior to each batch in addition to duplicate analysis after every 12th sample and quality control reference CO₂ samples, with precision of greater than or equal to ± 0.2 ‰.

PLFA and δ¹³C-PLFA analyses

Microbial community composition was determined by analysis of PLFA biomarkers extracted from soils after the 7 day experimental period. A subset of soils were extracted for PLFAs due to time and cost restrictions. Soils from Wayqecha (montane forest; 3025 m) and Tambopata (lowland forest; 210 m) which had been amended with
xylose plus N/P/control and with hemicellulose plus N/P/control were selected, to identify how the addition of +N or +P affected the assimilation of simple and more complex C substrates by different microbial functional groups (to address H3). For these assays, three out of four replicate soils were chosen randomly. Untreated control soils (no added C, no added nutrient treatment) were also extracted for microbial PLFAs to determine the natural abundance of $^{13}\text{C}$-PLFA in soils. Phospholipids were extracted as part of the total lipid extract from 0.50 g freeze-dried and ground samples of soil, by a Bligh-Dyer extraction method (Frostegård et al., 1993, Bardgett et al., 1996), described fully in Appendix B. Individual PLFAs were identified by gas chromatography-mass spectrometry (GC-MS) using an Agilent Technologies 5973 Mass Selective Detector coupled to an Agilent Technologies 6890 GC. Concentrations of identified PLFAs were determined via an internal standard (methyl nonadecanoate C19:0, Sigma Aldrich, UK) and calculated on a soil mass basis ($\mu\text{g PLFA g}^{-1}\text{ dwt soil}$).

To assess differences in microbial community composition in soils amended with different C substrates and nutrient treatments, gram positive bacteria (GP bacteria), gram negative bacteria (GN bacteria) and fungi (F) were identified by specific biomarkers indicative of these functional groups. PLFAs were defined by standard nomenclature, as described by Frostegard et al. (1993). Gram-positive bacteria were identified by the terminal and mid-chain branched fatty acids $i$-$15:0$, a-$15:0$, i-$16:0$, i-$17:0$, a-$17:0$ and GN bacteria by cyclopropyl saturated 7,cy-$17:0$ and 7,8cy-$19:0$ and mono-saturated 16:1$\omega7$ and 18:1$\omega7$ fatty acids (Rinnan and Bååth, 2009). Saprotrophic fungi were identified by 18:2$\omega6,9$ and 18:1$\omega9$ fatty acids (Kaiser et al., 2010, De Deyn et al., 2011). Although the 18:1$\omega9$ marker can also be present in bacteria (Schoug et al., 2008, Wallander et al., 2013), 18:1$\omega9$ and 18:2$\omega6,9$ markers tend to be correlated in forest soils (Frostegård et al., 2011). Here, the two markers were strongly correlated (Spearman’s coefficient = 0.83, $n = 42$; Supplementary Figure S6.1) and as such both markers were used to indicate fungal abundance. The ratios of F:B and GP:GN bacteria were calculated to represent the relative abundance of these functional groups. The total concentration of PLFAs ($\mu\text{g PLFA g}^{-1}\text{ dwt soil}$) in samples were calculated from all identified PLFAs (14:0, 15:0, 16:0, 16.1, 16:1$\omega5$, 17:0, 17:1$\omega8$, 10Me17:0, 17:1$\omega7$, 18:0, 10Me18:0 and18:1$\omega5$), in addition to those listed above as biomarkers for fungal and bacterial functional groups.
δ¹³C values of individual PLFAs were analysed using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Compounds were separated using an Agilent Technologies 6890 GC (splitless mode) with helium carrier gas. The GC effluent was diverted via a heart split union to a ceramic combustion furnace packed with a double copper wire/platinum wire catalyst which was heated at 860 °C. Prior to analysis, copper wires were oxidised overnight in an oxygenated stream of helium. Water was subsequently removed from the combustion products by passing the effluent through a nafion membrane, prior to the CO₂ entering the IRMS (Isoprime Ltd, UK). A sample of the methanol used for PLFA extractions was measured by continuous flow-elemental analyser-IRMS (CF-EA-IRMS) to determine its δ¹³C value. This value was used to back-correct the PLFA δ¹³C values for the addition of the extra C atom introduced to the molecule during methylation, by application of a mass balance equation (Jones et al., 1991; see ¹³C-PLFA calculations).

**Stable isotope calculations**

Enrichment of ¹³C in CO₂ and PLFAs were expressed as δ¹³C (‰), defined by equation 6.1, where R represents the ratio of ¹³C:¹²C in the sample relative to the PDB standard (0.0112372) (Coleman and Fry, 1991).

\[
\delta^{13}C \text{ (‰)} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000
\]

(Eq. 6.1)

**¹³C-CO₂ calculations**

The percentage of respired CO₂ from added C substrates was calculated for treated soils at 24, 48 and 168 hours, according to equation 6.2 (Nottingham et al., 2012b), derived from a mixing model (equation 6.3). δC is the δ¹³C value from the soil source, determined from Keeling plots as the intercept (x = 0) of the linear regression between
1/CO₂ concentration and δ¹³C (for untreated control soils, no added C or nutrient treatment) (Tu and Dawson, 2005). δL is the δ¹³C value of the labelled substrate (10.8 % atom ¹³C enrichment, δL = 9774.6), and δT is the δ¹³C value of respired CO₂ from treated soils at each time point. These data were used to calculate cumulative substrate derived C respired after 24, 48 and 168 hours for all soil/C substrate/nutrient treatments, reported as μg CO₂-C g⁻¹ soil C, using the approach described by Holland et al. (1999).

\[
\text{% C substrate derived} = \frac{\delta C - \delta T}{\delta C - \delta L} \times 100
\]

(Eq. 6.2)

The mixing model (equation 6.3) assumes that the δ¹³C in treated soils (δT) is equal to the sum of δ¹³C from soil (δC) and δ¹³C from the substrate (δL), whereby the proportion derived from soil and substrate are equal to 100 %.

\[
(Soil + Substrate)(\delta T) = (Soil)(\delta C) + (Substrate)(\delta L)
\]

(Eq. 6.3)

The change in mineralisation of pre-existing SOM following the addition of C substrates and nutrient treatments, hereafter referred to as primed C, was calculated after 24, 48 and 168 hours using a mass balance approach. Primed C (μg CO₂-C g⁻¹ soil C) was estimated from total measured respiration (CO₂ ppm) in treated soils, minus substrate derived respiration (CO₂ ppm) minus basal respiration (CO₂ ppm from control untreated soils). For soils which were only amended with nutrient treatments (no added C substrate), the change in mineralisation of SOM (hereafter referred to as primed C; μg CO₂-C g⁻¹ soil C) was calculated as total measured respiration (CO₂ ppm) in treated soils minus basal respiration (CO₂ ppm from control soils). Primed C could be positive or negative, where positive priming represented additional mineralisation of pre-existing SOM following the addition of C substrates and/or
nutrient treatments and negative priming represented reduced mineralisation of pre-existing SOM following the addition of C substrates and/or nutrient treatments. Substrate derived C and primed C were calculated on a soil C mass basis (to normalise for differences in C content among soils) separately for each bottle, and reported as mean ± 1SE for the 4 replicates for each soil/C/nutrient treatment.

**13C-PLFA calculations**

Isotopic enrichment of individual PLFAs were expressed as $\delta^{13}C_{\text{PLFA}}$ values after correction for the methyl-group added during methanolysis, by equation 6.4, where $n_{\text{PLFA}}$ is the number of C-atoms of the PLFA molecule, $\delta^{13}C_{\text{FAME}}$ is the $\delta^{13}C$ value of the FAME after methylation and $\delta^{13}C_{\text{MeOH}}$ is the $\delta^{13}C$ value of the methanol used for methanolysis ($\delta^{13}C = -54.15 \%o$).

$$\delta^{13}C_{\text{PLFA}} = \frac{[\text{(nPLFA + 1)} \delta^{13}C_{\text{FAME}} - \delta^{13}C_{\text{MeOH}}]}{n_{\text{PLFA}}}$$

(Eq. 6.4)

To determine the assimilation of added C substrates by different microbial functional groups, the percentage of substrate derived C within individual PLFAs was calculated by a modified version of equation 6.2, multiplied by the abundance of the specific PLFA ($\mu g \text{ PLFA-C g}^{-1} \text{ dwt soil}$) (Nottingham et al., 2009), where $\delta C$ is the $\delta^{13}C$-PLFA value from untreated control soils (no added C, no added nutrient treatment), $\delta L$ is the $\delta^{13}C$ value of the labelled substrate (10.8 % atom $^{13}C$ enrichment, $\delta L = 9774.6$), and $\delta T$ is the $\delta^{13}C$-PLFA value from treated soils. These data were used to calculate the actual incorporation of C substrates into individual PLFAs, from which the total C substrate incorporation into all PLFAs ($\mu g \ ^{13}C \text{ PLFA-C g}^{-1} \text{ soil dwt}$) was determined. The proportional substrate incorporation (% $^{13}C$) into different microbial functional groups (F, GP bacteria, GN bacteria, and unspecified PLFAs) were also calculated, as a percentage of the total $^{13}C$ substrate incorporated into all PLFAs, in order to identify if P and N treatments influenced the assimilation of simple and more complex C by
bacteria and fungi respectively (H3). Microbial substrate-carbon use efficiency (CUE) was calculated as the total $^{13}$C-substrate incorporated into PLFAs (used for growth) relative to total $^{13}$C-substrate consumed ($^{13}$C-incorporated + $^{13}$C-respired) (Keiblinger et al., 2010). Finally, to examine the incorporation of soil-derived C by different microbial functional groups in response to C and nutrient treatments, concentrations of excess soil-derived C in PLFAs (µg soil C g$^{-1}$ soil) were determined by a mass balance approach, as the total concentration of PLFA-C in treated soils, minus PLFA-C in untreated control soils, minus substrate-derived PLFA-C (Nottingham et al., 2009). Positive excess soil-derived C in PLFAs represented increased assimilation of C from SOM and negative excess soil-derived C represented reduced assimilation of C from SOM, relative to untreated control soils.

**Data analysis**

Statistical analyses were performed using R, version 3.2.1 (R Core Team, 2015). Main and two-way interactive effects of ‘Soil’, ‘C Substrate’ and ‘Nutrient Treatment’ on respired CO$_2$, substrate derived C and primed C were tested by analysis of variance (ANOVA). To assess if the availability of P and N affected the assimilation of C substrates by different microbial functional groups (H3), main and two-way interactive effects of ‘Soil’, ‘C Substrate’ and ‘Nutrient Treatment’ on microbial incorporation of $^{13}$C substrates were also tested by ANOVA. Data analysed included actual $^{13}$C incorporation into total PLFA (µg $^{13}$C PLFA-C g$^{-1}$ soil dwt), microbial substrate-CUE, and the relative proportion (as % of total incorporated) of $^{13}$C incorporated into fungi, GP and GN bacteria. To meet the assumptions of ANOVA, prior to analysis, dependent response variables were first tested for normality using the Shapiro-Wilk test and square-root transformed where necessary. Pair-wise comparisons of significant effects were conducted using Tukey’s HSD post hoc tests, with significant differences identified where p < 0.05.
6.3 Results

Soil biotic and abiotic properties

Soil properties (organic soil) measured from sub-samples prior to the start of the experiment are summarised in Table 6.2. Total soil C was lowest in the lowland forest soil (13.3 %) and greatest in the upper montane forest soil (47.7 %). Total N and P concentrations were also lowest in soil from the lowland forest (0.7 % and 0.011 % respectively), with the greatest concentration of N (2.3 %) in soils from both 1500 m and 3025 m elevations and greatest concentration of P (0.134 %) in the soil from 1500 m. The concentration of total N and P in these soils does not, however, directly correspond to the availability of inorganic N and P. Extractable inorganic PO₄-P is low in the lowland forest soil, and typically increases with increasing elevation, but is also very low in the montane grassland soil. Conversely, mineralised N (NH₄ + NO₃) is higher in soils from the lowland and lower montane forest, compared to soils from the upper montane forest and montane grassland (Nottingham et al., 2015a). Soil pH did not vary markedly among soils from the three forest sites (210 m, 1500 m, 3025 m; pH 3.6-3.9) but was higher in the grassland soil (3644 m; pH 4.9).
Table 6.2: soil properties (organic soil). Data represent mean (1SE), where \( n = 4 \) from 4 subplots at each elevation site.

<table>
<thead>
<tr>
<th>Elevation</th>
<th>Soil pH</th>
<th>Total C</th>
<th>Total N</th>
<th>Total P</th>
<th>C:N</th>
<th>C:P</th>
<th>N:P</th>
<th>(^a)Extractable P</th>
<th>(^b)Mineralised N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tambopata</td>
<td>210</td>
<td>3.6 (0.1)</td>
<td>13.3 (1.1)</td>
<td>0.7 (0.1)</td>
<td>0.011 (0.005)</td>
<td>17.9 (1.0)</td>
<td>491 (110)</td>
<td>27.4 (5.4)</td>
<td>4.39 (110)</td>
</tr>
<tr>
<td>San Pedro</td>
<td>1500</td>
<td>3.8 (0.1)</td>
<td>33.9 (3.6)</td>
<td>2.3 (0.1)</td>
<td>0.134 (0.013)</td>
<td>14.5 (0.8)</td>
<td>268 (57.0)</td>
<td>18.1 (2.8)</td>
<td>44.70 (57.0)</td>
</tr>
<tr>
<td>Wayqecha</td>
<td>3025</td>
<td>3.9 (0.1)</td>
<td>47.7 (1.0)</td>
<td>2.3 (0.1)</td>
<td>0.081 (0.003)</td>
<td>21.0 (0.8)</td>
<td>591 (19.2)</td>
<td>28.3 (1.2)</td>
<td>81.99 (19.2)</td>
</tr>
<tr>
<td>Tres Cruces</td>
<td>3644</td>
<td>4.9 (0.1)</td>
<td>23.7 (0.7)</td>
<td>1.6 (0.1)</td>
<td>0.085 (0.003)</td>
<td>14.6 (0.2)</td>
<td>282 (15.1)</td>
<td>19.3 (1.0)</td>
<td>0.88 (15.1)</td>
</tr>
</tbody>
</table>

\(^a\)Extractable P (organic soil horizon) measured by A. Nottingham (unpublished data)

\(^b\)Mineralised N (0-10 cm depth) determined by Nottingham et al. (2015a and unpublished data for Tres Cruces)
Microbial mineralisation of $^{13}$C substrates and priming of SOM

Basal respiration (CO$_2$ flux in the absence of added C and nutrient treatments) under controlled temperature and soil moisture conditions varied 3.2-fold among soils when compared on a soil dry mass basis (Supplementary Figure S6.2) and 2.7-fold among soils when compared on a soil C mass basis (Figure 6.2). On a soil dry mass basis, the lowest flux was from the lowland forest soil ($33.9 \pm 6.2 \mu$g CO$_2$-C g$^{-1}$ soil dwt day$^{-1}$) and the highest flux from the montane grassland soil ($110.8 \pm 4.0 \mu$g CO$_2$-C g$^{-1}$ soil dwt day$^{-1}$), whereas on a soil C mass basis the lowest flux was measured from lower montane forest soil ($172.6 \pm 12.5 \mu$g CO$_2$-C g$^{-1}$ soil C day$^{-1}$) and the highest flux from the montane grassland soil ($469 \pm 28.5 \mu$g CO$_2$-C g$^{-1}$ soil C day$^{-1}$). Henceforth, C fluxes (total respired C, substrate C, primed C) will be presented on a soil C basis, to normalise for differences in C content among soils.

Respired CO$_2$ from soils following amendment with C substrates in combination with nutrient treatments are illustrated in Figure 6.2. A significant interactive effect of ‘Soil x C Substrate’ on respired CO$_2$ was identified by ANOVA (Table 6.3), with post-hoc tests revealing that, typically, respired CO$_2$ was significantly greater following amendment with xylose compared to un-amended (no added C) soils. This was with the exception of the soil from Tres Cruces (3644 m) where there was no significant difference among un-amended and xylose-amended soils (Figure 6.2). Respired CO$_2$ from the Tambopata (210 m) soils amended with hemicellulose were also significantly greater compared to un-amended soils, but this relationship did not hold for the other soils. Although no significant effect of nutrient treatment on respired C was identified by ANOVA (Table 6.3), post-hoc tests revealed that amendment of soils from Tres Cruces (3644 m) with N (both alone and in combination with C) significantly reduced respired CO$_2$-C relative to controls (Figure 6.2).
Total resired CO$_2$-C was partitioned into that derived from added C substrates (substrate C) and from mineralisation of pre-existing SOM (primed C). Following amendment of soils with xylose and hemicellulose, substrate-derived C tended to increase rapidly between 0-48 hours, before the rate reduced up to 168 hours as the substrate was depleted (Figure 6.3). The cumulative amount of substrate-derived C resired after 168 hours varied among soils and C substrates, with a significant ‘Soil x C Substrate’ interaction (Table 6.4). After 168 hours, resired C from xylose (simple C) was significantly greater than the amount resired from hemicellulose (complex C), for all four soils. This relationship (xylose $>$ hemicellulose) was consistent among soils, despite significant differences in the overall amount of substrate C resired among soils (Figure 6.3). After 168 hours, the lowest substrate-respired C was from the upper montane forest soils (Wayqecha; 3025 m) in response to hemicellulose (hemicellulose +NP; 396 ± 51 μg CO$_2$-C g$^{-1}$ soil C) with the highest from the lowland forest soils (Tambopata; 210 m) amended with xylose (xylose + NP; 1309 ± 130 μg CO$_2$-C g$^{-1}$ soil C). There was no significant effect of nutrient treatments on substrate-respired C across all soils.

Table 6.3; main and two-way interactive effects of ‘Soil’, ‘C Substrate’ and ‘Nutrient Treatment’ on resired CO$_2$, tested by ANOVA.

<table>
<thead>
<tr>
<th>Term</th>
<th>d.f.</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respired CO$_2$ CO$_2$-C μg g$^{-1}$ soil C day$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil (S)</td>
<td>3</td>
<td>130.1</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>C Substrate (C)</td>
<td>2</td>
<td>47.9</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Nutrient Treatment (NT)</td>
<td>3</td>
<td>0.8</td>
<td>ns</td>
</tr>
<tr>
<td>S x C</td>
<td>6</td>
<td>2.6</td>
<td>0.02</td>
</tr>
<tr>
<td>S x NT</td>
<td>9</td>
<td>0.6</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>6</td>
<td>0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

Significant effect identified where $p < 0.05$, not significant (ns) where $p > 0.05$. 
Figure 6.2; respired CO$_2$-C (µg g$^{-1}$ soil C day$^{-1}$) from four soils incubated for 7 days at standard temperature (16 °C) and moisture (80 % maximum water holding capacity) following amendment with C substrates; no added C (control), hemicellulose (H) and xylose (X), in combination with nutrient treatments; control (no added nutrient), +N, +P, +NP. Bars represent mean ± 1SE (n = 4).
Figure 6.3 a-d; cumulative substrate respired C (µg CO$_2$-C g$^{-1}$ soil C) from four soils (Tres Cruces, Wayqecha, San Pedro, Tambopata) incubated at standard temperature (16 °C) and moisture (80% maximum water holding capacity) over 168 hours following amendment with xylose (X) and hemicellulose (H) in combination with nutrient treatments (control, +N, +P, +NP). Data represent mean ± 1SE (n = 4). Note differences in y-axis scales.
The direction and magnitude of primed C after 168 hours varied among soils, C substrates and nutrient treatments, with significant ‘Soil x C Substrate’ and ‘Soil x Nutrient Treatment’ interactions identified by ANOVA (Table 6.4). Soils from lower elevations (210 m and 1500 m) exhibited positive or negligible priming responses, dependent on the complexity of added C substrates, whereas soils from higher elevations (3025 m and 3644 m) typically displayed negative priming responses, the magnitude of which was dependent on nutrient treatments.

Amendment of the upper montane forest (Wayqecha, 3025 m) and grassland (Tres Cruces, 3644 m) soils with C substrates had a relatively minor influence on primed C, with no significant difference among priming responses to control (no added C), hemicellulose and xylose treatments (Figure 6.4 a-b). Instead, for these soils, priming responses varied significantly among nutrient treatments, where priming following the addition of +N and +NP (both alone and in combination with C) was more negative compared to untreated controls (in the absence of added nutrients). There was no significant difference among +N and +NP treatments, nor no significant difference in the response to +P and control treatments. Yet, despite generally consistent trends observed among these higher-elevation soils to C substrates and nutrient treatments, the negative priming responses measured from the Tres Cruces (3644 m) soil were typically four-times the magnitude of the responses measured from the Wayqecha (3025 m) soils, when compared on a soil-C mass basis (Figures 6.4 a-b). Furthermore, amendment of the Tres Cruces soil with xylose in combination with +P enhanced the mineralisation of SOM (positive priming), compared to the negative priming response induced by xylose alone (Figure 6.4a), a response not evident from the Wayqecha soil. In contrast to priming responses from the higher elevation soils, the magnitude of priming from the lower montane forest (San Pedro, 1500 m) and lowland forest (Tambopata, 210 m) soils were dependent on the complexity of added C substrates, whereby xylose stimulated significantly greater priming of SOM compared to hemicellulose and untreated controls (no added C; Figure 6.4 c-d). Amendment of these lower elevation soils with labile nutrients however, had no significant additional effect on priming responses, with no significant differences among control, +N, +P and +NP treatments.
Partitioning of gross respired CO$_2$ fluxes (Figure 6.2) revealed that increased CO$_2$-C from soils following amendment with xylose and hemicellulose was almost entirely derived from the added C substrates (Figure 6.3), with SOM a small source of additional C, primarily from the lower elevation soils (210 m and 1500 m) following amendment with the simple C substrate (xylose; Figure 6.4 c-d). Strong negative priming responses (reduced mineralisation of SOM) were evident from the two higher elevation soils (3025 m and 3644 m), particularly following amendment with +N and +NP treatments, both alone and in combination with C substrates (Figure 6.4 a-b). These responses were reflected by generally lower gross CO$_2$ fluxes when C was added in combination with +N and +NP, relative to gross respired CO$_2$-C in response to C substrates added alone (without nutrient treatment) for these soils (Figure 6.2).
Figure 6.4 a-d: cumulative primed C (µg CO₂-C g⁻¹ soil C) from four soils (Tres Cruces, Wayqecha, San Pedro, Tambopata) measured after 168 hours incubation at standard temperature (16 °C) and moisture (80% maximum water holding capacity) following amendment with C substrates (no added C, xylose and hemicellulose) in combination with nutrient treatments (control, +N, +P, +NP). Bars represent mean ±1 SE. Note different y-axis scales. Primed C after 24 hours, 48 hours and 168 hours following amendment illustrated in Supplementary Figure S6.3. Two-way ANOVA is presented in Table 6.4.
Table 6.4: main and two-way interactive effects of ‘Soil’, ‘C substrate’ and ‘Nutrient Treatment’ on substrate resired C and primed C after 168 hours, tested by ANOVA.

<table>
<thead>
<tr>
<th>Term</th>
<th>d.f.</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substrate Respired C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μg CO₂-C g⁻¹ soil C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil (S)</td>
<td>3</td>
<td>116.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C substrate (C)</td>
<td>1</td>
<td>166.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nutrient Treatment (NT)</td>
<td>3</td>
<td>0.09</td>
<td>ns</td>
</tr>
<tr>
<td>S x C</td>
<td>3</td>
<td>5.62</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x NT</td>
<td>9</td>
<td>0.18</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>3</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Primed C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μg CO₂-C g⁻¹ soil C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>3</td>
<td>82.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>18.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NT</td>
<td>3</td>
<td>15.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S x C</td>
<td>6</td>
<td>2.89</td>
<td>0.01</td>
</tr>
<tr>
<td>S x NT</td>
<td>9</td>
<td>10.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C x NT</td>
<td>6</td>
<td>1.85</td>
<td>ns</td>
</tr>
</tbody>
</table>

Significant effect identified where p < 0.05, not significant (ns) where p > 0.05.

Microbial assimilation of ¹³C substrates and excess soil C

Microbial abundance and community composition in untreated soils varied distinctly between the lowland forest and upper montane forest (Supplementary Figure S6.4), with soils from Wayqecha (3025 m) exhibiting a higher concentration of total PLFAs, higher F:B ratio and lower GP:GN bacteria ratio compared to soil from Tambopata (210 m). The total incorporation of ¹³C substrates in microbial PLFAs varied significantly among soils but not among C substrates nor nutrient treatments (Table 6.5). More ¹³C substrate was incorporated into microbial PLFAs in soil from Wayqecha (3025 m) than into PLFAs in soil from Tambopata (210 m; Figure 6.5). Microbial substrate-CUE varied significantly among soils (Table 6.5), with greater CUE in soils from Wayqecha (3025 m; Figure 6.5). A marginal difference (p = 0.06) in substrate-CUE dependent on the complexity of C substrates was also identified, where CUE of hemicellulose was marginally greater compared to xylose, with this difference more evident in the lowland forest soil.
Table 6.5: main and two-way interactive effects of ‘Soil’, ‘C Substrate’ and ‘Nutrient Treatment’ on incorporation of $^{13}$C by different microbial functional groups (actual incorporation $^{13}$C into total PLFA, microbial substrate-carbon use efficiency (CUE), and relative % $^{13}$C incorporated by fungi, gram positive (GP) bacteria and gram negative (GN) bacteria, as a proportion of total $^{13}$C incorporated), tested by ANOVA.

<table>
<thead>
<tr>
<th>Term</th>
<th>Term d.f.</th>
<th>Term F value</th>
<th>Term P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total $^{13}$C PLFA-C</td>
<td>Soil (S)</td>
<td>1</td>
<td>125.7</td>
</tr>
<tr>
<td>Actual $^{13}$C C Substrate (C)</td>
<td>1</td>
<td>1.12</td>
<td>ns</td>
</tr>
<tr>
<td>Incorporation (µg C g$^{-1}$ soil dwt)</td>
<td>Nutrient Treatment (NT)</td>
<td>2</td>
<td>0.26</td>
</tr>
<tr>
<td>S x C</td>
<td>1</td>
<td>1.67</td>
<td>ns</td>
</tr>
<tr>
<td>S x NT</td>
<td>2</td>
<td>0.26</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>2</td>
<td>0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Microbial Substrate-CUE S</td>
<td>1</td>
<td>63.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>3.77</td>
<td>0.06</td>
</tr>
<tr>
<td>NT</td>
<td>2</td>
<td>0.42</td>
<td>ns</td>
</tr>
<tr>
<td>S x C</td>
<td>1</td>
<td>1.29</td>
<td>ns</td>
</tr>
<tr>
<td>S x NT</td>
<td>2</td>
<td>0.34</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>2</td>
<td>0.10</td>
<td>ns</td>
</tr>
<tr>
<td>% $^{13}$C Fungi S</td>
<td>1</td>
<td>24.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>12.1</td>
<td>0.002</td>
</tr>
<tr>
<td>NT</td>
<td>2</td>
<td>0.25</td>
<td>ns</td>
</tr>
<tr>
<td>S x C</td>
<td>1</td>
<td>0.91</td>
<td>ns</td>
</tr>
<tr>
<td>S x NT</td>
<td>2</td>
<td>0.01</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>2</td>
<td>0.04</td>
<td>ns</td>
</tr>
<tr>
<td>% $^{13}$C GP bacteria S</td>
<td>1</td>
<td>28.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>167.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NT</td>
<td>2</td>
<td>0.32</td>
<td>ns</td>
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<tr>
<td>S x C</td>
<td>1</td>
<td>5.84</td>
<td>0.02</td>
</tr>
<tr>
<td>S x NT</td>
<td>2</td>
<td>0.08</td>
<td>ns</td>
</tr>
<tr>
<td>C x NT</td>
<td>2</td>
<td>0.14</td>
<td>ns</td>
</tr>
<tr>
<td>% $^{13}$C GN bacteria S</td>
<td>1</td>
<td>9.52</td>
<td>0.005</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>13.2</td>
<td>0.001</td>
</tr>
<tr>
<td>NT</td>
<td>2</td>
<td>0.13</td>
<td>ns</td>
</tr>
<tr>
<td>S x C</td>
<td>1</td>
<td>12.5</td>
<td>0.002</td>
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<tr>
<td>S x NT</td>
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<tr>
<td>C x NT</td>
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<td>0.14</td>
<td>ns</td>
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Significant effect identified where p < 0.05, not significant (ns) where p > 0.05.
Figure 6.5; assimilation of $^{13}$C-substrates into microbial PLFAs, and microbial substrate-carbon use efficiency (CUE) in two forest soils (Tambopata; 210 m and Wayqecha; 3025 m), following amendment with C substrates (xylose; X and hemicellulose; H) in combination with nutrient treatments (control; no added nutrient treatment, +N, +P). Data represent mean ± 1SE ($n = 3$), two-way ANOVA is presented in Table 6.5.
The relative proportion of $^{13}$C-substrates incorporated by different microbial functional groups varied significantly among ‘Soils’ and ‘C substrates’ for % $^{13}$C assimilated by fungi, with a significant interactive effect of ‘Soil x C substrate’ on % $^{13}$C assimilation by GP and GN bacteria, identified by ANOVA (Table 6.5). In both soils, fungi assimilated a greater proportion of xylose relative to hemicellulose, although fungi in the lowland forest soil typically incorporated a greater proportion of C substrates compared to C substrate incorporation by fungi in the upper montane forest soils (Figure 6.6). Gram-positive bacteria assimilated a greater proportion of hemicellulose compared to xylose in both soils, with GP bacteria in the Wayqecha (3025 m) soil incorporating a greater proportion of hemicellulose than in the Tambopata (210 m) soil. In the Wayqecha soil, GN bacteria assimilated a much greater proportion of xylose than hemicellulose, whereas C-substrate assimilation by GN bacteria in the Tambopata soil was low, with no significant difference in the relative proportion of xylose and hemicellulose assimilated (Figure 6.6).

Nutrient treatments had no significant effect on total incorporation of $^{13}$C substrates in microbial PLFAs, nor the relative proportion of substrate-C assimilated by different functional groups (Table 6.5). However, nutrient treatments did influence microbial assimilation of excess soil derived-C in some cases (Figure 6.7). In the Wayqecha soil (3025 m), GP bacteria exhibited increased incorporation of soil-derived C following amendment with xylose alone, while incorporation of excess soil-derived C in all microbial functional groups was reduced when xylose was added in combination with +N (Figure 6.7a). In response to hemicellulose, microbial incorporation of soil-derived C was reduced, most strongly in response to hemicellulose alone, and to a lesser degree when hemicellulose was added in combination with +N or +P (Figure 6.7b). In the case of the Tambopata (210 m) soil, amendment with xylose typically had a negligible influence on incorporation of soil-derived C in PLFAs (Figure 6.7c), while amendment with hemicellulose appeared to marginally increase assimilation of soil-derived C in microbial PLFAs (Figure 6.7d), with no clear differences in soil-C incorporation dependent on added nutrient treatments.
Figure 6.6; relative proportion (as % of total substrate incorporation into all PLFAs) of $^{13}$C substrate assimilated into PLFA biomarkers indicative of key microbial functional groups (fungi, gram positive bacteria; GP, gram negative bacteria; GN and unspecified PLFAs), in (a) lowland forest (Tambopata; 210 m) and (b) upper montane forest (Wayqecha; 3025 m) soils after amendment with xylose (X) and hemicellulose (H) in combination with nutrient treatments (control; no added nutrient, +N, +P). Data represent mean ($n = 3$), two-way ANOVA is presented in Table 6.5.
Figure 6.7 a-d; concentrations of $^{13}$C-substrate-derived C ($\mu$g substrate-C g$^{-1}$ dwt soil) and excess soil-derived C ($\mu$g soil-C g$^{-1}$ dwt soil) in microbial PLFAs (gram-positive bacteria; GP, gram-negative bacteria; GN, and fungi) in soils from Wayqecha (3025 m) and Tambopata (210 m) following amendment with C substrates (xylose or hemicellulose) alone and in combination with +N or +P. Microbial groups which appear above the dashed line ($y=0$) exhibited increased incorporation of soil-derived C, whereas those which appear below the dashed line exhibited reduced incorporation of soil-derived C, relative to PLFAs in untreated control soils. Data represent mean ± 1SE ($n=3$). Note different axis scales among panels.
6.4 Discussion

Across all soils, respired C derived from xylose (simple C) was greater than that derived from hemicellulose (more complex C), although the addition of nutrients (+N, +P, +NP) in combination with these C substrates did not increase the amount of C substrate respired for any of the soils, contrary to H1. Nutrient treatments did however influence the magnitude and direction of priming responses, in some cases. Strong negative priming of SOM in high elevation grassland and upper montane forest soils was induced by +N and +NP treatments individually and in combination with C substrates (Figure 6.4), suggesting that the turnover of SOM in these soils was regulated by microbial demand for N (in support of H2). Conversely, nutrient treatments alone and in combination with C did not influence priming responses from lower-montane and lowland forest soils. This was contrary to the hypothesis (H2), that P addition would alleviate P-limitation in lowland soils (Nottingham et al., 2015a) and stimulate negative priming of SOM due to reduced mining for P. Instead amendment of the lower-montane and lowland soils with C substrates induced positive priming of SOM, most strongly in response to xylose (simple C), suggesting that microbial activity in these soils was constrained by the availability of labile C rather than nutrients (Wardle, 1992, Heuck et al., 2015). The chemical complexity of added substrates determined the relative incorporation of substrate C by different microbial functional groups (Figure 6.6), however nutrient treatments had no significant additional influence on substrate C assimilation, contrary to H3.

Microbial mineralisation and assimilation of $^{13}$C-substrates

Despite predicting that the degree of C substrate mineralisation would depend on the availability of limiting nutrients (H1; hypothesised as N and P for montane and lowland soils respectively), substrate respired CO$_2$-C was unaffected by amendment with additional nutrient treatments (Figure 6.3). This was in disagreement with other tropical studies whereby mineralisation of substrate C was typically greater when C was added in combination with inorganic nutrients (Nottingham et al., 2012b, Nottingham et al., 2015b), although the difference among outcomes may reflect differences in the concentration and stoichiometry of nutrient treatments used among
studies, with lower concentrations applied here. Although in all studies nutrient treatments were added in a fixed ratio with added C, to approximately correspond to microbial biomass stoichiometry (Cleveland and Liptzin, 2007), here soils were amended with C at a rate of 0.2 mg C g\(^{-1}\) fwt soil (equivalent to 0.45-0.70 mg C g\(^{-1}\) dwt, with C:N:P 60:7:2) whereas in the studies by Nottingham et al. (2012b, 2015b), soils were amended with a higher concentration of C (1.0 mg C g\(^{-1}\) dwt, with C:N:P 60:6:6). As such, concentrations of nutrients added here were more moderate, with the concentration of N and P used by the contrasting studies approximately two and five-times greater respectively. In this study, labile C from substrates may have therefore been used in preference to comparably recalcitrant soil C without nutrient-constraint, whereby even in the absence of additional nutrient treatment microorganisms could acquire sufficient nutrients from the soil to mineralise the relatively low concentration of substrate C, with any resultant nutrient-constraint instead manifesting in the mineralisation of SOM (Figure 6.4). A similar response was observed in a temperate-region study, where addition of N did not affect the mineralisation of added sucrose C, but did influence the magnitude of SOM mineralisation (Chen et al., 2014).

Although nutrient treatments had no significant influence on microbial incorporation of C substrates (contrary to H3), the complexity of C substrates was an important determinant of assimilation by different microbial groups (Figure 6.6). Gram-negative and GP bacteria are associated with the mineralisation of labile and more complex C sources respectively (Fierer et al., 2007, Kramer and Gleixner, 2008), in broad agreement with my findings where GP bacteria (in both soils) assimilated a greater proportion of hemicellulose and GN bacteria (in the Wayqecha soil) assimilated a greater proportion of xylose, with low incorporation of both C substrates by GN bacteria in the Tambopata soil (Figure 6.6). While fungi have diverse capabilities for C-mineralisation (Kjøller and Struwe, 2002) and are often associated with the degradation of more complex C-compounds (de Boer et al., 2005, Cusack et al., 2011), some fungal taxa have been shown to target more labile C sources (Hanson et al., 2008, van der Wal et al., 2013), as evident here where fungi assimilated a greater proportion of the simple C substrate, particularly in the lowland forest soil (Figure 6.6). These results therefore demonstrate that the pathway for microbial C use is strongly shaped by the chemical complexity of organic matter and the functional
capacity of microbial taxa, rather than the availability of nutrients. Nutrient availability may instead play a role in shaping the fundamental composition of the microbial community (Fierer et al., 2009), as previous studies have reported microbial community shifts in response to fertilisation with N and P (Güsewell and Gessner, 2009, Liu et al., 2013, Fanin et al., 2015b, Fanin et al., 2015c). While no change to microbial composition was evident 7 days after amendment with nutrient treatments here (Supplementary Figure S6.4), the duration of the study may have been too short to observe a response, or the nutrient treatments too moderate compared to the inherent nutrient status of the soil to drive a compositional change. Longer-term studies are therefore required to evaluate the extent to which altered nutrient supply might modify microbial community composition in these soils.

Microbial priming of SOM and assimilation of excess soil-derived C

Contrasting priming responses were evident among soils from higher (3644 m and 3025 m) and lower (1500 m and 210 m) elevations, where the direction and magnitude of priming was most dependent on the availability of N in higher elevation soils and labile-C in lower elevation soils (Figure 6.4). In the upper montane forest and grassland soils, amendment with C substrates alone had a relatively minor influence on priming of SOM, but a strong reduction in the mineralisation of SOM was evident in response to +N and +NP treatments (Figure 6.4 a-b), indicating reduced mining of SOM for nutrient acquisition when these nutrients were exogenously supplied (Dijkstra et al., 2013). Given that priming responses to +N and +NP were generally not significantly different, it is reasonable to assume that responses to both treatments were driven by N, in support of H2 and consistent with previous studies of the Peruvian gradient which have identified high microbial demand for N at higher elevations (Fisher et al., 2013, Nottingham et al., 2015a). Negative priming responses to N have been widely reported (Craine et al., 2007, Janssens et al., 2010, Bird et al., 2011, Nottingham et al., 2015b), with reduced mineralisation of SOM under elevated N availability attributed to preferential use of the exogenous N source rather than co-mineralisation of SOM to liberate N to meet microbial nutrient demand (Fontaine et al., 2011, Chen et al., 2014). However, despite results indicating high microbial
demand for N, microorganisms in these higher elevation soils did not appear to be constrained by the availability of inorganic N per se, as amendment with +N did not increase mineralisation of added C substrates (Figure 6.3) nor the assimilation of substrate-C by different microbial groups (Figure 6.6). These results therefore suggest that while the availability of inorganic N in soils from higher elevations is low (Table 6.2), microbial demand for N is met by mineralising SOM (N-mining).

Differences in microbial incorporation of excess soil-derived C among treatments in the Wayqecha soil provides further evidence that mineralisation of SOM was regulated by microbial demand for N. Assimilation of soil-derived C by all microbial groups was lower in the xylose + N treatment (compared to xylose alone; Figure 6.7a), consistent with the negative priming response identified from the change in CO₂ production (Figure 6.4b). In a temperate study, K-strategists were identified as responsible for priming of SOM for N-mining (Chen et al., 2014) and while the microbial functional divisions used here do not directly correspond to the spectrum of r- K- growth strategies, GP bacteria and fungi are often recognised as K-strategists (oligotrophs), which slowly and efficiently target more recalcitrant C-sources (Fierer et al., 2007, Dungait et al., 2013). Indeed, following amendment of the upper montane forest soil with xylose alone (without additional nutrient treatment), GP bacteria exhibited increased incorporation of soil-derived C (Figure 6.7a). Fungi, however, did not appear to be associated with positive priming of SOM (for N-mining) following amendment with xylose alone, as incorporation of excess soil derived C in fungal biomarkers was negligible (Figure 6.7a).

While results indicate that microbial demand for N regulated the turnover of organic matter in soils from higher elevations (Figure 6.4 a-b), in the montane grassland soil very low concentrations of inorganic P (extractable PO₄) have been measured (Table 6.2). Despite this, microbial demand for N prevailed, as negative priming of SOM (reduced nutrient-mining) was observed in response to +N rather than +P (Figure 6.4a). The intensity of the negative-priming responses to +N in the grassland soil (approximately four-times as negative as those measured from the upper montane forest soil; Figure 6.4 a-b), indicates that grassland microorganisms intensely mineralise SOM to liberate N, resulting in the substantial reduction in SOM mineralisation observed when +N was externally supplied. Taken together, low
availability of PO₄ but high apparent demand for N may signify that N is intensely mined to support the production of extracellular phosphatase enzymes associated with the acquisition of P (Nannipieri et al., 2011), with this mechanism observed in tropical soils elsewhere (Treseder and Vitousek, 2001, Wang et al., 2007, Marklein and Houlton, 2012). Therefore, while results support the prevailing theory that microbial demand for N regulates microbial activity and the turnover of organic matter in tropical montane soils (Cusack et al., 2011, Fisher et al., 2013, Nottingham et al., 2015a), the underlying drivers contributing to high demand for N in grassland soils (which also exhibit low concentrations of inorganic P) warrants further investigation.

Soils from the lower montane (1500 m) and lowland (210 m) forests exhibited positive priming of SOM following amendment with C substrates, the magnitude of which was greatest in response to xylose (Figure 6.4 c-d). Consistent with this, inputs of labile C were previously found to induce positive priming from tropical lowland soils in Peru (Whitaker et al., 2014a) and Panama (Nottingham et al., 2012b, Nottingham et al., 2015b). Yet despite predicting that P addition would retard mineralisation of SOM in lowland soils due to reduced mining for P (H2), amendment with C substrates in combination with P had no significant additional influence, suggesting that microorganisms in these soils were primarily constrained by availability of labile C (Wardle, 1992, Wild et al., 2014). A recent temperate study also found that microorganisms were most strongly constrained by C, even in P-poor soils (Heuck et al., 2015) while a subtropical study across a substrate-age gradient found no relationship between soil P availability and priming responses (Sullivan and Hart, 2013). However, microbial P acquisition can be decoupled from the mineralisation of organic matter (Craine et al., 2007, Dijkstra et al., 2013), with organic-P released by biochemical hydrolysis (not associated with production of CO₂) rather than biological oxidation (McGill and Cole, 1981). Microbial mining of nutrients from organic matter may therefore be more important for N rather than P acquisition, hence why no change in the mineralisation of SOM was observed when additional P was supplied (Figure 6.4d). Moreover, inputs of labile C may have been used as a source of energy to mobilise P from organic and inorganic sources. For example, in soils where sources of organic P are low, when provided with sufficient energy, microorganisms may be able to liberate weakly adsorbed inorganic P (Walker
and Syers, 1976, Johnson et al., 2003) by producing chelating agents (Richardson, 2001, Oberson et al., 2005). Labile C may have also been invested in the synthesis of phosphatase enzymes, which liberate P from organic matter (Allison and Vitousek, 2005, Nannipieri et al., 2011). High phosphatase activity has been measured in lower elevation soils from the studied transect (Nottingham et al., 2015a), therefore a fundamental constraint by P may explain the high microbial demand for labile C in these soils.

As the intensity of priming from lower elevation soils was not mediated by nutrient treatments (Figure 6.4 c-d), the positive priming responses induced by added C substrates likely arose by stimulating the activity of microorganisms which target more recalcitrant sources of C (K-strategists), with these microbes continuing to mineralise SOM as the substrate was depleted (Fontaine et al., 2003, Fontaine and Barot, 2005, Pascault et al., 2013). Based upon this mechanism, we may expect to observe increased incorporation of soil-derived C into microbial PLFAs, particularly into GP bacterial and fungal biomarkers, functional groups associated with the K-growth strategy (De Vries and Shade, 2013, Dungait et al., 2013). While a small increase in soil derived C within PLFAs was evident in the lowland soils amended with hemicellulose (Figure 6.7d), consistent with the small positive priming effect identified from CO₂-production (Figure 6.4d), in the lowland soils amended with xylose, excess soil-derived C incorporation by all microbial groups was typically negligible (Figure 6.7c), surprising given the magnitude of positive priming induced by the xylose treatment (Figure 6.4d). Several factors may contribute to this apparently incongruous result. Firstly, low microbial substrate CUE was observed for these lowland forest soils (Figure 6.5), favouring respiration of mineralised substrate C rather than assimilation into biomass (Manzoni et al., 2012). Given this, primed soil C mineralised by these microorganisms would be mostly respired, with a very low proportion incorporated into PLFAs for growth, perhaps explaining why no increase in soil C assimilation was detected here. Secondly, labile C substrates may have been used to support the synthesis of extracellular oxidative enzymes which target recalcitrant soil C, with this increased enzymatic activity responsible for the enhanced mineralisation of SOM (Carney et al., 2007, Zhu et al., 2014), an explanation also proffered by other studies where positive priming was seemingly unrelated to
microbial growth dynamics (Wild et al., 2014, Rousk et al., 2015). While these mechanisms are both plausible explanations, it is also possible that a proportion of primed C was derived from increased turnover of microbial biomass (apparent priming) rather than from increased mineralisation of pre-existing SOM (Blagodatskaya and Kuzyakov, 2008), hence explaining why no clear increase in soil-derived C incorporation into microbial PLFAs was evident. Apparent priming may be particularly prevalent in response to more labile inputs of C (Wu et al., 1993) which trigger rapid growth and turnover of bacterial biomass (Blagodatsky et al., 2010). However, there was no significant difference in microbial abundance (indicated by total PLFAs) among untreated and C-nutrient treated soils (Supplementary Figure S6.4) and evolution of primed-C from these lowland forest soils continued to increase with time up to 7 days (Supplementary Figure S6.3d), rather than ceasing after 1-3 days, both factors indicative of real rather than apparent priming responses (Blagodatskaya et al., 2007, Blagodatskaya and Kuzyakov, 2008).

Although the direction of priming responses observed here were generally consistent with those identified by a previous study of the same Peruvian gradient, in the previous study, amendment with a nitrogenous C-substrate (an amino acid, glycine C2H5NO2) resulted in strong positive priming from both lowland and montane forest soils (Whitaker et al., 2014a). In contrast, addition of C substrates in combination with N (as NH4-NO3) here significantly reduced mineralisation of pre-existing SOM in higher elevation soils (Figure 6.4 a-b) and had no significant effect on soils from lower elevations (Figure 6.4 c-d). These differences may be attributed to the different sources by which C and N were supplied. Amendment of soils with trace amounts of amino acids has been shown to trigger activation of soil microorganisms (De Nobili et al., 2001, Mondini et al., 2006) and, as such, glycine may have stimulated microbial activity and growth, with microbes continuing to mineralise SOM after the substrate was depleted (Fontaine and Barot, 2005, Wild et al., 2014). Whereas here, microorganisms in the higher elevation soils appeared to use the exogenous supply of N instead of mineralising SOM as a means to acquire N. Differences in the stoichiometry of C and N inputs may have also influenced the direction of the priming response, as a recent subtropical study observed a small negative priming response when the C:N ratio of inputs was low (60:48) but strong positive priming of SOM.
when the ratio of C:N was increased (up to 60:0.2) (Qiao et al., 2016). However, despite the stoichiometry of C:N in glycine (60:36) being lower than the ratio of C:N added here (60:7), glycine elicited a strong positive priming response, inverse to the trend observed by the subtropical study. Alternatively, small changes in the soil physio-chemical environment stimulated by the different treatments may have also contributed to the contrasting responses, as an Arctic study found that glycine significantly increased soil pH, while treatment with N (as NH₄-NO₃) in some cases acidified soils (Hartley et al., 2010). However, concentrations of glycine (15 mg C g⁻¹ soil C) and N (8.75 mg N g⁻¹ soil C) used in the Arctic study were considerably greater compared to glycine (c. 2 mg C g⁻¹ soil C) used by Whitaker et al. (2014a) and the +N (c. 0.23 mg N g⁻¹ soil C) treatment used here. Furthermore, the +N treatment here did not elicit a systematic response across all soils, with no significant effect of +N on priming from the lower elevation soils (Figure 6.4 c-d), which would be expected if the treatment had substantially acidified soils. It is therefore reasonable to assume that changes in pH induced by the different treatments were relatively minor, highlighting that the direction of priming may be sensitive to the chemical composition of N inputs, further understanding of which will be important for predicting responses to increased N availability as a consequence of global change.

**Potential consequences for C mineralisation in tropical soils**

While inferences based upon short-term controlled laboratory experiments must be drawn with caution, findings presented here suggest potentially contrasting implications of altered C and nutrient supply on future soil C stores across higher and lower elevation tropical ecosystems. Increased inputs of plant-derived C in response to warming and elevated atmospheric CO₂ (Wood et al., 2012, Cernusak et al., 2013), especially of labile root exudates composed largely of simple sugars (Grayston et al., 1997), may induce positive priming of SOM across lowland and lower montane forest soils, resulting in enhanced C loss to the atmosphere. In contrast, for upper montane forest and grassland soils, increased inputs of N (of the form NH₄-NO₃) from atmospheric deposition (Hietz et al., 2011), reported even in remote Andean forests (Fabian et al., 2005, Boy et al., 2008), might reduce microbial mineralisation of SOM, stabilising C stocks and increasing net C sequestration in these higher elevation
systems. Longer-term and larger-scale *in-situ* experiments are required to substantiate these findings, however evidence from long-term experimental studies to date suggest that priming responses to altered C and nutrient supply can persist with time. In one tropical lowland forest, positive priming of SOM in response to experimentally increased litterfall was sustained over six years (Sayer et al., 2011). Likewise, long-term negative priming following N fertilisation was evident in two forests in Finland, whereby reduced CO$_2$ production from fertilised plots relative to untreated controls was observed 7 and 14 years after N fertilisation had ceased (Martikainen et al., 1989).

### 6.5 Conclusion

The mineralisation of simple and complex C substrates was not affected by nutrient availability in contrasting soils from a 3400 m tropical elevation gradient, however the direction and intensity of priming responses varied among soils and was in some cases dependent on the availability of N. Strong negative priming responses from upper montane forest and grassland soils to exogenously supplied N suggest that turnover of SOM in these soils was regulated by microbial demand for N. In contrast, microbial activity in lower montane and lowland forest soils was not influenced by N nor P availability, and appeared to be primarily constrained by the availability of labile C. These results suggest that modified C and nutrient supply to soils as consequences of global change could have opposing implications for future soil C storage across higher and lower elevation tropical ecosystems. Inputs of N, from atmospheric deposition, may constrain the turnover of SOM (due to reduced N mining) and stabilise C stocks in soils at higher elevations, while increased inputs of labile plant-derived C, as a consequence of elevated atmospheric CO$_2$ and climatic warming, may stimulate positive priming and induce C loss from lower elevation tropical soils.
Supplementary Figure S6.1: Relative concentration of two PLFA bio-markers (18:2 ω 6,9 and 18:1 ω 9) in soils from Tambopata (210 m) and Wayqecha (3025 m). Spearman’s rank correlation coefficient = 0.83 (p < 0.001, n = 42).
**Supplementary Figure S6.2**: respired CO$_2$-C (µg g$^{-1}$ soil dwt day$^{-1}$) from four soils incubated for 7 days at standard temperature (16 ºC) and moisture (80 % maximum water holding capacity) following amendment with C substrates; no added C (control), hemicellulose (H) and xylose (X), in combination with nutrient treatments; control (no added nutrient), +N, +P, +NP. Bars represent mean ± 1SE (n = 4).
Supplementary Figure S6.3 a-b: cumulative primed C (µg CO₂-C g⁻¹ soil C) from soils incubated at standard temperature (16 °C) and moisture (80% maximum water holding capacity) measured over 168 hours following amendment with C substrates (no added C, Xylose; X, Hemicellulose; H) in combination with nutrient treatments (+N, +P, +NP). Data represents mean ± 1SE (n = 4). Note different y-axis scales.
Supplementary Figure S6.3 c-d; cumulative primed C (µg CO$_2$-C g$^{-1}$ soil C) from soils incubated at standard temperature (16 °C) and moisture (80% maximum water holding capacity) measured over 168 hours following amendment with C substrates (no added C, Xylose; X, Hemicellulose; H) in combination with nutrient treatments (+N, +P, +NP). Data represents mean ± 1SE (n = 4). Note different y-axis scales.
Supplementary Figure S6.4; total PLFA (µg PLFA g\(^{-1}\) dwt soil), fungal: bacterial (F:B) ratio and gram positive: gram negative bacteria (GP:GN) ratio in untreated control and C-nutrient treated (Xylose control/+N/+P and Hemicellulose control/+N/+P) soils from lowland forest (Tambopata, 210 m) and upper montane forest (Wayqecha, 3025 m). Bars represent mean ± 1SE (n = 3).
Chapter 7

Synthesis and conclusions

The overarching aim of this thesis was to contribute towards a better understanding of the factors that regulate soil carbon (C) cycling in tropical lowland forests, montane forests and grasslands. To address this, large-scale manipulation experiments were conducted to examine the climatic, abiotic and biotic factors which influence the rate of soil C cycling (decomposition and soil respiration) across a 3400 m tropical elevation gradient extending from the Peruvian Amazon to Andes. Findings from these in-situ field experiments were combined with those from controlled laboratory studies to further examine the underlying microbial-scale mechanisms and multi-factor interactions which collectively determine the ecosystem-scale processes observed in the field. Here I review and synthesise the key findings from the research papers presented in Chapters 3-6 to answer the four principal science questions identified at the start of this thesis;

(1) How does the chemical composition of plant inputs influence soil C dynamics?
(2) Is there a shift with elevation in the most limiting nutrient to microbial activity and hence decomposition, from phosphorus (P) in lowland forests to nitrogen (N) in montane forest and grassland soils?
(3) Do differences in microbial community composition among soils (with elevation) influence soil C dynamics?
(4) How may these factors together influence the observed, apparent temperature sensitivity of soil C dynamics to climatic warming?

The wider implications of this research in the context of global change is then evaluated, followed by recommendations for future work and concluding remarks.
7.1 Key Findings

How does the chemical composition of plant inputs influence soil carbon dynamics?

- Leaf chemical traits strongly influence rates of leaf decomposition, particularly in lowland forests
- The chemical complexity of carbon inputs determines the magnitude of increased soil carbon turnover (by priming)
- Changes to root communities and root inputs to soil (associated with climatic warming) could stimulate soil carbon loss, especially in montane systems

Leaf chemistry was an important determinant of the rate of leaf decomposition ($k$) in both *in-situ* (Chapter 3) and controlled-microcosm (Chapter 4) experiments. The chemical complexity of added C substrates (as surrogates of plant-derived C) also strongly influenced both the magnitude of C substrate mineralisation and the mineralisation of pre-existing soil organic matter (SOM) in the $^{13}$C-tracer experiment (Chapter 6), while root-derived C inputs stimulated net C loss from montane soils following translocation downslope to the lowland forest site (Chapter 5).

The leaf translocation study presented in Chapter 3 investigated the role of multiple climatic, abiotic and biotic controls on decomposition, one of few such studies which exist for the tropics (Powers et al., 2009, Dale et al., 2015). While climate is often considered the dominant control regulating rates of decomposition, in this study a strong role for leaf chemical traits was also evident. Indeed, across the elevation gradient, leaf chemical traits explained a greater proportion of total variance in $k$ compared to temperature (24.0 % and 17.7 % total variance explained by leaf traits and temperature respectively; Table 3.5). Greater concentrations of N and cellulose in leaves enhanced the rate of decomposition, whereas greater concentrations of total C and lignin in leaves constrained the rate of decomposition. Leaf chemistry was a particularly important determinant of decomposition at the lowland forest sites, while
temperature explained a greater proportion of variance in $k$ across the higher-elevation montane sites. This finding was in agreement with that previously reported from a meta-analysis of tropical forest studies, whereby temperature strongly regulated rates of decomposition across tropical montane forests (MAT < 20 ºC) but did not affect $k$ at warmer lowland sites, where instead the effect of leaf chemistry most strongly prevailed (Waring, 2012). Changes to the chemistry of leaf inputs could therefore influence future rates of decomposition, particularly at lower elevations where climate is already favourable for decomposition (Prescott, 2010).

The chemical composition of plant inputs also strongly regulated rates of decomposition under controlled climatic conditions. In the $^{13}$C-tracer experiment (Chapter 6), the chemical complexity of added C substrates (as surrogates of plant-derived C) determined the degree of substrate mineralisation, with consistently greater mineralisation of the simple C substrate (xylose) compared to the more complex C substrate (hemicellulose; Figure 6.3). Carbon chemistry was also an important determinant of leaf decomposition in the microcosm study (Chapter 4) where leaf cellulose concentration explained the greatest proportion of variance in $k$ (38.4 %), while leaf N concentration had a smaller effect (6.9 % variance explained). In the in-situ study however, foliar C and N concentration most strongly regulated the rate of decomposition (together explaining 27.2 % variance in $k$; Chapter 3) with a much smaller secondary influence of C chemistry (cellulose and lignin content; together explaining only 1.9 % variance). Several factors may explain this difference among studies. In the controlled experiment (Chapter 4), the role of leaf C and lignin content were not directly assessed, as these traits co-varied with foliar cellulose concentration and, as such, part of the variance explained by cellulose in this study may be attributed to leaf C and lignin concentration. Alternatively, some studies have reported that the best leaf trait predictor of $k$ can vary with the completeness (stage) of decomposition (Melillo et al., 1989, Loranger et al., 2002, Li et al., 2011). Thus, although rates of decomposition in both in-situ and microcosm studies were determined over c. 300 days, differences in climatic conditions (temperature, soil moisture) among the studies will have led to differences in the stage of decomposition measured at this time point. Therefore, while both foliar nutrient concentration and C chemistry regulate rates of decomposition (as evident from findings in Chapters 3 and 4), their relative importance...
might vary dependent on the stage of decomposition, which requires further investigation. Nonetheless, together these findings demonstrate that the inherent chemical composition of plant inputs to soil strongly influences the rate at which they are decomposed. There is therefore much potential for a traits-based approach to modelling the biosphere-atmosphere C flux associated with the decomposition of plant residues in tropical systems.

There was also strong evidence, from both controlled laboratory and in-situ studies that, dependent on their chemistry, plant-derived C inputs can enhance the mineralisation of pre-existing SOM (by ‘priming’) and contribute to substantial loss of C from soils. In the $^{13}$C-tracer experiment (Chapter 6), amendment of the lower montane and lowland forest soils with simple and more complex C substrates accelerated the mineralisation of SOM (positive priming), with the degree of priming dependent on the substrate complexity (simple > complex; Figure 6.4 c-d). Amendment of upper montane forest soils with C substrates also induced a small positive priming response, although when C was added in combination with N (as NH$_4$-NO$_3$), reduced mineralisation of SOM prevailed (negative priming; Figure 6.4b). Although priming responses were only measured over 7 days during this controlled laboratory study, findings suggest that more labile inputs of plant-derived C could stimulate net C loss from forest soils across the elevation gradient. Root exudates are an important source of labile C in soils (Grayston et al., 1997, Jones et al., 2009), readily mineralised by soil microorganisms. In the soil translocation experiment (Chapter 5), root-soil interactions stimulated substantial loss of C from the montane forest and grassland soils following translocation downslope to the lowland forest site, as total C was significantly more depleted in soils in which root ingrowth was permitted compared to translocated control soils where root growth was excluded (Figure 5.5). Positive priming of SOM stimulated by root-derived inputs of labile C, as observed in the $^{13}$C-tracer experiment (Chapter 6), therefore provides a viable mechanism for this response, with soil C loss stimulated by root growth (rhizosphere priming) also reported by studies elsewhere (Dijkstra and Cheng, 2007, Bird et al., 2011, Cheng et al., 2014).

There were however some inconsistencies among the responses to C inputs determined by the controlled $^{13}$C laboratory study (C-substrate mediated responses)
and soil translocation study (root mediated responses). Firstly, in the controlled laboratory study, amendment of the lowland forest soil with labile C induced strong positive priming of SOM (Figure 6.4d), whereas in the soil translocation study roots did not appear to alter the turnover of organic matter in lowland soils, with no significant difference in total C among ingrowth and control soils after 24 months (Figure 5.5d). Secondly, in the case of the montane grassland soil, amendment with C substrates in the $^{13}$C-tracer study resulted in negligible to small negative priming responses (reduction in SOM mineralisation; Figure 6.4a) whereas root-soil interactions following soil translocation appeared to induce substantial soil C loss (Figure 5.5a). These emerging differences in soil C dynamics in response to inputs of plant-derived C among studies may be explained by several factors. Soil from the organic horizon was used in the controlled laboratory study, with responses measured over only 7 days under controlled temperature and soil moisture conditions. Whereas in the soil translocation experiment, monoliths of mineral soil were translocated and exposed to prevailing temperature and precipitation regimes in-situ, with responses measured over 24 months. Organic and mineral soils may exhibit differences in their amenability to priming, with subtropical (Qiao et al., 2016) and Arctic (Wild et al., 2014) studies also reporting priming responses to differ among organic and mineral soils. Furthermore, we know that soil from the studied lowland forest site has less organic matter (in the mineral horizon) physically and chemically unprotected from microbial decomposition, compared to the montane forest soils (Zimmermann et al., 2012). Thus, the potential for long-term root-mediated decomposition of SOM may have been constrained by lower concentrations of organic matter in the mineral soil amenable to microbial decomposition. Potential differences in priming responses from organic and mineral soils across the Peruvian elevation gradient requires further investigation, to more comprehensively understand how plant-derived C inputs influence soil C turnover. However, irrespective of these differences, the apparent long-term responses to root inputs evident from montane soils in the in-situ study, 24 months following translocation (Chapter 5), indicate that priming responses to C inputs are sustained with time. Under a scenario of increased plant productivity and altered root communities, as a consequence of climate change, rhizosphere priming could therefore represent a quantitatively important mode of C loss from these
systems. Plant-mediated soil C loss must therefore be included in future soil C budgets for the tropics.

**Is there a shift with elevation in the most limiting nutrient to microbial activity and hence decomposition, from phosphorus in lowland forests to nitrogen in montane forest and grassland soils?**

- **Microbial demand for nitrogen regulates the turnover of SOM in upper montane forest and grassland soils**
- **No evidence that the availability of phosphorus constrains microbial activity in lowland forest soils**

In soils from upper montane forest and montane grassland sites, microbial demand for N regulated the turnover of SOM (Chapter 6), in support of the prevailing theory. There was however, no clear evidence that microbial activity in lowland soils was constrained by the availability of P. Elevated availability of inorganic P had no significant influence on soil C dynamics in the controlled laboratory study presented in Chapter 6, where instead microbial activity in the lower montane and lowland forest soils appeared to be constrained by the availability of labile C. For leaf decomposition, the concentration of N in leaves (rather than P) strongly influenced rates of decomposition across both lowland and montane systems (Chapter 3).

From the $^{13}$C-tracer experiment presented in Chapter 6, results suggest that microbial demand for N strongly regulates the turnover of SOM in higher elevation upper montane forest and montane grassland soils. Microbial mineralisation of SOM was substantially reduced when N was externally supplied (as NO$_3$-NH$_4$ ; Figure 6.4 a-b), attributed to reduced mining for N from organic matter (Janssens et al., 2010, Bird et al., 2011). High investment in enzymes associated with the acquisition of N (relative to P) has been previously reported for higher elevation soils from the studied Peruvian gradient (Nottingham et al., 2015a), in support of high microbial demand for N and the findings from Chapter 6. Yet, microorganisms in these
soils did not appear to be constrained by the availability of N \textit{per se}, as amendment of soils with N did not increase the mineralisation of added C substrates (Figure 6.3 a-b) nor the assimilation of added C by different microbial functional groups (Figure 6.6). These findings therefore suggest that, while the availability of inorganic N in soils from higher elevations is low, microbial demand for N is met by mineralising N from organic matter.

For old, strongly weathered and leached tropical lowland soils, the prevailing theory states that microbial activity will be most strongly constrained by the availability of P (Walker and Syers, 1976, Reed et al., 2011a). However, amendment of lowland forest soils with inorganic P (NaH2PO4), both alone and in combination with organic C substrates, had no significant influence on the mineralisation of added C substrates (Figure 6.3d) nor pre-existing SOM (Figure 6.4d). This was contrary to expectation, and in disagreement with previous findings from other tropical lowland forests, whereby fertilisation with P stimulated microbial growth (Turner and Wright, 2014), decomposition of SOM (Cleveland et al., 2002) and soil respiration (Cleveland and Townsend, 2006). However, a previous in-situ study focused on the same lowland forest in Peru found no significant difference in microbial respiration among control and P-fertilised plots (Fisher et al., 2013), consistent with results from the 13C-tracer study (Chapter 6), suggesting that microbial activity in this lowland system is not constrained by the availability of P. Instead, amendment of lowland and lower montane forest soils with C substrates enhanced the mineralisation of SOM (Figure 6.4 c-d), with the simple C substrate eliciting the strongest response, indicating that microorganisms in these soils were primarily constrained by the availability of labile C.

Whether or not a fundamental constraint by P underlies this high demand for labile C however remains uncertain as, when provided with sufficient energy, microorganisms can acquire P from inorganic and organic sources. For example, microorganisms can produce chelating agents to liberate weakly adsorbed inorganic P (Richardson, 2001, Oberson et al., 2005). Microorganisms can also liberate P from organic matter through the synthesis of phosphatase enzymes (Nannipieri et al., 2011), although enzyme production is energy and N intensive (Allison and Vitousek, 2005). Labile C in the 13C-tracer experiment may have therefore been used as a source of
energy, to mobilise P, especially as high phosphatase activity in these lowland forest soils has been previously reported (Nottingham et al., 2015a). High microbial demand for N, to support enzyme production, was also proffered to explain why foliar N concentration (rather than P) most strongly regulated rates of decomposition across the lowland forest sites (Chapter 3). Overall, therefore, while there was no direct evidence that low availability of P constrained microbial activity across these lowland forest sites, high demand for N (Chapter 3) and labile C (Chapter 6) in these soils may be indicative of a fundamental constraint by P, if labile C and N was used to acquire P from other organic or inorganic sources.

**Do differences in microbial community composition among soils influence soil carbon dynamics?**

- **Microbial community composition did not influence rates of leaf decomposition**
- **However, functional differences among microbial groups were evident, whereby gram-negative (GN) bacteria and fungi use more simple carbon and gram-positive (GP) bacteria use more complex carbon**

Despite marked differences in the relative abundance of different microbial functional groups among soils across the elevation gradient (Figure 4.1), microbial community composition (the ratio of fungi:bacteria; F:B, and GP:GN bacteria) did not have a significant influence on rates of leaf decomposition in the controlled microcosm study (Chapter 4). In the $^{13}$C-tracer study presented in Chapter 6, however, clear functional differences among different microbial groups were evident (Figure 6.6). In the upper montane forest soil, GN bacteria assimilated a greater proportion of the simple C substrate, while assimilation of both simple and complex C substrates by GN bacteria in the lowland forest soil was low. In contrast, GP bacteria in both soils assimilated a greater proportion of the more complex C substrate, with GP bacteria also associated with positive priming of SOM in the upper montane soil (following amendment with xylose; Figure 6.7a). In agreement with these findings, GN and GP
bacteria have been previously associated with the mineralisation of labile and more recalcitrant sources of C respectively (Fierer et al., 2007, Kramer and Gleixner, 2008). Fungi are often assumed to have the capacity to degrade a wide range of simple to more complex substrates (Kjøller and Struwe, 2002), such that faster decomposition has been reported on soils with greater fungal dominance (García-Palacios et al., 2013b, He et al., 2016). However, other studies have reported fungi to be inefficient decomposers, with some fungal taxa targeting more labile sources of C (Osono and Takeda, 2002, Hanson et al., 2008). In the $^{13}$C-tracer study (Chapter 6), fungi assimilated a greater proportion of the simple C substrate, a trend evident in both lowland and upper montane forest soils (Figure 6.6). Given this, it is perhaps less surprising that the F:B ratio did not appear to influence rates of leaf-decomposition (Chapter 4). If fungi in these soils use more labile sources of C, then greater fungal abundance is unlikely to result in faster decomposition of comparatively complex leaf material.

While there was no evidence that microbial community composition influenced rates of decomposition, differences in functional breadth among forest and grassland communities may have contributed to the slower rates of decomposition on the grassland soil (Chapter 4). Temperate region studies have observed decomposition of leaves (sourced from trees) to be constrained on grassland soil, compared to forest soils, attributed to differences in microbial functional breadth dependent on the history of plant inputs (Keiser et al., 2011, Fanin et al., 2015a). As such, microbial adaptation to energy-rich and less chemically complex plant material may have constrained the ability of grassland microorganisms to degrade more chemically complex leaves (Van Der Heijden et al., 2008). If true, this could have important implications for future rates of decomposition across montane grassland systems, if the treeline advances into the grassland (Lutz et al., 2013, Rehm and Feeley, 2015), markedly altering the chemistry of plant-inputs to soil. This theory of functional breadth therefore merits further investigation, especially in the context of treelines on tropical mountains, through higher resolution analysis of microbial diversity and function in relation to the decomposition of simple and more chemically complex plant material (Fierer et al., 2012).
The decomposer community did, however, have a discernible influence in the in-situ leaf translocation study (Chapter 3). Invertebrate macrofauna accelerated leaf decomposition across the two lowland forest sites (Figure 3.2), whereas across the higher elevation montane sites (above 1500 m) there was no significant difference in the rate of decomposition measured from litterbags which excluded and permitted macrofaunal access. This was most likely due to low macrofaunal abundance at higher elevations (Palin et al., 2011), indicating that changes to macrofaunal ranges as a consequence of climatic warming could influence future rates of decomposition in tropical systems (Colwell et al., 2008, Wall et al., 2008).

How may these factors (plant inputs, soil fertility, and microbial community composition), together influence the observed, apparent temperature sensitivity of soil carbon dynamics to climatic warming?

- Temperature-related changes to the chemistry of leaf inputs will modulate the observed sensitivity of leaf decomposition to future warming, although the direction of the response is uncertain
- Upslope extension of macrofaunal ranges in response to warming will increase the observed temperature sensitivity of leaf decomposition across mid-elevation montane forests
- Warming-related increases in plant (root) productivity will stimulate soil carbon loss and increase the observed temperature sensitivity of soil respiration in montane systems

Climatic warming is likely to directly influence microbial activity and this could accelerate rates of decomposition (Davidson and Janssens, 2006, Lu et al., 2013), however, we lack understanding of how non-climatic factors will interact to determine the observed response to increased temperature. In this thesis, the temperature sensitivity ($Q_{10}$) of leaf decomposition (Chapter 3) and soil respiration (Chapter 5) were estimated using data collected from large-scale leaf and soil translocation
experiments conducted across 3400 m of elevation in the Peruvian Andes. In both cases, decomposition and soil respiration appeared to be more sensitive to temperature at higher elevations. The $Q_{10}$ of leaf decomposition across cooler mid-to-high elevation sites ($Q_{10} = 2.3-3.7$) was greater compared to the $Q_{10}$ derived across lower montane to lowland forest sites ($Q_{10} = 1.0-1.3$; Figure 3.4). The $Q_{10}$ of soil respiration (Rh + Rr) was also greater for soils originating from the montane forest and grassland sites ($Q_{10} = 2.9-4.6$) compared to the $Q_{10}$ of respiration for soil originating from the lowland forest ($Q_{10} = 1.8$; Figure 5.6). Together, these results suggest a greater vulnerability of montane systems to climatic warming, a potentially important finding given that montane soils contain more C compared to soils at lower elevations (Nottingham et al., 2015c). However, these experimentally derived estimates of temperature sensitivity may be strongly influenced by other factors which differ among sites across the gradient, and co-vary with temperature. Here I review the factors which may contribute to the observed, apparent temperature response, and evaluate how these factors could interact to influence the observed temperature sensitivity under future climatic warming.

In the case of the leaf translocation experiment (Chapter 3), greater macrofaunal abundance at the lowland forest sites (Palin et al., 2011) likely increased the apparent temperature sensitivity of decomposition. Macrofaunal abundance has been reported to be strongly influenced by temperature (Wall et al., 2008), with upslope extension of macrofaunal ranges expected in response to climatic warming (Colwell et al., 2008). As such, increased abundance of macrofaunal decomposers at higher elevations would increase the observed temperature sensitivity of leaf decomposition above that which might be expected as a direct response to temperature alone. Difference in the rate of leaf-decomposition on grassland and forest soils, identified from the microcosm study under controlled temperature and moisture conditions (Chapter 4) may have also influenced the observed temperature sensitivity of decomposition estimated from the translocation experiment (Chapter 3). In the microcosm study, leaves decomposed more slowly on the grassland soil relative to the forest soils, with differences in microbial functional breadth offered as one potential explanation (Fanin et al., 2015a). Irrespective of the underlying cause, this may have
contributed to increasing the apparent temperature sensitivity of decomposition estimated across the grassland-upper montane forest ecotone (Figure 3.4).

Results from in-situ (Chapter 3) and laboratory-based (Chapter 4) studies demonstrated that leaf chemical traits are important in determining rates of decomposition independently of climate. The apparent $Q_{10}$ of decomposition determined from the leaf translocation study was derived using the same eight species of leaves, such that differences in plant community composition across the gradient did not confound my findings. However, these results suggest that if plant inputs were to change differentially, either in response to temperature or through warming-related shifts in species distributions, then this could strongly influence the observed temperature sensitivity of decomposition. For example, if warming increases the inherent decomposability of plant inputs, then the observed temperature sensitivity of decomposition may be higher than predicted by the direct effect of increased temperature alone. Whereas if warming reduces the inherent decomposability of plant inputs, then the observed temperature sensitivity may be dampened. Understanding how the chemical composition of plant inputs will change in response to warming, however, remains a challenge. One tropical study reported a positive relationship between leaf N concentration and temperature (Silver, 1998) whereas another found leaf N concentration to decline with small increases in temperature (Tully and Lawrence, 2010). Up-slope shifts in plant-species ranges may increase the decomposability of leaf inputs, as species from lower elevations along the studied transect have generally greater concentrations of foliar N (van de Weg et al., 2009). However, this premise depends on the extent to which foliar traits are maintained or are altered in response to warming and the new up-slope environment and requires further investigation. Understanding how the chemistry of plant inputs will be modified by climate change, will therefore be important in predicting future rates of leaf decomposition in tropical systems.

In the case of the soil translocation experiment, differences in plant community composition and primary productivity across the gradient likely strongly influenced the observed temperature sensitivity of soil respiration ($R_h + R_r$; discussed in Chapter 5). Briefly, root productivity is positively related to temperature across the studied gradient (Girardin et al., 2010) and, as such, greater root productivity (and root
exudation) at the warmest, lowest elevation site likely increased the Rr-component of soil respiration, in turn increasing the observed, apparent sensitivity of soil respiration (Rh + Rr) to temperature (compared to the $Q_{10}$ of Rh alone; Table 5.5). Differences in the $Q_{10}$ of Rh + Rr among soils was also evident, with greater temperature sensitivities estimated for the montane soils compared to the lowland soil. These differences may be explained by greater amounts of chemically and physically unprotected SOM in these montane soils (Zimmermann et al., 2012) making montane soils more amenable to root-mediated decomposition. Future warming-related changes to root productivity, exudation and/or the species composition of plant communities could therefore stimulate considerable net C loss from montane forest and grassland soils, increasing the observed sensitivity to temperature.

While reasonable to assume that the same microbial community and enzyme assemblages originated in soil cores sourced from the same elevation (prior to translocation), over the 24 months following translocation across gradient (spanning 20 °C MAT), temperature-related community shifts may have occurred, influencing the observed temperature sensitivity of soil respiration. Although not examined here, a previous experimental study using soils from the Arctic to the Amazon (including soils from the studied Peruvian gradient) investigated how microbial community-level responses to prolonged (90 days) temperature change (cooling) influenced the temperature sensitivity of respiration (Karhu et al., 2014). From this study, microbial responses more often enhanced the temperature sensitivity of respiration, compared to the $Q_{10}$ derived as an instantaneous response to temperature. Moreover, the greatest enhancing responses were observed for soils originating from cooler systems and for soils with greater ratios of C:N. Given this, differences in microbial community-level responses to long-term temperature change over the course of the study (imposed by translocation up/downslope) may have contributed to differences in the observed temperature sensitivity of respiration (Rh) among soils (Table 5.5). However, irrespective of whether microbial community responses enhanced or reduced the temperature sensitivity of soil respiration estimated from this study, these observed temperature sensitivities may be more representative of long-term responses to climatic warming, compared to those estimated from short-term instantaneous responses to temperature (Appendix C).
7.2 Wider implications

Global change has the potential to strongly influence future soil C dynamics across tropical ecosystems. Here I evaluate the implications of my findings, in the context of global change.

Climatic warming

Climatic warming of 1.8-5.1 °C is projected to occur in the tropical Andes over the course of this century (Urrutia and Vuille, 2009, IPCC, 2013), which could both directly and indirectly influence the rate of C loss from tropical soils. While there is consensus that increasing temperature will stimulate microbial activity and this could directly accelerate rates of C turnover (Davidson and Janssens, 2006, Conant et al., 2011, Lu et al., 2013), findings from this thesis indicate that the magnitude of this response may be strongly influenced by warming-related changes to plant community composition and productivity. Firstly, given that the chemistry of plant inputs to soil strongly regulates the rate of leaf decomposition (Chapters 3 and 4), changes to the composition of plant inputs, whether in response to increasing temperature or through climate-related shifts in plant species distributions, could have important implications for the rate of C cycling and storage across these ecosystems. Whether these changes increase or decrease the inherent decomposability of plant inputs, and hence will enhance or reduce the observed temperature sensitivity of decomposition, however remains unknown. Secondly, climatic warming coupled with elevated concentrations of atmospheric CO$_2$ will increase plant productivity (Wood et al., 2012, Cernusak et al., 2013, Giardina et al., 2014), in turn increasing the quantity of plant-derived C inputs to soils. Increased root productivity could stimulate considerable net C loss from montane soils (Chapter 5), while increased inputs of labile C may also enhance the decomposition of pre-existing SOM in lowland forests (Chapter 6), leading to elevated effluxes of CO$_2$ from soils independently of the direct response to warming.

Climate-related shifts in macrofaunal ranges could also influence future rates of C cycling across tropical systems. Invertebrate macrofauna accelerated rates of decomposition in lowland forests (Chapter 3) but had no significant influence on rates of decomposition at higher elevation sites (above 1500 m) where macrofaunal
abundance is currently constrained by cool temperatures (Wall et al., 2008, Palin et al., 2011). Increased macrofaunal abundance at higher elevations because of climate-related upslope-extension of macrofaunal ranges (Colwell et al., 2008, Moret et al., 2016) therefore has the potential to accelerate rates of decomposition at mid-elevation montane forest sites, above that directly stimulated by increased temperature alone.

Together, these examples illustrate the potential for indirect effects of climatic warming to increase the sensitivity and vulnerability of soils to substantial C loss under future temperature regimes. The extent to which increased C assimilation by plants will balance soil C losses in response to elevated temperature remains uncertain (Wood et al., 2012). However, these results demonstrate that to more comprehensively predict the fate of large soil C stocks in the tropics, in response to future climatic warming, we must integrate how parallel warming-related changes to plant and biotic community composition will modulate the direct response to temperature.

**Atmospheric nitrogen deposition**

Nitrogen deposition is increasing in some areas of the tropics (Hietz et al., 2011), with high N deposition reported even across remote Andean systems (Boy et al., 2008). This will increase the availability of inorganic N in soils and, in turn, increase concentrations of foliar N (Homeier et al., 2012). Nitrogen deposition therefore has the potential to strongly influence rates of soil C cycling across tropical ecosystems. Firstly, increased availability of N in soils could accelerate rates of leaf decomposition, especially in ecosystems characterised by leaves rich in accessible C (Figure 4.3). Secondly, given that leaf N concentration strongly regulated rates of leaf decomposition (Chapters 3 and 4), increased concentrations of foliar N would likely increase the decomposability of leaves and accelerate rates of C and nutrient cycling from plant residues. Yet, in higher elevation montane forest and grassland systems, N deposition could also reduce the mineralisation of SOM (Figure 6.4 a-b), if microorganisms use the externally supplied source of N rather than mining N from organic matter to meet demand. Reduced mineralisation of SOM would therefore reduce the efflux of CO₂ from these soils and stabilise the large soil C stocks across higher elevation montane systems. While warming-related changes to plant (root)
communities and productivity could stimulate substantial C loss from montane soils (as indicated from the study presented in Chapter 5), under a global change scenario of increased temperature and N deposition, results suggest a possible countervailing effect may be imposed on warming-accelerated decomposition across upper montane forest and montane grassland systems, if increased N availability reduces microbial mineralisation of SOM.

7.3 Future Research Direction

Work presented in this thesis highlights the dynamic and complex nature of soil C cycling in tropical ecosystems. While these studies have considerably advanced our understanding of how multiple interacting factors regulate rates of C cycling in tropical soils, and how non-climatic factors may modulate the direct response to climatic warming, further research is required in order to resolve some of the new questions raised by the studies presented in Chapters 3-6. These future research recommendations are summarised below.

The rhizosphere priming effect

Whilst identifying that root-soil interactions stimulated substantial loss of C and N from soils following translocation downslope (Chapter 5), akin to positive priming responses induced by inputs of labile C identified from the $^{13}$C-tracer experiment (Chapter 6), it remains necessary to more closely examine the mechanisms which underlie this response. Firstly, analysis of microbial abundance and enzymatic activities within the rhizosphere soil (soil samples retained for future use) would yield important insights into microbial growth dynamics stimulated by the supply of labile C from root exudation. We might expect to observe greater microbial abundance in the rhizosphere soil, compared to bulk soil from the control soil cores where root growth was excluded. Furthermore, shifts in microbial community composition may also be evident, perhaps with increased abundance of GP bacteria in rhizosphere soils, microorganisms associated with positive priming of SOM in Chapter 6 and rhizosphere priming in a subtropical grassland study (Bird et al., 2011). Secondly,
analysis of mineralised N and P among control and ingrowth cores (by extraction of ion exchange resin bags installed within the soil cores over the duration of the experiment) would reveal whether root-soil interactions increased the mineralisation of nutrients, thereby increasing the availability of nutrients available for uptake by plants (Drake et al., 2011, Murphy et al., 2015). Differences in the flow of photosynthates to roots across the elevation gradient, proffered to explain differences in the intensity of root-mediated soil C loss among the lowland and montane sites, could also be investigated using isotopic partitioning to differentiate between sources of respired C (SOM or root-derived), as used in Chapter 6. To employ this approach *in-situ*, a pulse of $^{13}$C would be supplied to plants, by either exposing a plant (within a sealed chamber) to a pulse of $^{13}$C-CO$_2$ for uptake during photosynthesis (De Deyn et al., 2011) or by injecting a $^{13}$C-labelled substrate directly into the xylem (Churchland et al., 2012). The $^{13}$C would then be rapidly transferred to the soil through root exudation and this could be detected in the soil CO$_2$ efflux, allowing quantification of plant-derived $^{13}$C to the soil, to identify differences with elevation and over the seasonal cycle.

**Labile carbon and nitrogen used for phosphorus acquisition in lowland soils?**

Despite the prevailing theory that low P-availability strongly constrains microbial activity in tropical lowland soils (Walker and Syers, 1976, Nottingham et al., 2015a), there was no clear evidence to support this based upon findings presented in this thesis. Microbial activity (and decomposition of SOM) in lowland soils instead appeared to be constrained by the availability of labile C (Chapter 6), while leaf N concentration (rather than P concentration) most strongly regulated rates of leaf decomposition across lowland forest sites (Chapter 3). However, high microbial demand for labile C and N could be indicative of a fundamental constraint by P, if used as a mechanism by which to mobilise P from inorganic and organic sources (Richardson, 2001, Oberson et al., 2005, Nannipieri et al., 2011). Whether or not P limitation lies at the heart of the observed responses is relevant to our interpretation of the classical theory of Walker
and Syers (1976), although it may be sufficient to use C and N availability to parameterise current soil C dynamics models.

**Further focus on montane grassland systems**

Tropical montane-grassland systems are understudied but important ecosystems in terms of their contribution to the terrestrial C budget, storing as much C belowground as adjacent tropical montane forests (compared on an area basis) (Zimmermann et al., 2010b) as well as being highly productive (Oliveras et al., 2014). The temperature sensitivity of decomposition and soil respiration in these Peruvian montane grassland systems had not previously been assessed, yet $Q_{10}$ coefficients estimated from leaf and soil translocation experiments were high (compared to the $Q_{10}$ of 2 typically assumed for biological processes), indicating a great vulnerability of these soils to increased C loss in response to future climatic warming. From the leaf translocation experiment, for decomposition across the grassland-upper montane forest ecotone, a $Q_{10}$ of 2.3 – 2.7 was estimated (Chapter 3). However, this observed, apparent temperature sensitivity may have been confounded by differences among the grassland and forests soils, as observed in the microcosm experiment presented in Chapter 4. In this case, under controlled temperature and moisture conditions, rates of leaf decomposition were slower on grassland soils, relative to rates of decomposition on the forest soils. Differences in microbial functional breadth among the soils may explain this, however this theory to date has only been directly tested in temperate regions. Improved understanding of montane grasslands, and the extent to which a functionally narrow microbial community might constrain future rates of decomposition will be particularly important, given the potential for climate-driven advancement of the treeline (Lutz et al., 2013, Rehm and Feeley, 2015), which would markedly alter the chemistry of plant inputs to the soil.

From the soil translocation study (Chapter 5), while the $Q_{10}$ of Rh alone was relatively low (1.9 ± 0.1) compared to the other montane soils, the $Q_{10}$ of Rh + Rr was very high (4.2 ± 0.2). Following translocation downslope to the lowland forest site (experimental warming treatment), total C was significantly more depleted in soil in which root ingrowth was permitted compared to the translocated control soil where
root growth was excluded (Figure 5.5a), indicating that warming-related changes to belowground root communities and increased root productivity could stimulate net C loss from these grassland soils, increasing the observed sensitivity to temperature. However, in the controlled study presented in Chapter 6, amendment of these grassland soils with N (alone and in combination with C), resulted in a substantial reduction in the decomposition of SOM (negative priming). The intensity of the negative priming response was markedly greater compared to the equivalent response induced in the upper montane forest soils, suggesting that relatively moderate increases in N availability (through atmospheric N deposition) could substantially reduce soil C turnover, to a greater degree than in the adjacent upper montane forests. The future stability of C stocks in these montane grassland soils is therefore likely to be dependent on the balance between changes to plant-derived C and atmospheric N inputs, dictating whether either substantial C loss or stabilisation prevails. Given the amount of C stored by montane grassland soils (Zimmermann et al., 2010b), further investigation is required to assess the long-term stability of these soil C stocks to changes in C and N supply, as the response could represent an important feedback to climate change.

**In-situ warming experiments**

Translocation experiments across elevation gradients (as employed by Chapters 3 and 5) offer a valuable opportunity to investigate how future climatic warming may influence soil C dynamics in tropical systems. However, to fully understand the net effects of warming on the soil C cycle, in-situ soil-warming experiments in tropical forests must be a priority for future research (Cavaleri et al., 2015, Nottingham et al., 2015c). This is most important for lowland forests, where translocation experiments do not offer the opportunity to impose an experimental warming treatment, only cooling by translocation upslope. As a consequence, estimates of the apparent temperature sensitivity of soil C dynamics in lowland forest are subject to greater uncertainty, as we assume that the temperature-dependence of soil C dynamics determined by cooling, is also sufficient to describe the temperature-dependence of processes to warming.
7.4 Concluding Remarks

This thesis has yielded important insights into the factors which regulate soil C dynamics across tropical lowland and montane systems, with results from *in-situ* translocation experiments and controlled laboratory studies illustrating that complex interacting processes will determine the future soil C balance in these ecosystems. Evidence suggests that the influence of temperature on the rate of soil C cycling will be strongly affected by the composition of plant-derived and atmospheric nutrient inputs, the principal constraints varying with elevation. The high observed temperature sensitivity of decomposition and soil respiration across montane systems was coupled with high microbial demand for N which regulates the turnover of organic matter in upper montane forest and grassland soils. While, in contrast, at lower elevations leaf decomposition was strongly influenced by chemical traits and accelerated by active macrofaunal breakdown, while microbial decomposition of SOM was constrained by the availability of labile C.

Under a global change scenario of increased temperature and N deposition, these results suggest that: (i) modified foliar chemistry will influence rates of leaf decomposition independently of climate; (ii) increased availability of labile plant-derived C will lead to more rapid decomposition of SOM at lower elevations; (iii) higher (root) productivity will stimulate net C loss from montane soils, increasing the observed sensitivity of soil respiration to elevated temperature, but (iv) across upper montane forest and grasslands, a possible countervailing effect may be imposed on warming-accelerated decomposition if increased N availability reduces microbial mineralisation of SOM. The net effects on the ecosystem C budget will depend on the overall balance of C gain from primary productivity, and C loss from soils. However, the results presented here suggest that the large soil C stores in higher-elevation montane regions are particularly vulnerable to substantial reductions under exposure to short and medium-term climatic warming.
References


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Appendix A

Soil and plant material analyses; quality control

Carbon and nitrogen concentration

The concentration of carbon and nitrogen in soil and plant samples was determined using a TruSpec elemental analyser (LECO, USA). The instrument was set up on each day of analysis. First, 10 blanks were run, with the mean of the final 3 blank values used for a blank correction. Next, 5 samples of certified LECO standard were run, with these values used to calibrate the instrument. Each sample of soil or plant material was analysed twice, with a third analysis undertaken if inconsistency arose. Repeat standards were run after every 20 samples, to check for drift, with drift correction applied if necessary.

Phosphorus concentration

Total phosphorus concentration in soil and plant samples was determined by sulphuric acid-hydrogen peroxide digestion, followed by colorimetric analysis (Bran and Luebbe AutoAnalyser 3, Germany). Two blank tubes were included with every batch of digested samples, with the values from these tubes used for a blank correction. Results were calibrated using a five-point calibration curve (1-5 ppm PO₄).

Soil pH

Soil pH was measured in water (soil: H₂O, 1:2.5 w: v) using a Hanna pH meter (Hanna Instruments, USA). Prior to use, the instrument was calibrated using buffers of pH 4, 7 and 10. For each measurement, the glass electrode was immersed in the soil-water solution for at least 30 seconds, or until the reading stabilised (varied less than 0.02 units over a 5 second period). The electrode was thoroughly rinsed with a stream of de-ionised water between each sample, ensuring the probe was cleared of soil particles. After every 20 samples, the pH of buffers was re-measured, with calibration repeated if the measured value deviated by more than 0.02 units from the correct value.
Appendix B

Protocol for extraction of phospholipid fatty acids (PLFAs) from soil

This appendix describes the method used for the extraction of microbial PLFAs, undertaken as part of the work presented in Chapter 6.

Phospholipids were extracted as part of the total lipid extract from freeze-dried and ground samples of soil, by a Bligh-Dyer extraction method.

**Stage 1; Extraction**

Freeze-dried samples of soil (0.50 g) were accurately weighed into 50 ml chloroform rinsed tubes. An additional blank tube was included with every batch of 17 samples.

Bligh and Dyer extractant was prepared by mixing 135 ml chloroform, 270 ml methanol and 108 ml freshly prepared citrate buffer (0.15 M; 15.76 g citric acid dissolved in 500 ml milli-q water). To each tube, 1.5 ml freshly prepared citrate buffer was added, followed by 1.9 ml chloroform, 3.8 ml methanol and 2.0 ml Bligh and Dyer extractant. Tubes were vortexed for one minute and then covered with foil (to prevent fatty acids being degraded by light) and left to separate for 2 hours.

After 2 hours, tubes were vortexed again for one minute and centrifuged at 2000 rpm for 10 minutes. The supernatant was transferred to a new chloroform rinsed tube using a Pasteur pipette. The remaining soil pellet was rinsed using 2.5 ml Bligh and Dyer extractant, vortexed for one minute, centrifuged at 2000 rpm for 10 minutes and then the supernatant transferred to the new tube as previously described. The phases were split by adding 3.1 ml chloroform and 3.1 ml citrate buffer to each tube, before tubes were vortexed for one minute and left overnight to separate.

The following day, 3 ml of the lower phase from each tube was transferred to a clean chloroform rinsed tube. Tubes were placed in a sample concentrator and samples were evaporated under a stream of nitrogen at 35 ºC.
Stage 2; Lipid Fractionation

Isolute columns (unbonded silica sorbent, used to isolate polar analytes from non-polar matrices) were placed in a holder on a metal tray to catch the waste solvent. Columns were activated by adding 2.5 ml chloroform. The dry material from stage 1 was dissolved in 0.5 ml chloroform and carefully transferred to the column using a glass pipette. Each tube was rinsed a further two times with 0.5 ml chloroform, with washings transferred to the column each time. 5 ml of chloroform was then added to each column to elute the neutral lipids (including hydrocarbons, free fatty acids and sterols). Once all of the solvent had run to waste, 20 ml acetone was added to each column in 5 ml portions to elute the glycolipids. Once all the acetone had run to waste, the stand containing the isolute columns was removed from the metal tray and placed over a test tube rack containing clean, methanol rinsed tubes, such that the end of each column was positioned in the top of the corresponding tube. Polar lipids (phospholipids) were eluted by addition of 5 ml methanol to each column. The solvent was evaporated under a stream of nitrogen at 35 ºC in the sample concentrator, leaving the dried phospholipid fraction.

Stage 3; Mild Alkaline Methanolysis

Two internal standards were prepared: C13 standard methyl tridecanoate and C19 standard methyl nonadecanoate. 30 µl of each internal standard (C13 and C19) were carefully added to each tube, ensuring that no bubbles were in the pipette. The sample was dissolved in 1 ml methanol: toluene (1:1 v/v) before 1 ml freshly prepared potassium hydroxide (0.2 M KOH in methanol) was added to each tube. Samples were incubated in a water bath at 37 ºC for 15 minutes. After the period of incubation, 2.0 ml hexane: chloroform (4:1 v/v), 0.3 ml acetic acid and 2.0 ml Milli Q water were added to each tube, before tubes were vortexed for 1 minute and centrifuged at 2000 rpm for 5 minutes. The upper organic phase was transferred to new hexane rinsed tubes using glass pipettes. The remaining lower layer was rinsed with a further 2.0 ml hexane: chloroform, before tubes were vortexed and centrifuged as previously described. The upper phase was again transferred to the new tube before samples were evaporated.
under a stream of nitrogen at 35 °C in the sample concentrator. Samples were frozen at -20 °C until required for GC analysis.

**Re-suspending samples for GC Analysis**

Frozen samples were allowed to thaw in the dark for an hour before 150 µl hexane was added to each tube. Tubes were allowed to stand for 10 minutes before they were gently vortexed for one minute. The samples were then transferred to labelled GC vials using a glass syringe. The syringe was rinsed with hexane twice between each sample transfer to avoid contamination among samples. Suspended samples were stored in a fridge at 4 °C for a maximum of 3 days before GC analysis.

Individual PLFAs were identified by gas chromatography-mass spectrometry (GC-MS) using an Agilent Technologies 5973 Mass Selective Detector coupled to an Agilent Technologies 6890 GC. Concentrations of identified PLFAs were determined via the internal standard (methyl nonadecanoate C19:0, Sigma Aldrich, UK) and calculated on a soil mass basis (µg PLFA g⁻¹ dwt soil).
Appendix C

Soil CO$_2$ effluxes; diurnal variation, temperature dependence and sensitivity

This appendix presents additional data collected to evaluate diurnal variation in soil CO$_2$ effluxes across the elevation gradient, and the temperature dependence of soil CO$_2$ effluxes derived from the instantaneous response to temperature variation over the diurnal cycle. At four sites across the 3400 m elevation gradient (210 m, 1500 m, 3025 m, 3644 m), intensive measurements of soil CO$_2$ effluxes were conducted over 24 hour periods, using the same soil cores and collars installed as part of the translocation experiment presented in Chapter 5.

Method

Automated continuous measurements of CO$_2$ efflux were made every 30 minutes over 24 hour periods (48 continuous measurements) during the middle of the dry season in July 2014, using a closed chamber system with a 10 cm diameter survey chamber and portable Li-Cor 8100-IRGA (LI-COR Biosciences, USA). At each time point, soil temperature (0-10 cm depth) and soil moisture (0-10 cm depth) were also measured (externally adjacent to the soil core so as not to impede the automated soil respiration measurements). At each elevation site (210 m, 1500 m, 3025 m, 3644 m), these intensive measurements were conducted for three soil cores;

1. Undisturbed soil collar; representing total soil respiration (heterotrophic (Rh) plus root-rhizosphere (Rr) respiration, including the organic soil horizon)
2. Control core (mineral soil only, with root growth excluded) - soil excavated and reinstalled at same elevation site (Rh)
3. Ingrowth core (mineral soil only, with root growth permitted)- soil excavated and reinstalled at same elevation site (Rh + Rr)

See Chapter 5 for full explanation of experimental procedure
Data analysis

To determine the temperature dependence of soil CO$_2$ effluxes, first-order exponential curves of the form displayed in equation C.1 were fitted; where $R$ is the soil CO$_2$ efflux, $T$ is soil temperature (measured 0-10 cm depth), and $a$ and $b$ are fitted constants. These exponential curves were fitted to soil temperature-CO$_2$ efflux data collected over the diurnal cycle, in each case separately by soil and core type.

$$R = ae^{bT}$$

(Eq. C.1)

Regression-derived estimates of CO$_2$ effluxes were used to calculate the temperature sensitivity ($Q_{10}$) of soil respiration (according to equation C.2), where the $Q_{10}$ was defined as the factor by which respired CO$_2$ ($R$) changed with a 10 °C change in soil temperature ($T$).

$$Q_{10} = \left[\frac{R_2}{R_1}\right]^{\frac{10}{(T_2-T_1)}}$$

(Eq. C.2)

The data represent the metabolic response of the same microbial community to a change in temperature over a short period of time during which C-substrate availability may not vary markedly. It must be noted however that, in some cases, particularly where diurnal temperature variation was very low (< 2 °C), the relationship between soil temperature and CO$_2$ efflux was too weak ($R^2 < 0.10$) to derive a reliable $Q_{10}$ coefficient (Table C.1).
Results

Diurnal variations among soil CO$_2$ effluxes are illustrated in Figure C.1, with the temperature dependence ($R^2$) and temperature sensitivity ($Q_{10}$) summarised in Table C.1. At the forest sites (210 m, 1500 m, 3025 m), there was minimal variation in soil temperature over the diurnal cycle (typically less than 2 °C range; Figure C.1 b-d), with soil CO$_2$ effluxes also showing relatively low variation. In contrast, at the montane grassland site (3644 m), much greater variation in temperature over the diurnal cycle was evident (c. 6-11 °C; Figure C.1a), with associated soil CO$_2$ effluxes tending to increase from 06:00 hours and peaking at approximately 13:00-14:00 hours. The proportion of variance in soil CO$_2$ efflux explained by temperature for the grassland soils (25-51 %) was much greater than that typically explained by temperature for the forest soils, where in several cases temperature explained less than 10 % of the observed variance in soil CO$_2$ effluxes (Table C.1). The narrow diurnal temperature range at the forest sites may have contributed to the difficulty in deriving a clear temperature response, and thus the associated $Q_{10}$ coefficients are subject to relatively large uncertainty.

The $Q_{10}$ coefficients derived from these diurnal measurements are not directly comparable with the main dataset presented in Chapter 5 ($Q_{10}$ values estimated from among-site temperature variation) because of the narrow temperature range and associated uncertainty. Nonetheless, in the case of the Tres Cruces soil (3644 m), the estimated $Q_{10}$ of Rh + Rr (7.2) was greater than the $Q_{10}$ for Rh alone (5.6), consistent with the trend observed in the main dataset reported in Chapter 5 and, despite uncertainty, the $Q_{10}$ of Rh + Rr was also greater than Rh in the soils from the montane forest sites (1500 m and 3025 m; Table A2.1). However, in the lowland forest soil (210 m), the estimated $Q_{10}$ of Rh + Rr (2.6) was markedly lower than that estimated for Rh alone (3.7).
Table C.1: temperature dependence ($R^2$) and sensitivity ($Q_{10}$) of total soil respiration ($Rh + Rr$ including organic soil), heterotrophic respiration ($Rh$; from control soil core, mineral soil) and heterotrophic plus root-rhizosphere respiration ($Rh + Rr$; from ingrowth soil core, mineral soil) for four soils (Tambopata, San Pedro, Wayqecha, Tres Cruces; elevation in parentheses) derived from temperature variation over the diurnal cycle (corresponding to data illustrated in Figure C.1 a-d).

<table>
<thead>
<tr>
<th>Derived from within site, diurnal temperature variation</th>
<th>Tambopata (210 m)</th>
<th>San Pedro (1500 m)</th>
<th>Wayqecha (3025 m)</th>
<th>Tres Cruces (3644 m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature Dependence ($R^2$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Resp. (inc. organic soil)</td>
<td>0.03*</td>
<td>0.08*</td>
<td>0.09*</td>
<td>0.51</td>
</tr>
<tr>
<td>$Rh$ (mineral soil)</td>
<td>0.19</td>
<td>0.03*</td>
<td>0.09*</td>
<td>0.25</td>
</tr>
<tr>
<td>$Rh + Rr$ (mineral soil)</td>
<td>0.47</td>
<td>0.04*</td>
<td>0.23</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Temperature Sensitivity ($Q_{10}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Resp. (inc. organic soil)</td>
<td>(1.3)</td>
<td>(4.1)</td>
<td>(6.0)</td>
<td>3.3</td>
</tr>
<tr>
<td>$Rh$ (mineral soil)</td>
<td>3.7</td>
<td>(1.9)</td>
<td>(2.1)</td>
<td>5.6</td>
</tr>
<tr>
<td>$Rh + Rr$ (mineral soil)</td>
<td>2.6</td>
<td>(2.1)</td>
<td>2.8</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*where temperature dependence of soil CO$_2$ efflux was low ($R^2 < 0.10$), $Q_{10}$ coefficients (in parentheses) have greater uncertainty
Figure C.1 a-b; temperature dependence ($R^2$) of soil respiration at (a) 3644 m and (b) 3025 m measured over the diurnal cycle.
Figure C.1 c-d; temperature dependence ($R^2$) of soil respiration at (c) 1500 m and (d) 210 m measured over the diurnal cycle.

Closed black circles indicate soil respiration with roots excluded (control core, mineral soil; Rh).

Open circles indicate soil respiration including the contribution from roots and the rhizosphere (ingrowth core, mineral soil; Rh + Rr).

Grey closed circles illustrate undisturbed total soil respiration including the organic soil horizon and contribution from roots and the rhizosphere.

Black dashed line indicates soil temperature ($^\circ$C; secondary axis).