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The response of rainforest trees to climate
warming along an elevational gradient in the
Peruvian Andes



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The response of rainforest trees to climate warming along an elevational gradient in the Peruvian Andes

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The enduring Trocha Union and the battle of the three crosses

The Trocha Union winds along the ridge, snaking down into the Kosñipata valley, looped by the road and rivers. This old trail connects the lush lowland Amazon forest in the East to the dry Andean Highlands of Central Peru. It is home to a series of 8 study plots; the tropical montane cloud forest section of a long and diverse elevational transect spanning from 200 to 3600 m asl. In 2015 my team spent two weeks at these plots; measuring leaf traits of different tree species in an effort to better understand upslope migration in these populations. The battle of the three crosses refers to the highest point of the trail- Tres Cruces- and the struggle to walk there.

Paintina submitted 2016 for the Geosciences' art and science exhibition

Abstract

The tropical rainforests of the Peruvian Andes are some of the most biodiverse and most vulnerable to climate warming in the world. The Andes are predicted to experience substantial increases in warming of between +2 °C to +5 °C by the end of the century, in addition to an increase in the frequency of high temperature extremes, drought and flood events. The response of these forests to climate change over the next century has global relevance, due to the high levels of endemic species present and the potential role these areas will play as refugia for lowland species. Despite this, the response of tropical montane forests (TMCFs) to climate change remains under-studied.

Our current understanding of how Andean species will respond to climate change is based on studies of past compositional changes. Upslope shifts in plant communities of approximately 1.2 - 2.0 m·y⁻¹ have been observed along elevational gradients within Central and South America over the last decade. Based on these migration rates, it has been estimated that the majority of communities will lag behind increases in temperature by 5.5 °C by the end of the century. The implications of this for populations at the trailing range edge is unclear, due to a lack of mechanistic data concerning the acclimatory limits of rainforest species.

When faced with rapid warming plant species will need to rapidly adapt, acclimate or migrate in order to survive. In the case of Andean species, migration rates may not be sufficient for a species to remain within its optimal thermal niche and adaptive responses will likely be too slow to be effective, hence individuals will have to acclimate *in situ* to prevent a decline in performance. The acclimatory ability of species can be quantified by measuring changes in performance, leaf physiology and anatomy in response to experimental manipulations of climate, however such studies are rare within the tropics.

Here we carried out a seedling transplant experiment, utilising an extensive 400- 3500 m asl elevational gradient in the Peruvian Andes, to simulate climate warming and upslope migration of tree seedlings under real-world conditions. To provide context for the transplant study, natural variation in leaf anatomical traits and physiological stress were explored for twelve species belonging to lowland (LF), mid-elevation (LMF) and tropical montane cloud forest communities. Adults and seedlings from the centre and furthest-most extent of each species' elevational range were studied and compared. Seedlings of each elevational forest

community were transplanted downslope and upslope of their local elevational range by the equivalent of ± 2 °C and ± 4 °C in mean annual temperature. The experiment followed the transplanted seedlings of eleven species over a one year period, monitoring survival, growth and physiological stress (F_v/F_m) of individuals. The acclimatory ability of a subset of these species was quantified by measuring changes in photosynthetic capacity (V_{cmax} and J_{max}), respiratory capacity (R_d) and anatomical traits (N_a , P_a , LMA , $LDMC$) in response to transplantation.

The results showed that within the natural population there was little evidence of leaf trait acclimation to elevational shifts in climate, but also little evidence of physiological stress at the trailing range edge. There were however differences in the leaf trait strategies employed by each elevational community, increasing in abiotic stress-tolerance with elevation. Physiological stress was greatest in the seedling population and, unlike the adult population, increased slightly at the trailing edge. This indicated that seedlings were more vulnerable to warming than their adult counterparts and at mid-elevations TMCF seedlings were more vulnerable than LMF seedlings.

Seedling survival and growth declined in response to transplantation away from the home elevation for the majority of species, with upslope declines as a result of abiotic limitations, and downslope declines due to biotic limitations. All seedlings were found to be able to acclimate their respiratory capacity in response to transplantation, however this was not the case for photosynthetic capacity. LMF species performed significantly better than TMCF seedlings with transplantation, demonstrating a greater acclimatory capacity for photosynthesis. LMF species were able to adjust J_{max} in order to maintain rates at ambient temperatures, but were not able to upregulate V_{cmax} upslope, whereas TMCF species were not able to respond in either transplant direction.

Overall, these findings suggest that under moderate warming scenarios LMF species will have a competitive advantage over TMCF species at mid-elevations, gradually expanding their range into TMCF species' habitat over the next century. As a result of this and due to the slow pace of upslope migration, we predict that TMCF species will undergo range retractions and possible extinctions. The speed of this response will be determined by the trajectory of future warming and the frequency of extreme climatic events.



Location, location, translocation- a doomed move or a forever home?

These seedlings have been transplanted along an elevational gradient in the Neotropics to mimic migration, due to climate warming. How will their new home affect their health and survival? What are the implications for their species and others like them, if they cannot survive in a changing environment?

Painting submitted 2015 for the Geosciences' art and science exhibition

Lay Summary

The rainforests of the Peruvian Andes are some of the most biodiverse, yet threatened of the natural world. They are home to an estimated 45,000 plant species, of which 20,000 are unique, this represents 15% of global plant species in only 1% of the world's land area. These rainforests are under threat from deforestation as a result of increasing agriculture and industry, but they are also vulnerable to changes in climate. The Peruvian Andes have been described as a climate change hotspot, with predicted rates of warming higher than the global average. The region is also predicted to experience a higher frequency of extreme weather events, such as heatwaves, droughts and floods. The future of Andean montane forests and their lowland neighbours is difficult to predict, due to a lack of scientific studies investigating the response of rainforest trees to climate change.

Our current understanding of how rainforest trees will respond to climate change is based on studies that track populations over time. Along elevational gradients spanning from lowland rainforest to montane cloud forests, the centre of forest communities have been found to shift upslope at a rate of a few metres per year. This is as a result of mortality at warmer downslope sites and seedling and tree growth at cooler upslope sites. From these studies it has been estimated that the majority of these forest communities will not be able to move upslope quickly enough to keep up with current rates of warming. The implications for populations at the downslope sites is unclear, as we do not fully understand the underlying mechanisms behind mortality at these sites. Similarly we do not know if trees will be able to continue moving upslope, as these sites may become increasingly unfavourable.

When faced with rapid warming plant populations respond by migration, adaptation (progeny become gradually better suited to their environment than parents over many generations) or acclimation (adjusting leaf function and anatomy to prevailing conditions). In the case of Andean species, acclimation is the only viable strategy to respond to short-term rapid climate change. However little is known about the extent to which rainforest trees can adjust their leaf function and anatomy in response to climate change.

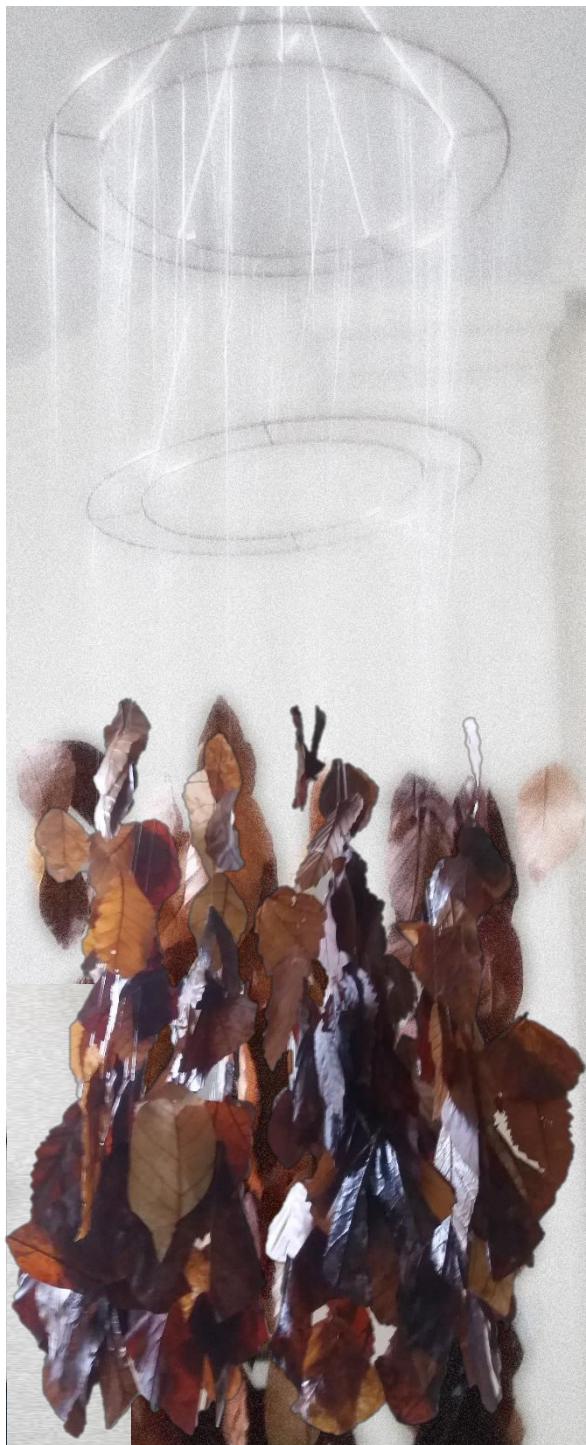
Here we used an elevational gradient spanning 3000 metres along the eastern slopes of the Peruvian Andes, to test how rainforest tree seedlings respond to climate change. We chose seedlings to represent lowland, lower montane and upper montane forest communities. We grew the seedlings at the centre of their forest community (home site) and then transplanted

some of them away. We moved seedlings downslope to simulate end-of-century levels of warming and upslope to simulate the rate of migration required to keep up with current rates of warming. We monitored the performance of the seedlings over a one year period, recording survival, growth and a measure of seedling health. For a subset of these seedlings we measured changes in underlying plant function (the capacity of individuals to photosynthesise and respire) and anatomy (leaf nutrients, thickness, and mass to area relationships) in response to transplantation. In order to interpret the results of the transplant experiment we also measured the natural variation in rainforest species health and anatomy. We compared the variation in both adult trees and seedlings across the whole distribution of the plant populations. In this way, we would be able to discover evidence of upslope migration i.e. declines downslope and growth upslope.

The results showed that across each forest community there was little evidence of changes in leaf anatomy or health. Seedlings were more vulnerable to declines in health than their adult counterparts, particularly at downslope sites, indicating that they were more vulnerable to warming. We also found that where upper and lower montane communities overlapped, the former were healthier than the latter.

The results of the transplant experiment showed that seedling survival and growth was lower away from the home elevation both downslope and upslope. The mechanism behind downslope declines were related to biological factors, such as increases in defoliation by pests and diseases. In contrast, upslope declines were related to decreases in the favourability of the environment, such as low temperatures and low light levels, due to increased cloud cover. The forest communities differed in their ability to adjust their underlying leaf function in response to transplantation. Lowland and lower montane species were able to optimise their leaf function at lowland and home sites, however they were not able to optimise their leaf function fully at upslope sites. Upper montane species were unable to optimise their function at any sites away from their home elevation.

Overall, these findings suggest that by the end of the century, at current rates of warming, lowland and lower montane species will be able to migrate upslope and begin to replace upper montane species. This will result in declines and possible extinctions of the upper montane population. The rate at which these changes will occur depends upon how climate change unfolds, nevertheless the impact will be substantial for montane biodiversity



Uniting the pieces

Each piece is made from an impression of a leaf fragment collected during my fieldwork in Peru. My research uses anatomical and physiological leaf traits to understand how Andean tree species respond to climate change. The leaves represented here are taken from the survivors of a seedling transplant experiment simulating climate warming and upslope migration.

Mobile submitted 2017 for the Geosciences' art and science exhibition

Declaration

The seedling experiment presented in this thesis was part of a collaborative project, funded by the National Science Foundation (NSF). The project was proposed by Miles Silman (Wake Forest University) and Ken Feeley (Florida International University) in collaboration with my supervisor Patrick Meir (Australian National University, formerly University of Edinburgh). A pilot experiment, using fewer species and subplots, was set-up according to this proposal during 2013- 2014 by Rachel Hillyer (PhD candidate, Wake Forest University) with contributions from myself concerning subplot design and the positioning of microclimate sensors. Based on data I collected from this pilot study (2014), I further re-designed aspects of the set-up to facilitate measurements of seedling physiology. This resulted in the implementation of additional subplots for my use during 2015 and 2016.

I worked with Rachel Hillyer to select species for the experiment, based on the needs of this study and the wider project. Together we designed the methodology to monitor seedling growth and survival of seedlings across the wider project, but the monthly measurements of survival and physiology were of my own design (Chapter 4). Rachel Hillyer and Catherine Bravo (PhD candidate, Florida International University) were responsible for the set-up and running of all censuses. They, along with Cassie Freund (PhD candidate, Wake Forest University), were involved in data-entry and initial data-quality assessments of microclimatic and census data, whereas I was responsible for the methodological design, collection and analysis of all data relating to anatomical and physiological traits.

I declare that I composed this thesis and that the work presented here is my own, except where acknowledged above. This work has not been submitted, either whole or in part, for any other degree or professional qualification.



Philippa Stone,
September 2017

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Throughout the many ups and downs of my PhD I received a great deal of help from many people. I am most grateful for the generous guidance and encouragement I have received from my supervisors. For Patrick's boundless enthusiasm that stretched across oceans, for John's abundant stimulating conversation and for Caroline's unwavering support in all things. Under your direction I have grown in confidence, taken ownership of the project and produced a thesis that I am proud of.

I feel lucky to have learnt from some of the best plant physiologist: Yann Salmon who introduced me to photosynthesis measurements on the LI-6400XT during fieldwork in Barcelona; Owen Atkin, Keith Bloomfield and John Egerton who trained me at the FACE site near Sydney; and finally Patrick Meir and Lucy Rowland who helped me to troubleshoot during my own fieldwork. Without their assistance I would never have completed my data collection.

The mega seedling transplant experiment would not have been possible without many years of collaboration between members of the Andes Biodiversity and Ecosystem Research Group (ABERG), or the dedication of a small number of PhD students and research assistants who kept the project running over many months. I will be eternally grateful to Rachel Hillyer, who taught me so much about tropical fieldwork, Catherine Bravo, who saved my LI-6400XT and for all the small miracles performed by: Judith, Alex, Adan, Ed, Rosa, Omayra, Carlos, Ted, Amalia, John, Claudia, Dino, Andy, Cameron, Gabriel, Rossibelt, Paul, Lucero, Rudy and Jhonatan. Thank you for the fieldwork memories!

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Finally, I am so very thankful for the love and support that I have received from my husband David, my family and closest friends over so many years. They gave me the belief to aim high and the encouragement to keep on writing.

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List of acronyms

| | |
|------------------------------------|--|
| A/A_n | Light-saturated photosynthesis |
| AIC | Akaike Information Criterion |
| A_{sat} | Area-based net photosynthesis measured at saturating light, ambient CO ₂ (400 µmol mol ⁻¹) and ambient temperature |
| asl | Above sea level |
| A_{max} | Area-based maximum photosynthesis measured at saturating light, saturating CO ₂ (1500µmol mol ⁻¹) and ambient temperature |
| C_i | Intercellular CO ₂ concentrations |
| CUE | Carbon use efficiency |
| ENSO | El- Niño southern oscillation |
| F_o | Minimum fluorescence (dark) |
| F_m | Maximum fluorescence (dark) |
| F_v | Maximum variable fluorescence (dark) |
| F_{m'} | Maximum fluorescence (light) |
| F_t | Steady state fluorescence |
| F_v/F_m | Potential quantum efficiency of PSII (dark) |
| GLLM | General linear mixed effect model |
| GLM | General linear model |
| GPP | Gross primary productivity |
| g_s | Stomatal conductance |
| J_{max} | Maximum rate of Ribulose-1,5-bisphosphate regeneration at ambient temperature |
| J_{max25} | Maximum rate of Ribulose-1,5-bisphosphate regeneration adjusted to 25 °C |
| LAI | Leaf area index |
| LDMC | Leaf dry matter content |
| LF | Lowland forest |
| LMA | Leaf mass per unit area |
| LMF | Lower montane Forest |
| L_{th} | Leaf thickness |
| MAT | Mean annual temperature |
| N_a | Leaf nitrogen content on an area basis |
| N_{mass} | Leaf nitrogen content on a mass basis |
| NDVI | Normalised difference vegetation index |
| NPP | Net primary productivity |
| NPQ | Non-photochemical quenching |
| P_a | Leaf phosphorus content on an area basis |
| PAR | Photosynthetically active radiation |
| ΦPSII | Quantum yield of photosystem II |
| Q₁₀ | Change in rate with a change in 10 °C |
| R_a | Autotrophic respiration |
| R_d | Dark leaf respiration measured at ambient leaf temperature |
| R_{d25} | Dark leaf respiration adjusted to 25 °C |
| SLA | Specific leaf area |
| T_{leaf} | Leaf temperature |
| TMCF | Tropical Montane Cloud Forest |
| T_{opt} | Temperature optimum |
| TPU | Triose phosphate utilisation |
| T_{soil} | Soil temperature |
| V_{cmax} | Maximum rate of Rubisco carboxylation at ambient temperature |
| V_{cmax25} | Maximum rate of Rubisco carboxylation adjusted to 25 °C |
| VPD | Vapour pressure deficit |

Chapter 1. General Introduction to Thesis

1.1 Tropical Andes biodiversity hotspot

The tropical Andes are one of the most understudied regions in the world (Stroud and J. Feeley 2017), despite their status as a high-priority biodiversity hotspot. The Andes contain approximately 20,000 endemic plant species (6.7% of global plant species) currently at risk of extinction from land-use changes such as agriculture, deforestation, industry and mining (Myers et al. 2000, Suarez et al. 2011). In addition the tropical Andes are vulnerable to and are currently experiencing rapid climate change (Marengo et al. 2011, Russell et al. 2017). This is predicted to have large negative impacts on existing biodiversity and ecosystem services such as water resources, soil stability and carbon stores (Anderson et al. 2011).



Figure 1-1 Location of elevational study transect (yellow circle) within the tropical Andes' biodiversity hotspot (red area) (Myers et al. 2000).

The biodiversity hotspot (Figure 1-1, red area) incorporates a wide geographic and elevational range across the breadth of the Andes including vegetation ranging from tropical wet forest in the east (Los Yungas) to desert in the west (Puna Xerofítica), with highland grass and scrubland in between (Puna Húmeda) (Josse et al. 2009). The elevational transect under study is located within the Peruvian Yungas (Figure 1-1) and spans the following elevational forest communities: lowland, below 500-800 m asl (metres above sea-level); lower montane

between 500-800 and 1800-1900 m asl; and montane between 1800-1900 and 2900-3200 m asl (Figure 1-2).

Elevational forest community groups differ in a number of key functional characteristics related to structure and composition. The most distinctive structural changes with increasing elevation are a decrease in forest stature with fewer emergent trees, a decline in overall canopy height and an increase in the number of polycormic trees (Figure 1-2, b-e) (Schwarzkopf et al. 2010, Asner et al. 2013, Girardin et al. 2014, Fahey et al. 2016). There is also a marked increase in the abundance of epiphytes at mid elevations within the cloud immersion zone (Figure 1-2, b-c) (Küper et al. 2004, Krömer et al. 2005). The functional traits of community groups shift gradually with increasing elevation from acquisitive and fast-growing strategies to conservative and slow growing strategies (Loehle 1998, Wright et al. 2004, Read et al. 2014, Vitasse et al. 2014), which partially explains the low productivity of high montane forests (van de Weg et al. 2014, Malhi et al. 2016, Fyllas et al. 2017).

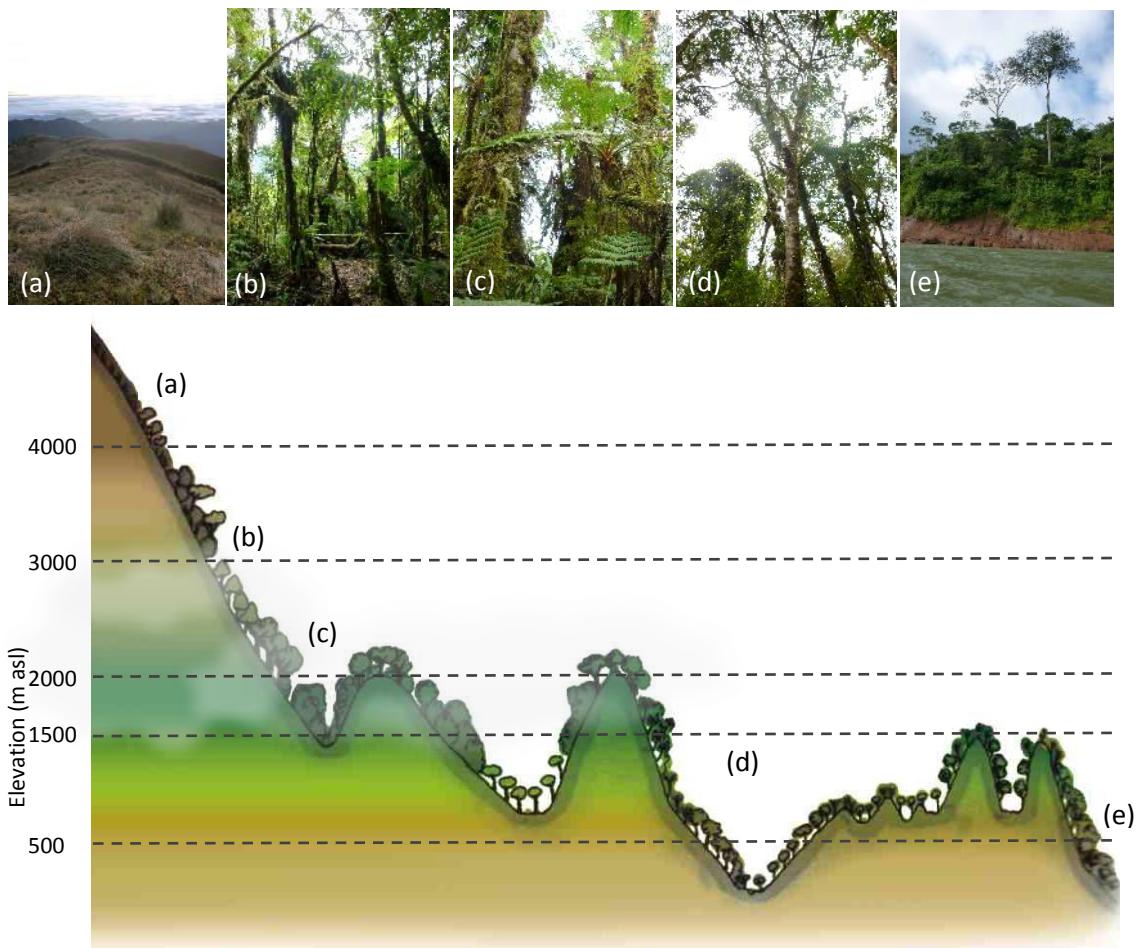


Figure 1-2 Major vegetation groups along the eastern slopes of the Peruvian Andes with decreasing elevation. (a) Puna húmeda, (b) high montane forest, (c) montane forest, (d) premontane/ lower montane forest, (e) lowland Amazon transitional forest, based on figure from Josse et al. (2009)

1.2 Climate change in the Andes

By the end of the century, climate forecasts predict a substantial increase in warming of between +1.7 °C to +6.7 °C, plus an increase in the frequency and severity of high temperature extremes across the Neotropics (Magrin et al. 2014). Changes in precipitation have also been predicted, but long-term trends are often obscured by inter-annual fluctuations in temperature and rainfall as a result of the El- Niño southern oscillation (ENSO). The general trend has been for reduced rainfall within the central Andes (up to 15% reduction by 2100) and Amazonia, but with an increase in extreme events over the last two decades: the most recent droughts were during 2005, 2010 and 2016 (in eastern Amazonia), and floods during 2009, 2012 and 2014 (south western Amazonia) (Lewis et al. 2011, Marengo et al. 2011, Magrin et al. 2014, Jimenez-Munoz et al. 2016, Marengo and Espinoza 2016).

Amazonia and the Andes have been identified as climate change hotspots using projections from the CMIP5 global climate model ensemble (Diffenbaugh and Giorgi 2012) and regional climate models (Russell et al. 2017). Andean forests are currently warming by between +0.1 °C and +0.6 °C per decade (Marengo et al. 2011, Vuille et al. 2015), with increases in temperature predicted from a wide range of models of between +2 °C to +5 °C by the end of the century (Magrin et al. 2014). As climate warming is expected to increase with elevation, projected values of warming vary along the elevational study transect; +2.5 °C to +3.5 °C at 2000 m asl relative to +3.0 °C to +5.0 °C at 4000 m asl (Bradley et al. 2006, Urrutia and Vuille 2009). However, according to satellite observations over a 25 year period, cloud frequency has tended to decrease and drought intensity has tended to increase in the lowlands at the base of the Andes (Halladay et al. 2012), exacerbating current warming.

1.3 Migratory response to climate change

There is consistent evidence of migration in response to climate change across most communities, but with few studies within the tropics (McCarty 2001, Parmesan 2006, Kelly and Goulden 2008, Chen et al. 2011, Feeley et al. 2017). The first notable example of climate-induced whole species range shifts was documented by Parmesan et al. (1999), showing that butterfly species in Europe have migrated poleward in response to increasing temperatures. Within the tropics, species are more likely to migrate along elevational rather than latitudinal gradients, as the former offers greater environmental gradients over much shorter distances, therefore allowing more rapid range shifts (Colwell et al. 2008).

Within montane cloud forest in Costa Rica, a decrease in the frequency of cloud immersion and rainfall at lower elevations was found to drive upslope migration in bird species and declines in lizard and Anuran populations (Pounds et al. 1999, Pounds et al. 2005). Along an elevational gradient in Madagascar, lizards and amphibians were found to migrate upslope, keeping pace with climate warming (Raxworthy et al. 2008), but with declines and extinctions predicted in the future. This was because, as in many tropical montane forests, high levels of endemism exist in locations with no future climate analogues i.e. above the treeline and beyond (Rull and Vegas-Vilarrubia 2006). At lower elevations where future climate analogues are present, many are unsuitable for species establishment due to poor habitat quality, or cannot be reached due to migration barriers such as changing land-use and deforestation (Feeley and Rehm 2012). Where migration corridors exist there is increased competition for decreasing habitat areas upslope, with lowland species increasingly encroaching on tropical montane species' habitat (Foster 2001, Feeley and Silman 2010).

Shifts in plant distributions have only recently been studied within the tropics. They require long-term observational data-sets tracking shifts in species' ranges through repeated censuses of permanent forest plots along climatic gradients. Studies in Peru (Kosñipata gradient), Costa Rica and Colombia observed mean upslope movements in tree populations of around $1.2\text{--}2.5 \text{ m year}^{-1}$ over the last few decades with over half of plot communities lagging behind current rates of warming (Feeley et al. 2011, Feeley et al. 2013, Duque et al. 2015). Upslope shifts in communities were mainly driven by mortality at the lower range edge and were slowed by limitations to dispersal, establishment and growth at the upper range edge (Feeley et al. 2011, Feeley et al. 2013, Duque et al. 2015). This suggests that the majority of species' ranges are contracting from the lower range edge without compensatory expansions at the upper range edge.

1.4 Acclimatory response to climate change

In response to rapid warming plant species will need to rapidly adapt, acclimate or migrate in order to survive. Studies have mainly focused on the importance of migration and adaptation in species survival, but acclimation through phenotypic plasticity is equally important (Nicotra et al. 2010). For example, the extent to which a plant can acclimate to changing conditions will determine its fitness *in situ*. Recent studies have also highlighted that certain environment-induced changes may be heritable (epigenetic processes), thus

influencing the adaptive potential of a species (Bossdorf et al. 2008). These responses are connected, as successful migration requires sufficient phenotypic variability for individuals to be competitive under new environmental conditions and pressures (Almeida et al. 2013).

1.4.1 Response to warming by carbon-related plant physiological processes

The response of plants to temperature is complicated, being species specific and dependent on both the ecological adaptation of the individual and the co-occurring abiotic and biotic factors acting upon it (Ghannoum and Way 2011). Generally for tropical plants, photosynthesis responds positively to warming until the optimum temperature is reached at approximately 30 °C, above which photosynthesis tends to decline. Above 30 °C the risk of permanent damage to photosynthetic systems increases with proximity to a critical temperature threshold, which has been observed from 45 °C to 53 °C (Larcher 2003, Doughty and Goulden 2008, Vargas and Cordero 2013, Krause et al. 2014, Slot et al. 2016, O'Sullivan et al. 2017).

Photosynthetic machinery is most at risk from high temperatures, which directly affect the chloroplast membrane structure and all of the constituent biochemical reactions (Taiz and Zeiger 2010). The ability of photosynthesis to acclimate to warming through an increase in the thermal optimum is associated with the thermal stability of key enzymes (e.g. Rubisco activase) and related membranes, or an increase in underlying photosynthetic capacity (Sage and Kubien 2007, Wood et al. 2012). In particular, the overall temperature dependence of photosynthesis is most affected by changes in the activation energy of the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of RuBP (Ribulose-1,5-bisphosphate) regeneration (J_{max}), as well as the ratio of the latter to the former ($J_{max}: V_{cmax}$) (Hikosaka et al. 2006, Sage and Kubien 2007, Lloyd and Farquhar 2008, Taiz and Zeiger 2010).

The response of plant respiration to temperature has often been modelled using a constant Q_{10} (the change in rate with a change in temperature of 10 °C) of 2.0, with respiration peaking at temperatures of approximately 50 °C. However, it has since been established that Q_{10} varies seasonally and has been found to decelerate in response to warming in a way that is consistent across biomes and different plant functional types (Atkin et al. 2000, O'Sullivan et al. 2013, Heskell et al. 2016). At low temperatures, Q_{10} is thought to be limited by maximum catalytic enzyme activity, whilst at moderate temperatures substrate availability declines to

become limiting instead. At very high temperatures, enzyme activity again becomes limiting, due to increased deactivation of respiratory processes (Taiz and Zeiger 2010).

With the onset of warming it is unlikely that the ratio of photosynthesis to respiration will be maintained, especially under temperature extremes. Both photosynthesis and respiration are thought to exhibit greater acclimation to new temperatures in new leaves than pre-existing leaves (Atkin and Tjoelker 2003), but the extent of acclimation depends on the thermal regimes compared and species studied (Atkin et al. 2005). Dark respiration (R_d) has been found to acclimate more readily than photosynthesis across many functional groups, temperature, nutrient and drought regimes (Campbell et al. 2007, Metcalfe et al. 2010, Way and Oren 2010, Atkin et al. 2015, Rowland et al. 2015, Slot and Kitajima 2015, Rowland et al. 2016). Where acclimation of photosynthesis does occur, plant growth may still decrease: Way and Yamori (2014) describe this as a detractive acclimatory adjustment. This potential difference in constructive acclimatory potential between photosynthesis and respiration has implications for the carbon balance and performance of individuals and communities, potentially contributing to mortality under climate warming scenarios (Campbell et al. 2007, Way and Oren 2010, Slot and Winter 2016).

1.4.2 Response of functional traits to changing climate

Functional leaf traits have been widely used as a tool to explore ecosystem composition, function and processes, as well as to predict the response of communities to changing climate (Wright et al. 2005, Ordoñez et al. 2009, Kattge et al. 2011, Díaz et al. 2013). At large spatial scales advances in canopy-level mapping of leaf anatomical and physiological traits enable the efficient monitoring of inaccessible (Pieruschka et al. 2014, Jeong et al. 2017) and highly-species diverse communities over time (Lavorel and Garnier 2002, Ananyev et al. 2005, Messier et al. 2010, Asner et al. 2013, Joiner et al. 2014, Asner et al. 2016, Fyllas et al. 2017). Temporal variation in reflective vegetation indices and measures of physiological stress show canopy-level responses of the Amazon basin to seasonality of rainfall and cloud cover as well as extreme drought-related events, enabling detection of declines in photosynthetic productivity as a result of climatic stress (Asner and Alencar 2010, Lee et al. 2013, Guan et al. 2015, de Sousa et al. 2017).

At the plant level, acclimation to changing water and light stress are often evident in the variation within an individual's anatomical leaf traits. For example, shade leaves maximise

their ability to capture light, increasing their specific leaf area (*SLA*), whereas sun leaves have thicker leaves with greater investment in photosynthetic capacity and nutrients per unit leaf area (Jones 1992, Poorter et al. 2009). Leaf traits often vary more extensively among individuals and species, as changes in environmental stress alter the trade-off between conservative (stress-tolerant) and acquisitive (resource-competitive) resource allocation strategies as per the leaf economic spectrum (Wright et al. 2004). Where stress is greatest, traits are expected to be the most conservative, with lower *SLA* and higher leaf thickness and nutrients contents (area-basis) relative to their acquisitive counterparts (Loehle 1998, Wright et al. 2004, Read et al. 2014, Vitasse et al. 2014).

Under very high irradiances, shade leaves are able to avoid or rapidly disperse excess light; through leaf orientation, increased stomatal conductance and in the use of leaf pigments (Morales et al. 2006). However when these mechanisms are not sufficient, for example in combination with water deficits or under high ultraviolet (UV-B) radiation, individuals can suffer photoinhibition and long-term damage to the photosynthetic machinery (Jones 1992, Caldwell et al. 1995, Krause et al. 2003, Larcher 2003, Bader et al. 2007). Chlorophyll fluorescence analysis provides an effective tool to rapidly assess changes in the efficiency of photosystem II (PSII) that may indicate photoinhibition or long-term stress (Maxwell and Johnson 2000). Many parameters can be calculated from a single fluorescence trace (Figure 1-3). The most useful are the quantum yield of photosystem II (Φ_{PSII}), which is the proportion of quantum energy used in photochemistry (light-adapted) (Equation 1) and the potential quantum efficiency of PSII (dark adapted F_v/F_m) (Equation 2):

$$\Phi_{PSII} = \frac{(F_m' - F_t)}{F_m'} \quad (1)$$

$$F_v/F_m = \frac{(F_m - F_0)}{F_m} \quad (2)$$

Where F_m' and F_t are maximum and steady state fluorescence under light-adapted conditions and F_m , F_0 and F_v are maximum, minimum and variable fluorescence under dark-adapted conditions (Figure 1-3).

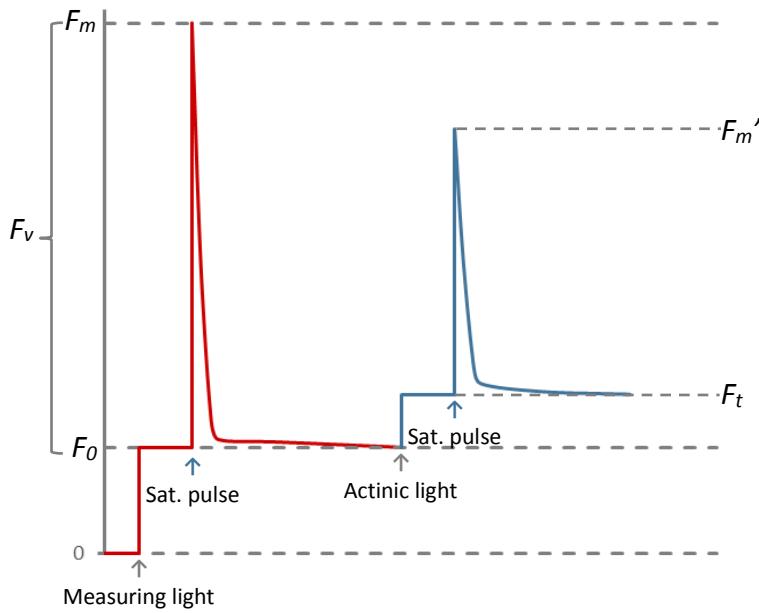


Figure 1-3 Fluorescence trace adapted from Murchie and Lawson (2013) using dark-adapted leaf material. Minimum fluorescence (F_0) was measured under a weak measuring light, followed by a saturating light pulse to measure maximum fluorescence (F_m). Variable fluorescence (F_v) is the difference between these parameters. After exposure to actinic light a saturating pulse is applied to obtain light-adapted maximum ($F_{m'}$), and steady state fluorescence (F_t).

Φ_{PSII} shows the extent of energy use by PSII and overall rates of photosynthesis at one time point and is commonly used to show reversible changes in leaf-level photosynthesis over diurnal cycles. F_v/F_m is useful in determining whether the plant is experiencing long-term stress, as it shows depressions in efficiency during a fully relaxed dark-adapted state. When a plant experiences thermal damage, minimum fluorescence (F_0) is found to increase whereas maximum fluorescence (F_m) decreases. Generally values of F_v/F_m above 0.8 indicate that the plant is not experiencing physiological stress, whereas values below 0.6 indicate long-term depressions in photosynthetic efficiency from which the individual is unlikely to fully recover (Krause et al. 2010, Murchie and Lawson 2013).

1.5 Vulnerability of tropical trees to climate change

1.5.1 Comparisons between functional groups

Relative to other functional groups, tropical forests are considered to be more vulnerable to changing climate. This is due to the narrower range of climatic conditions experienced during tropical species' evolutionary history and the large distances required for them to migrate to their climate analogues (Cunningham and Read 2003a, Cunningham and Read 2003b, Wright et al. 2009, Feeley and Rehm 2012). In a meta-analysis Way and Oren (2010) found that

tropical and subtropical trees were more likely to experience reduced growth as a result of higher temperatures (+3 to +6 °C) than trees from cooler environments. This could partly be explained by the proximity of each group to their optimum growth temperature; species from cooler climates were found to exist further from their optimum growth temperature than tropical species i.e. warming moves them closer to their optimum. Similarly tropical species are considered to be closer to their temperature optimum for photosynthesis, with observed declines in productivity linked to recent climatic warming and drought events (Clark 2004, Doughty and Goulden 2008, Lloyd and Farquhar 2008, Doughty 2011, Clark et al. 2013).

1.5.2 Comparisons between lowland and montane species

Montane species are adapted to tolerate the increasing environmental stress due to low temperatures, diurnal temperature differences and increased UV-radiation. However, the increasing tendency towards conservative traits with elevation (increases in leaf mass per unit area (*LMA*) and leaf nitrogen per unit area (*N_a*)) make montane species less able to respond to climate warming than lowland species (Read et al. 2014), as they are less able to compete with acquisitive, fast growing species that may migrate into their habitat as the climate warms (Way and Oren 2010). Based on historic range shifts in response to changing climate, montane species have previously been replaced by upslope encroachment of lower-elevation species during warmer and drier time periods (Bush et al. 2004, Colwell et al. 2008, Morueta-Holme et al. 2015).

Tropical lowland species are considered at greater risk of experiencing high temperature stress than montane tree species due to the proximity of their photosynthetic temperature optima to current and predicted extreme climatic events (Colwell et al. 2008, Doughty and Goulden 2008, Corlett 2011). Experimental warming studies demonstrate that montane species are more sensitive to warming than lowland species; although there was no difference in the temperature optima for *V_{cmax}* (~37 °C), they had lower temperature optima of photosynthesis for *J_{max}* (~30 °C relative to ~36 °C in lowland species) (Vårhammar et al. 2015). When tropical montane species have been grown under warmer temperatures (~+5 °C difference) they have been found to reduce their photosynthetic capacity at a given temperature (Dusenge et al. 2015). These studies remain very rare and the effect of these adjustments to warming on growth and survival were not investigated.

A moderate increase in night-time temperature (+3 °C to + 6 °C) has been found to increase the growth of lowland tropical seedlings, doubling seedling biomass, increasing leaf area and decreasing *LMA*, with some evidence of thermal acclimation of R_d under controlled conditions (Cheesman and Winter 2012). Slot and Winter (2017) showed that lowland and lower montane tropical species are able to maintain growth rates with a +5 °C increase in growth temperature by shifting their optimum temperature of photosynthesis to a warmer value and down-regulating their rates of R_d . However, thermal acclimation was imperfect, resulting in plants operating at above optimal temperatures for photosynthesis with greater levels of warming (+8 to +10 °C). Similarly, Cheeseman and Winter (2013) showed that a moderate increase in growth temperature (+ 3 °C) above ambient (~30 °C) elicited a similar growth response in populations of a lower montane and lowland early-successional species, whereas an increase of + 6 °C caused declines in growth and an increase of + 9 °C resulted in the death of the montane species. This was despite the lower montane species range extending into the lowlands, suggesting that the local montane population may have differed from the local lowland population, possibly through local epigenetic variation, developmental factors or genetic drift (Hargreaves et al. 2014).

1.6 Overview of thesis

1.6.1 Thesis aims

The overarching thesis aim is to explore whether Neotropical tree seedlings of different species are able to survive and grow under an experimental change in climate and to assess species' acclimatory abilities through the study of anatomical and physiological leaf traits. Upslope shifts in populations as well as community composition-related shifts in functional traits have been observed along the Kosñipata gradient; however, the mechanisms behind these shifts have not been explored. There is little understanding of how intra-specific functional traits change with elevation and ontogenetic stage (life-stage), or if these changes enhance individuals' fitness in response to climate change. In particular, this thesis addresses the following questions through both an observational study of trait distributions and a real-world seedling transplant experiment:

| CHAPTER AIMS | THESSIS Q.s | Can we use leaf traits to understand how species respond to climate change? | Do tree species differ in their response to climate change? | How do tree seedlings perform with a ± 2 °C and ± 4 °C change in climate? | Is there evidence that tree seedlings can acclimate to new transplant conditions? |
|--|---|---|---|---|---|
| 3. Can upslope shifts along an Andes-Amazon migratory corridor be explained by changes in leaf and physiological stress at the lower range edge? | To measure anatomical and physiological traits of seedlings and adults in response to elevational shifts in climate | 4. The response of Andean tree seedlings to a 100 year shift in climate warming To measure changes in seedling physiological stress in response to ± 2 and ± 4 °C transplantation To detect between-species differences in seedling response to transplantation | 5. Pushing the limits: using an elevational gradient to experimentally test the acclimation of Neotropical tree seedlings to changing climate To measure changes in seedling physiological and anatomical traits in response to transplantation To test for differences in response between species of different elevational groups | | |
| To test for intraspecific and interspecific differences in leaf traits at species' range edges | To assess the current extent of species' functional trait variation in response to elevational shifts in climate | To measure seedling survival, stress and growth response to transplantation over time To assess evidence for physiological stress, declines in survival and restrictions of growth after transplantation | To measure the response of seedling leaf physiology and anatomy to transplantation To assess the evidence of acclimatory shifts in seedling physiology and anatomy in response to transplantation | | |

Figure 1-4 Thesis questions and specific aims colour-coded to indicate thesis themes.

1.6.2 Thesis structure

Chapter 2- Seedling transplant experiment along the Kosñipata gradient

- An overview of the gradient, including abiotic and biotic trends with elevation
- The rationale behind the transplant experiment and approach taken
- Interpreting and predicting the response of seedlings to transplantation

Chapter 3- Can upslope shifts along an Andes-Amazon migratory corridor be explained by changes in leaf and physiological stress at the lower range edge?

- An overview of the importance of studying inter-specific and intra-specific leaf trait variation in the tropics
- Assessing intra-specific and ontogenetic differences in traits and stress at the extremes of a species' natural elevational range

Chapter 4- 100 years of climate warming: tracking the response of Neotropical tree seedlings to real world changes in climate

- Current predictions of the response of Andean trees to end-of-century warming
- Measuring the response of seedlings to transplantation in terms of their survival, physiological stress, and growth response

Chapter 5- Pushing the limits: using an elevational gradient to experimentally test the acclimation of Neotropical tree seedlings to changing climate

- The importance of understanding the mechanisms behind acclimatory responses to changing climate
- Assessing the acclimatory response of seedling's anatomical traits and carbon-related physiological processes

Chapter 6- Synthesis and Conclusions

- An overview of the key findings in relation to the thesis aims and questions

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Chapter 2. Seedling transplant experiment along an Andes to Amazon gradient

2.1 Study Site

2.1.1 Kosñipata valley location

This study takes place along a 3000 m elevational gradient extending from the Kosñipata

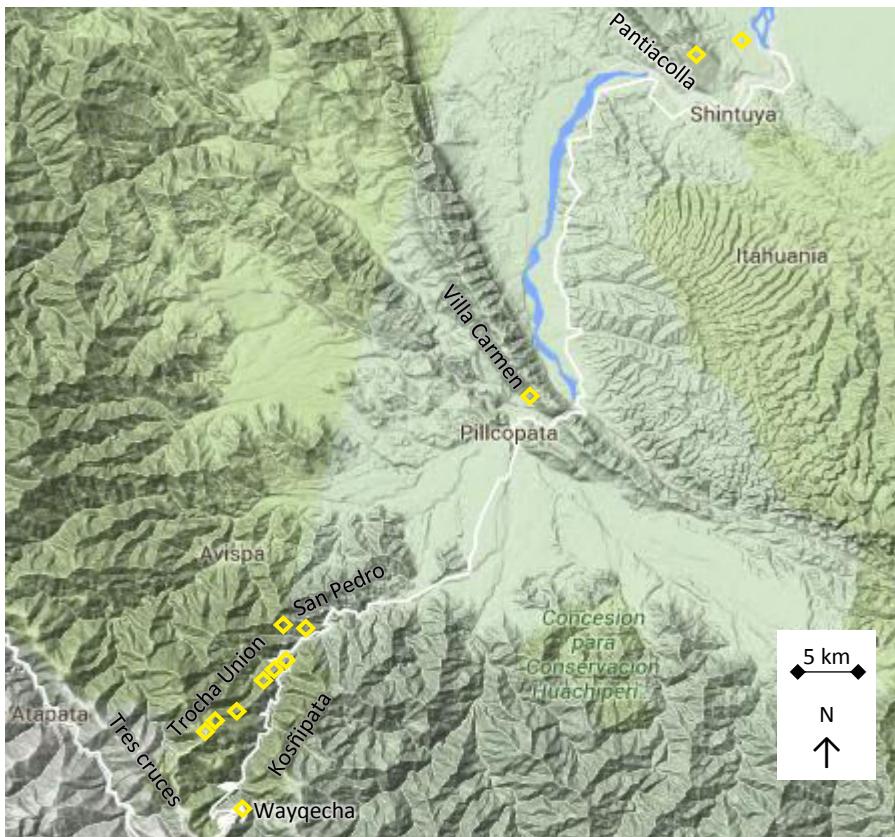


Figure 2-1 Relief map of study plots along the Andes-Amazon elevational gradient. Diamonds show the location of the study plots (Google 2017).

Valley of Manu National Park, along the south-eastern slopes of the Peruvian Andes, down to the Madre de Dios Amazonian region (Figure 2-1). The elevational gradient, hereafter referred to as the Andes-Amazon gradient, has been studied in detail for 10-15 years, with research focussed on a series of 1 ha permanent plots established by the Andes Biodiversity and Ecosystem Research Group (ABERG), at intervals from 200-3500 m (Zimmermann et al. 2009, Malhi et al. 2010, Rapp and Silman 2012, Girardin et al. 2013b, Nottingham et al. 2015b, Malhi et al. 2016). The montane sites were located along a 10 km stretch of the Andes-Amazon gradient: the top of the cloud immersion zone (2950 m asl); within the cloud immersion zone (2550 m asl, 2150 m asl and 1750 m asl); and at the lower edge of the cloud

base (1550 m asl) (Girardin et al. 2013a). The lowest sites (800 m asl and 450 m asl) were located in lowland forest near the town of Pillcopata and at the base of the Pantiacolla range (Figure 2-1).

2.1.2 Abiotic changes with elevation

The general abiotic trends associated with an increase in elevation are: a decrease in atmospheric pressure (and partial pressures of CO₂ and O₂); a decrease in temperature; an increase in radiation with clear skies; and an increase in UV-B relative to solar radiation (Körner 2007). The Andes-Amazon gradient follows these general trends, with a linear environmental lapse rate of approximately 5°C km⁻¹ (Rapp and Silman 2012, Malhi et al. 2016). The region has a particularly high UV-Index (Liley and McKenzie 2006), with the highest levels of UV-damage expected during clear-sky events at higher elevations (Rehm and Feeley 2013).

The most abrupt change in environmental conditions along the Andes-Amazon gradient occur at the transition zone between lowland forest and montane cloud forest (1500 - 2000 m asl), which is characterised by a sharp change in geology, soil properties, slope and radiation in the presence of the cloud base (Zimmermann et al. 2010, Halladay et al. 2012a, Rapp and Silman 2012, Whitaker et al. 2014, Malhi et al. 2016) (Table 2-1). Cloud dynamics drive many of the temporal and elevational changes in microclimate along the gradient; such as decreases in photosynthetically active radiation (PAR), vapour pressure deficit (VPD) and temperature in the presence of the cloud base. The footprint of the cloud base changes seasonally: during September and October the cloud base shifts upslope, so VPD in the transition zone exceeds 1-1.2 kPa for 20% of the time, whereas during May and June the cloud base shifts downslope exposing high elevations (Rapp and Silman 2012). Cloud frequency along the eastern slopes of the Andes correlates with Pacific and Indian Ocean sea surface temperatures and the occurrence of El Niño-Southern Oscillation (ENSO) events. An increase in the occurrence of El Niño events is predicted to decrease cloud frequency along the Andes-Amazon gradient and increase the risk of drought (Halladay et al. 2012b).

Total soil nutrients (N and P) and the N:P ratio are variable along the Andes-Amazon gradient, although N:P ratios tend to increase with elevation within the mineral soil but decrease in organic soil (Zimmermann et al. 2012, Whitaker et al. 2014, Nottingham et al. 2015a, Nottingham et al. 2015b). The availability of nutrients to plants decreases along the Andes-Amazon gradient due to a number of direct and indirect impacts of lower temperatures on

the rate of decomposition processes (Nottingham et al. 2015a). Extremes of soil moisture also limit soil processes: either water-logging as a consequence of low levels of evaporation, or shortages due to poor retention in shallow soils on slopes, although the evidence to date shows little indication of soil water limitation (Zimmermann et al. 2010) (Table 2-1).

Table 2-1 Summary of abiotic variables for plots at each transplant elevation. Where measurements were not made during the transplant experiment, data are provided from nearby 1 ha study plots a (Malhi et al. 2016), b (Whitaker et al., 2014), c (Nottingham et al. 2015)

| RAINFOR | PAN02 | NA | SPD02 | SPD01 | TRU07 | TRU05 | TRU03 |
|--|-------------------------|--------------|------------------------------|------------------------------|------------------------------------|--------------------------|--------------------------|
| Site name | Pantiacolla | Villa Carmen | San Pedro | San Pedro | Trocha Union VII | Trocha Union V | Trocha Union III |
| Elevation (m asl) | 426 (5) | 861 (8) | 1487 (80) | 1786 (17) | 2140 (25) | 2525 (14) | 2900 (15) |
| Longitude | -12.65250 | -12.86873 | -13.04774 | -13.04658 | -13.07729 | -13.09347 | -13.10825 |
| Latitude | -71.23777 | -71.40565 | -71.53608 | -71.54358 | -71.56247 | -71.57422 | -71.59618 |
| Slope (°) | na | na | 27.1 ^a | 30.5 | 23.0 (9.4) | 13.9 (6.6) | 18.8 (7.9) |
| Aspect (°) | na | na | 125 ^a | 117 | 83 (68) | 196 (123) | 117 (87) |
| Solar Radiation ($\text{GJ m}^{-2} \text{yr}^{-1}$) ^a | 3.82 | na | 4.08 | 4.36 | na | na | na |
| Precipitation (mm yr^{-1}) ^a | 2366 | 3087 | 5302 | 5302 | 1827 | na | 1776 |
| MAT (°C) ^a | 23.5 | na | 18.8 | 17.4 | 17.4 | na | 11.8 |
| Soil parent Material ^b | Alluvial | na | Plutonic intrusion (granite) | Plutonic intrusion (granite) | Palaeozoic shales-slates (granite) | Palaeozoic shales-slates | Palaeozoic shales-slates |
| Soil Type ^b | Plinthosol ^a | na | Cambisol | Cambisol | Cambisol | na | Umbrisol |
| Soil Organic Layer Depth (cm) ^b | NA | 3.6 | 16.0 | 9.6 | 16.8 | 13.6 | 17.2 |
| Total Soil C content (%) | 3.4 (1.3) | 6.9 (1.9) | 11.1 (6.6) | 26.0 (10.0) ^c | 37.0 (4.8) ^c | 25.8 (5.7) ^c | 27.1 (5.5) ^c |
| Total Soil N content (%) | 0.16 (0.10) | 0.42 (0.12) | 0.61 (0.50) | 1.56 (0.50) ^c | 2.0 (0.24) ^c | 1.73 (0.34) ^c | 1.57 (0.21) ^c |
| Total Soil P content (mg P g^{-1}) | 0.80 (0.14) | 0.66 (0.08) | 1.59 (0.40) | 1.44 (0.09) ^c | 0.71 (0.10) ^c | 0.98 (0.14) ^c | 0.92 (0.13) ^c |
| T_{leaf} (°C) | 29.7 (1.5) | na | 23.8 (1.9) | na | 21.5 (2.9) | na | 16.9 (1.4) |
| T_{soil} (°C) | 22.5 (0.3) | na | 17.2 (0.4) | na | 15.3 (0.6) | na | 10.6 (0.5) |
| Soil Moisture Content (%) | 42.4 (8.6) | na | 43.1 (5.9) | na | 40.5 (9.6) | na | 58.7 (11.3) |
| Canopy Openness (%) | | | | | | | |
| Open plot | 5.3 (2.8) | 10.3 (3.0) | 13.9 (3.4) | 20.7 (6.5) | 23.4 (8.2) | 16.2 (4.9) | 19.8 (8.7) |
| Shaded plot | 4.8 (1.2) | 10.6 (1.2) | 11.5 (2.1) | 13.2 (3.2) | 14.2 (2.8) | 13.4 (1.2) | 14.8 (2.9) |

MAT, mean annual air temperature; T_{leaf} , leaf temperature; T_{soil} , daytime soil temperature

2.1.3 Biotic changes with elevation

As previously described (section 1.3) the Andes-Amazon gradient has been identified as a migration corridor for tropical trees, with upslope migration rates estimated to be 2.5 m year⁻¹ ($\pm 95\%$ CI= +0.6 to +4.9 m year⁻¹) at montane sites (Feeley et al. 2011). In addition to this study, the ABERG group have reported on elevational changes in forest functional traits, canopy structure, productivity and biological interactions (Table 2-2).

Table 2-2 Reported biotic trends with increased elevation along the Andes-Amazon gradient.

| Elevation (m asl) | Significant decrease | Significant increase | No significant change | Author |
|----------------------|---|--|---|---------------------------|
| 194-3644 | Macrofaunal leaf decomposition | | | (Hicks 2016) |
| 120-3537 | GPP, LAI | | CUE, NPP allocation, R_a , V_{cmax} , J_{max} | (Malhi et al. 2016) |
| 132-3379 | Leaf N:P ratio | V_{cmax25} , J_{max25} , V_{cmax25} : N_a N fraction allocated to Rubisco and electron transport $Leaf N_a$, $Leaf P_a$, LMA | J_{max25} : V_{cmax25} , Total N fraction allocated to carbon metabolism and pigment-proteins | (Bahar et al. 2016) |
| 194-3400 | P constraint on microbial processes, total N mineralisation, enzymic activity | Enzymic N:P ratio N constraint on microbial processes | Microbial nutrient ratios | (Nottingham et al. 2015b) |
| 194-3644 | | Relative importance of fungal to bacterial microbial decomposers | | (Whitaker et al. 2014) |
| 200-3500 | Canopy height and NDVI | Gaps ha ⁻¹ , % understorey cover | | (Asner et al. 2013) |
| 210-3450 | Tree height, above-ground woody biomass, species richness (> 1500 m asl) | Species, genus and family richness (< 1500 m asl) | Stem density, basal area, species richness (peaks at 1000-1500 m asl) | (Girardin et al. 2014) |
| 1750-3400 | Tree growth between species, herbivory (unpublished) | | Tree growth within species | (Rapp et al. 2012) |
| Lowland lit.- 3025 | | R_{d25} , LMA | V_{cmax25} , J_{max25} , R_d | (van de Weg et al. 2012) |
| 194-3025 | Total NPP, Above ground NPP, below ground NPP, Above-ground woody biomass | Disturbance rates, belowground C residence time | Allocation of NPP, relative C stock shifts from above to below ground | (Girardin et al. 2010) |
| 194-3025 | Stem Respiration, tree growth rate | | | (Robertson et al. 2010) |

| | | | |
|-----------|-----------------|-----------------|---|
| 1000-3450 | Seed predation | Seed survival | (Hillyer and Silman 2010) |
| 200-3600 | Leaf N_{mass} | LMA, leaf P_a | Leaf N:P ratio, P_{mass} , N_a (van de Weg et al. 2009) |

GPP, Gross primary productivity; *LAI*, leaf area index; *CUE*, carbon use efficiency; *NPP*, net primary productivity; V_{cmax} , the maximum rate of Rubisco carboxylation at ambient temperature; J_{max} , the maximum rate of RuBP regeneration at ambient temperature; V_{cmax25} , the maximum rate of Rubisco carboxylation adjusted to 25 °C; J_{max25} , the maximum rate of RuBP regeneration adjusted to 25 °C; $J_{max25}:V_{cmax25}$, the ratio of the maximum rate of Rubisco carboxylation and the maximum rate of RuBP regeneration, adjusted to 25 °C; $V_{cmax25}:N_a$, the ratio of the maximum rate of Rubisco carboxylation adjusted to 25 °C per gram of leaf Nitrogen; R_a , autotrophic respiration; R_d , area-based respiration measured in the dark at ambient temperature; R_d , area-based respiration measured in the dark adjusted to 25 °C; *LMA*, leaf mass per unit area; N_a , leaf nitrogen content on an area basis; *NDVI*, normalised vegetation index; P_a , leaf phosphorus content on an area basis; N_{mass} , leaf nitrogen content on a mass basis; P_{mass} , leaf phosphorus content on a mass basis; N:P ratio, ratio of leaf nitrogen and leaf phosphorus.

The frequency of conservative leaf traits increase with elevation along the Andes-Amazon gradient in response to the increased environmental stressors such as UV-B radiation: *LMA* and N_a increase by approximately 50% from lowland to montane sites and leaf phosphorus content on an area basis (P_a) approximately doubles (Flenley 1995, van de Weg et al. 2009, Bahar et al. 2016). Although leaf N:P ratios have been found to decrease with elevation, they remain higher than the limiting threshold (<10) (Güsewell 2004), suggesting that cloud forest species are not nitrogen limited. Similarly, soil microbes within the montane forests exhibit traits that increase their nitrogen uptake (Nottingham et al. 2015b), yet they did not seem to be directly constrained by nitrogen availability (Hicks 2016). In contrast, leaf phosphorus content is thought to be limiting at some lowland sites (van de Weg et al. 2009, Fisher et al. 2013, Rowland et al. 2016); as evidenced in the correlation between both soil and leaf phosphorus content with photosynthetic capacity (Bahar et al. 2016). In terms of soil microbial activity, phosphorus has not been found to be directly limiting (Hicks 2016), but there may still be indirect effects of low phosphorus on microbial activity (Nottingham et al. 2015a, Nottingham et al. 2015b).

As elevation increases, the forest becomes shorter in stature and gaps become more frequent, reducing the normalised difference vegetation index (NDVI) and leaf area index (LAI) of the canopy (Asner et al. 2013, Girardin et al. 2013b, Malhi et al. 2016). There is also an observed decrease in tree growth (Robertson et al. 2010, Rapp et al. 2012), above-ground woody biomass and total net primary productivity (NPP) with increasing elevation (Girardin et al. 2010, Girardin et al. 2014, Malhi et al. 2016). The reasons for low productivity at higher elevations have evaded clear understanding for several decades (Bruijnzeel and Veneklaas 1998, Tanner et al. 1998, Vitousek 1998, Letts and Mulligan 2005, Fahey et al. 2016), with

multiple explanations surrounding temperature, light availability, leaf and soil wetness, nutrient availability and multiple combinations of these. A number of recent analyses of the forests in the region have progressed the debate, linking reduced productivity to low gross primary productivity (GPP) (van de Weg et al. 2014, Malhi et al. 2016, Fyllas et al. 2017).

Recent studies investigating productivity along the Andes-Amazon gradient have shown significant increases in temperature-adjusted values (adjusted to 25 °C) of R_{d25} (area-based respiration measured in the dark) (van de Weg et al. 2012), V_{cmax25} (the maximum rate of Rubisco carboxylation) and J_{max25} (the maximum rate of RuBP regeneration) in higher elevation communities (Bahar et al. 2016). This greater investment in physiological capacity enables higher metabolic rates at lower temperatures as experienced at high elevations. Consistent with this, measurements at leaf temperatures of 20 - 25 °C suggest that net photosynthetic rates of tropical montane cloud forests (TMCF) are similar to those of lowland forests (LF) (Malhi et al. 2016). However, montane daily temperatures fall far below these temperatures (9 °C -18 °C), so it may be that further testing under these lower ambient temperatures could reveal a different pattern (van de Weg et al. 2014). It is therefore still unclear whether the more conservative leaf trait strategies observed in upland species enable these individuals to maintain high levels of productivity at lower temperatures i.e. through the upregulation of photosynthetic capacity and N-use efficiency (van de Weg et al. 2009, van de Weg et al. 2012, van de Weg et al. 2014, Malhi et al. 2016).

In addition to the abiotic stress gradient along the Andes-Amazon gradient, there is evidence of a biotic stress gradient: as with other environmental gradients in the tropics (Krömer et al. 2005, Brehm et al. 2007, Rodríguez-Castañeda et al. 2010), species richness has been found to peak at mid-elevations (1000 – 1500 m asl), coinciding with the transition between lowland and montane forest (Girardin et al. 2014). With increased elevation beyond this peak a reduction in pressure from predation has been linked to an increase in seed survival (Hillyer and Silman 2010) and a decrease in herbivory (Rapp et al. 2012).

2.2 Rationale for transplant experiment

Although retrospective observational studies are the most common way to monitor species' range shifts over time (Sexton et al. 2009), correlative approaches are limited in their capacity to predict future responses to climate change. In comparison, controlled experiments are able to test the response of species to one or more environmental variables, such as warming

and drought, which can be used to identify species' fundamental niche. However, under real-world conditions, the distribution of a species is affected by a combination of both abiotic and biotic factors, e.g. competition, predation, parasitism and mutualism, termed the realised niche (Colwell and Rangel 2009). Transplant experiments incorporate both abiotic and biotic factors in their design, allowing more realistic space-for-time simulations of future climate change scenarios.

Limitations to tree species' distributions arise at the point of pollination, seed dispersal, germination, seedling establishment and juvenile to adult recruitment. The lifetime fitness of an individual is a combination of all these factors, and when taken across a population provides the best measure of species response. However, direct measures of lifetime fitness are impractical. Of the factors contributing to lifetime fitness, those affecting early ontogenetic stages are arguably the most important, as seedlings are most susceptible to environmental fluctuations (Ettinger and HilleRisLambers 2013) and thus experience the highest levels of density-dependent mortality (Grubb 1977, Ishida et al. 2005, Bell et al. 2006, Poorter 2007). So although seedling transplant experiments cannot provide a direct assessment of an individual's lifetime fitness (Hargreaves et al. 2014), indirect measures can be made from seedlings' performance, e.g. survival, growth or biomass accumulation.

2.3 Transplant approach

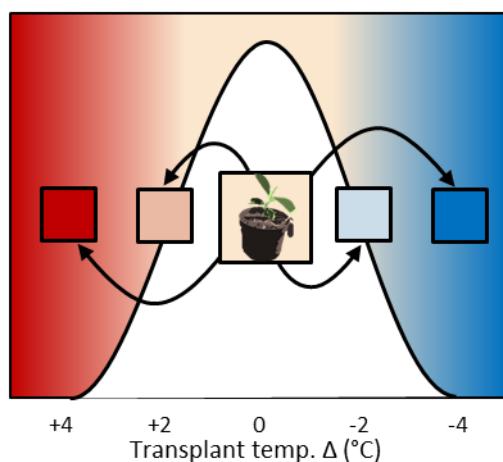


Figure 2-2 Transplant sites relative to a home transplant site within the species' range. Sites simulating climate warming were equivalent to +2 °C and +4 °C and upslope migration equivalent to -2 °C and -4 °C.

In order to keep pace with predicted warming over the next century, trees along the Andes-Amazon gradient need to migrate upslope by 300 - 1300 m, given a lapse rate of -5 °C km^{-1} (Rapp and Silman 2012). Based on current mean migration rates (2.5 m year^{-1} , $\pm 95\% \text{ CI} = +0.6$ to $+4.9 \text{ m year}^{-1}$) and moderate warming ($+4 \text{ °C}$) this is unattainable for the majority of species.

To predict the response of tree species to this end-of-century warming, we carried out a seedling transplant experiment moving individuals along the Kosñipata gradient away from the centre of each species' local distribution by the equivalent of +/- 2 °C and +/- 4 °C (Figure 2-2).

2.3.1 Species selection

Species were primarily chosen based on their local abundance and local and Neotropical range, incorporating species with different elevational range widths centred on three elevational communities; lowland forest (LF), lower/ sub-montane forest (LMF) and tropical montane cloud forest (TMCF). The observed response of each species to recent climate change was considered in the selection, i.e. fast or slow migrants, however no clear distinction could be made between species, due to small sample sizes at the species-level in the current census record (Feeley et al. 2011).

*Table 2-3 Tree species selected for transplant experiment by elevational community. Abundance and range widths are estimated from a series of 1 ha plots along the Andes to Amazon gradient (Feeley et al. 2011). *Hemiepiphytic species (Austin 1993). a (Stimm et al. 2008), b (Lütge 2007), c (Roth 1987), d (Graham 1984), e (Todzia 1988), f (Russo et al. 2005), g (Swaine and Beer 1977), h (Swamy 2008).*

| Species | Family | Abundance | Range width | Migratory class. | Dispersal |
|----------------------------------|----------------|-----------|-------------|------------------|--------------------------------------|
| <i>Clethra revoluta</i> | Clethraceae | 182 | 1250 | Stable | Wind ^a |
| <i>Clusia thurifera*</i> | Clusiaceae | 172 | 750 | Stable | Birds ^b |
| <i>Myrcia splendens</i> | Myrtaceae | 87 | 1905 | Stable | Birds ^c |
| <i>Alzatea verticillata*</i> | Alzateaceae | 423 | 750 | Change | Most likely by wind ^d |
| <i>Hedyosmum goudatianum</i> | Chloranthaceae | 96 | 1700 | Change | Birds ^e |
| <i>Schefflera patula*</i> | Araliaceae | 41 | 700 | na | Birds, bats and mammals ^c |
| <i>Tapirira cf. guianensis</i> | Anacardiaceae | 37 | 655 | Stable | Birds and mammals ^c |
| <i>Guatteria glauca</i> | Anonaceae | 47 | 550 | Stable | Birds, bats and mammals ^c |
| <i>Dictyocaryum lamarckianum</i> | Arecaceae | 5 | 550 | Change | Birds and bats ^c |
| <i>Cedrelinga cateniformis</i> | Fabaceae | 32 | 825 | na | Mammals ^f |
| <i>Theobroma cacao</i> | Malvaceae | na | na | na | Mammals ^h |
| <i>Iriartea deltoidea</i> | Arecaceae | 215 | 1325 | na | Mammals ^f |
| <i>Brosimum lactescens</i> | Moraceae | 7 | 1325 | na | Birds and mammals ^{fh} |
| <i>Hura crepitans</i> | Euphorbiaceae | na | na | na | Explosive ^g |
| <i>Celtis schippii</i> | Cannabaceae | na | na | na | Birds ^h |
| <i>Leonia glycyarpa</i> | Violaceae | 11 | 450 | na | Mammals ^h |

TMCF, Tropical montane cloud forest; LMF, lower montane cloud forest; LF, lowland forest.

2.3.2 Seedling translocation

Seeds and seedlings of each species were selected from the centre of their local elevational range. They were grown in herbivore-exclusion shade houses, near their elevational community centre (Figure 2-3, a), for at least two months prior to transplantation to sites within the forest. Species were allocated at random to 6 of up to 18 open plots at each transplant elevation, resulting in mixed plots of at least three species. Within each plot, subplots were chosen in open and more shaded environments, according to canopy openness (%) measurements, and cleared of understorey vegetation (Figure 2-3, b, c). These



Figure 2-3 Transplant set-up: (a) pre-transplant shade house at home elevation; (b) gap subplot; (c) shade subplot; (d) transplanted seedling *in situ*; (e) transplanted seedling *in pot*; (f) micrometeorological station.

subplots were then maintained at monthly intervals during the transplant experiment. Where seedling populations allowed (depending on germination success at the community centre), six individuals per species were randomly allocated to each subplot, totalling a maximum of 72 seedlings across 12 subplots and 6 plots at each transplant elevation.

Seedlings were transplanted into local soils, but without cleaning the roots. This was in order to maintain mycorrhizal community associations that are predicted to shift with changes in elevation along the Andes-Amazon gradient (Nottingham et al. 2015a). Seedlings were transplanted into well-draining plastic pots (3.77 l), which were buried to local soil level (Figure 2-3, d, e). This allowed seedlings to be transported in order for sensitive physiological measurements to be made efficiently and safely. Seedlings were observed to maintain their root system within the confines of the pots for the duration of the experiment.

2.3.3 Micrometeorology

In addition to seedling transplantation, micrometeorological stations were installed in the understorey (0.5 m above ground level) in one gap and one shade plot at each transplant elevation. Each station consisted of a temperature/ relative humidity smart sensor (HOBO, 12-bit, #S-THB-M002; resolution of 0.02 °C at 25 °C; temperature accuracy of +/- 0.21 °C from 0 to 50 °C; relative humidity accuracy of +/- 2.5 % from 10 % to 90 % humidity, mounted inside a protective enclosure), a soil moisture smart sensor (10HS #S-SMD-M005; resolution of 0.08 %; accuracy of +/- 3.3 % from 0 to 50 °C) and a photosynthetically active radiation (PAR) smart sensor (#S-LIA-M003; resolution of 2.5 µmol m⁻² s⁻¹; accuracy of +/- 5 µmol m⁻² s⁻¹ with an additional temperature-induced error +/- 0.75 µmol m⁻² s⁻¹ above 25 °C; Cosine corrected 0 - 80 °, 360 ° rotation). Data were logged every hour (HOBO U23 Pro v2 External Temperature Logger #U23-004, HOBO USB micro station data logger #H21-USB) during the set-up and throughout the transplant experiment (Figure 2-3, f).

2.3.4 Measuring seedling response to transplantation

The response of a species to transplantation is best evaluated by investigating the impact on an individual's lifetime fitness, defined as the cumulative result of traits expressed throughout an organism's life (Hargreaves et al. 2014), such as establishment, survival and reproduction. In the absence of these lifetime measures, performance may still be assessed through the study of these components at critical life-stages. In this study seedling survival

over a one year period was used as a direct assessment of fitness, and growth as an indicator of habitat quality and future performance. Where mortality occurred, evidence of biotic limitations such as leaf damage, were recorded. In addition, throughout the transplant period seedling physiological stress was monitored (section 1.4.2) as a dynamic measure of the abiotic limitations to seedling performance.

To unravel the acclimatory mechanism behind changes in seedling performance, measurements were made of key functional traits that have been observed to shift with elevation and warming, such as V_{cmax} , J_{max} , R_d , *leaf N_a* (leaf nitrogen content on an area basis), *leaf P_a* (leaf phosphorus content on an area basis) and *LMA* (leaf mass per unit area) (Table 2-2). Where adjustments to these trait values occurred, relative to the home transplant site, seedlings were considered to have acclimated to the new climatic regime. As outlined in section 1.4, acclimatory responses do not necessarily convey fitness advantages. Performance and acclimation were therefore considered together when assessing seedlings' overall response to simulated climate change scenarios.

2.4 Interpreting seedling response to transplantation

As illustrated in Figure 2-4, when transplanting seedlings to the edge of and beyond their local range, changes in and interactions between abiotic and biotic factors moderate the survival and growth response of individuals. Where there is little change in abiotic and biotic factors relative to the home transplant site, seedling response may not differ (survival and growth \geq home), so these transplant sites may offer no additional limitations to seedling fitness (Figure 2-4, b). Although, this may not be the case for an individual's lifetime fitness.

Where survival is immediately very low (survival ≈ 0) interpreting which of a range of abiotic and biotic factors are behind the decline becomes difficult (Figure 2-4, a). However where declines in survival are more gradual ($0 < \text{survival} < \text{home}$), concurrent declines in growth and increases in physiological stress response may indicate direct abiotic limitations such as high light, temperature or water stress (Figure 2-4, c). Where there is no indication of direct abiotic stress, a seedling's physiology may still be impacted by abiotic factors, such as non-optimal temperatures or light levels. Under this scenario individuals may not be able to acclimate, i.e. they are unable to optimise their performance under the new climatic regime (growth $< \text{home}$), thus leaving them at a competitive disadvantage (Figure 2-4, e).

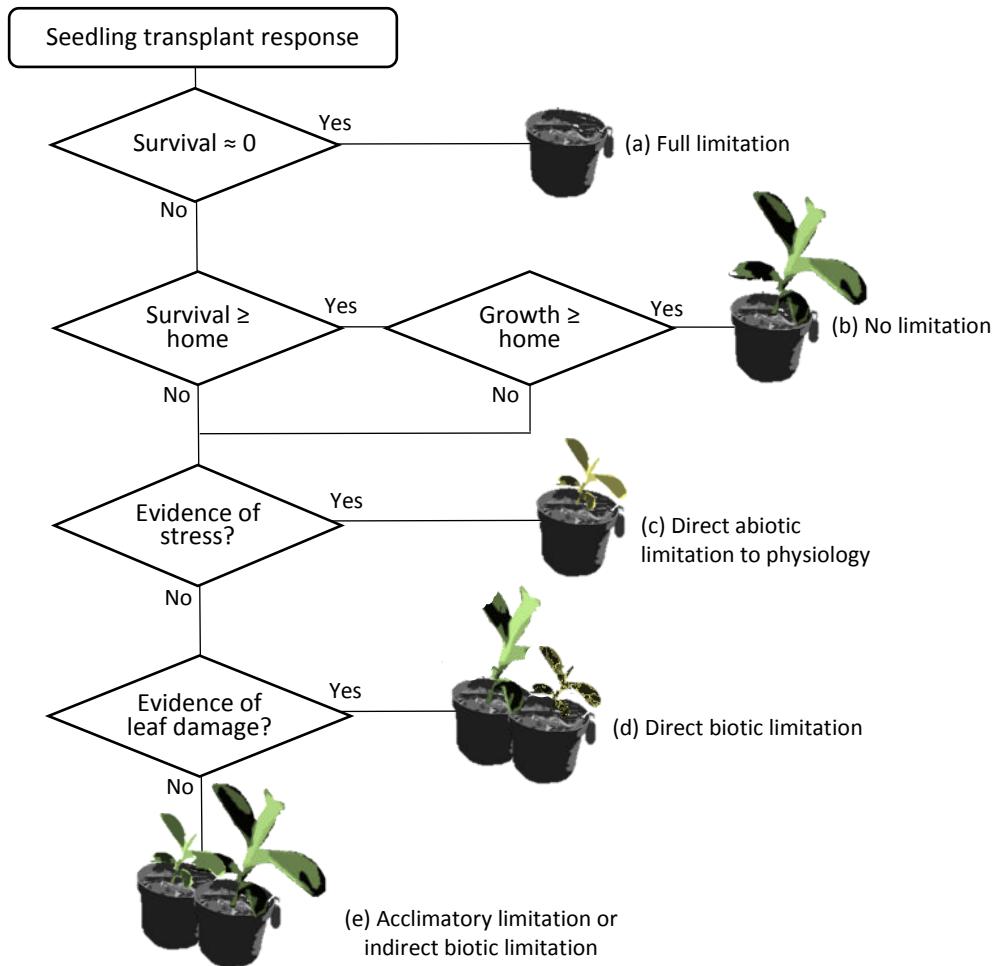


Figure 2-4 Possible survival and growth responses of transplanted seedlings relative to home transplant site. Potential scenarios are: (a) full survival limitation (abiotic and biotic); (b) no additional limitation (abiotic or biotic); (c) direct abiotic limitation to physiology; (d) direct biotic limitation such as herbivory or pathogens; (e) acclimatory limitation to physiology resulting in indirect biotic limitation (competition)

In the final seedling response scenario there is little or no physiological limitation ($\text{growth} \geq \text{home}$), but survival remains low ($0 < \text{survival} < \text{home}$). In this case biotic factors are likely to play a more dominant role, as transplanted seedlings may be able to withstand defoliation from increased pressure from herbivory or disease (Figure 2-4, d).

2.4.1 Predictions of seedling response

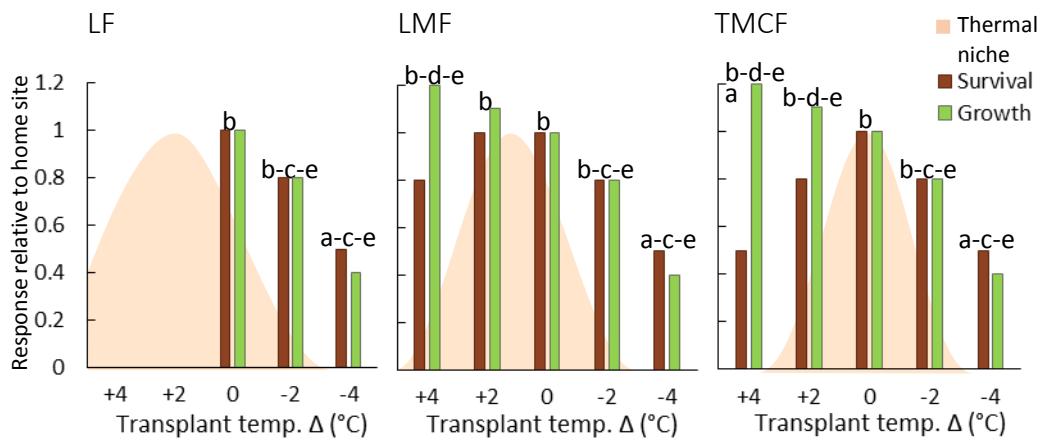


Figure 2-5 Predicted growth and survival response of transplanted seedlings relative to centre of species' thermal niche. LF, lowland species; LMF, lower montane species; TMCF, montane cloud forest species. (a) full limitation (abiotic and biotic); (b) no additional limitation (abiotic or biotic); (c) full abiotic limitation to physiology; (d) direct biotic limitation (herbivory or pathogens); (e) acclimatory limitation to physiology potentially resulting in indirect biotic limitation (competition).

As conservative or stress-tolerant strategies increase with elevation generally (section 1.5.2) and along the Andes-Amazon gradient (section 2.1.3), we predict that seedling survival response will decline upslope, with seedlings becoming increasingly stressed due to changing abiotic factors such as exposure to low temperatures and seasonally high UV-B at high elevations (section 2.1.2) (Figure 2-5 a-c-e). These abiotic factors may also impact on seedling growth rates, with transplantation into the cloud base reducing radiation, thus directly impacting on carbon gain and low temperatures potentially reducing physiological rates (section 1.4.1). We hypothesise that seedlings transplanted upslope but within or just outside their local range (estimated realised niche) may not differ in response from the home transplant site, as the observed lag in upslope migration (see 1.3) may be as a result of dispersal or establishment limitations rather than direct abiotic limits (Figure 2-5 b-c-e).

Transplantation downslope may result in equally stressful conditions for transplanted seedlings, as temperature and water stress is more likely beyond the cloud base particularly at sites within the transition zone (section 2.1.2) and the lowlands (1.2), (Figure 2-5 b-d-e). There is a particularly high risk of mortality when transplanting outside the local range at downslope sites, as seedlings may experience exceptional warming and drought events that could cause thermal damage to the photosynthetic machinery (sections 1.4.1 and 1.5.2). In addition montane species that are transplanted downslope may be susceptible to mortality due to greater biotic stress (section 2.1.3), having invested in abiotic stress-response rather

than leaf-defence traits (section 1.5.2) (Figure 2 5 b-d-e, a). Conversely, under normal non-drought conditions seedling growth is expected to increase with moderate warming ($\leq 4^{\circ}$ C) (section 1.5.2), so seedlings may equally thrive at the lower edge or just outside of their local range (Figure 2 5 b-d-e).

The acclimatory response of seedlings to transplantation is more difficult to predict. The best-evidenced acclimatory response to climate change is that of the upregulation of Rd in response to low temperatures, low soil fertility and drought conditions (section 1.4.1 and 1.5.1). We would therefore predict that Rd will upregulate with transplantation upslope and may also upregulate in response to water-stress downslope. The response of photosynthetic capacity to warming is less clear, but we do predict an increase in photosynthetic capacity with increased community elevation (Table 2 1) and tentatively predict an upregulation of capacity with warming (section 1.5.2). The response of anatomical leaf traits to warming is also unpredictable, as trends are often species and site-specific (section 1.4.2), however based on previous studies along the Andes–Amazon gradient we predict an increase in LMA (or decrease in SLA) and leaf nutrients on an area basis with increased transplant elevation.

2.5 Addressing transplant questions

In *Chapter 3* the following questions are addressed in relation to the study population, thus putting the transplant experiment into the context of the natural population along the Andes-Amazon gradient:

- 1) Is there evidence that abiotic stress is driving range contractions at the lower range edge?
- 2) Do seedlings differ from adults in their trait-response to an environmental gradient?
- 3) What is the natural leaf trait variation within the study population?

Chapter 4 investigates the performance of seedlings to transplantation, asking:

- 1) Can seedlings survive and grow with a ± 2 and ± 4 °C change in climate?
- 2) Is there evidence of abiotic or biotic limitations to growth and survival with transplantation?

Chapter 5 asks if seedlings can acclimatise to transplantation through specific hypotheses related to a number of leaf traits:

- 1) Photosynthetic capacity will decrease with simulated warming
- 2) Respiratory capacity will increase relative to photosynthetic capacity higher in TMCF species and will increase with upslope transplantation
- 3) The response of photosynthetic and respiratory capacity to transplantation will be affected by shifts in anatomical leaf traits

Chapter 4, *Chapter 5* and *Chapter 6* will address broader questions related to the response of elevational communities to climate change:

- 1) Are TMCF species more susceptible than LMF and LF species to climate change?
- 2) Is the acclimatory potential of tropical trees currently underestimated?
- 3) Can we use leaf traits to understand and predict the response of tropical trees to climate change?

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Chapter 3. Can upslope shifts along an Andes-Amazon migratory corridor be explained by changes in leaf traits and physiological stress at the lower range edge?

3.1 Introduction

Variable upslope shifts in tree species distributions have been reported along Neotropical elevational gradients in response to changing climate, with mortality at lower range edges identified as an important driver (Feeley et al. 2011, Feeley et al. 2013, Duque et al. 2015). Species that succumb to climatic stress at the lower range edge are likely to be limited by their current phenotypic plasticity or, over longer time-scales the rate of adaptability in their functional traits (Jump and Penuelas 2005, Nicotra et al. 2010, Asner et al. 2014). Investigating potential changes in plant physiological stress and associated functional traits at shifting range edges is therefore pertinent to our understanding of the response of communities to ongoing climate change (Funk et al. 2017). This study uses an Andes-Amazon elevational gradient spanning 400- 4000 m asl (above sea-level) to identify stress-related migratory shifts within pre-montane and cloud forest communities.

Along elevational gradients, leaf traits are thought to be mediated by the trade-off between conservative (stress-tolerant) and acquisitive (resource-competitive) resource allocation strategies (Loehle 1998, Wright et al. 2004, Read et al. 2014, Vitasse et al. 2014). As environmental stress increases and competition decreases with elevation, this favours conservative strategies, with lower specific leaf area (SLA) and higher leaf thickness (L_{th}) and nutrients contents compared to their lowland counterparts (Körner et al. 1983, Körner et al. 1986, Cordell et al. 1998). Shifts in trait frequency distributions along environmental gradients are driven by both interspecific (species turnover) and intraspecific trait variability. Studies are increasingly focussing on the relative contribution of the latter and its subsequent incorporation into frameworks that predict community assembly and responses to environmental change (Albert et al. 2010, de Bello et al. 2011, Lepš et al. 2011, Viole et al. 2012, Kichenin et al. 2013, Lajoie and Vellend 2015, Bastias et al. 2017).

Intraspecific variation in traits associated with the leaf economic spectrum, such as leaf mass per unit area (LMA) and leaf N content on an area-basis (N_a), are equally important as

interspecific variation in determining shifts along elevational gradients globally (Read et al. 2014). These traits demonstrate greater variability than those associated with leaf size (Siefert et al. 2015). Few tropical studies have explicitly investigated changes in intraspecific trait variation with elevation (Hulshof et al. 2013). It is therefore unclear whether intraspecific variation, specifically in traits related to leaf economics, will be as important as interspecific variation in determining leaf trait frequency distributions along tropical gradients (see conceptual framework, Lajoie and Vellend (2015)).

Along the Andes-Amazon gradient, elevation plays a major role in driving leaf trait distributions (Asner et al. 2016). The observed increase in leaf canopy trait variation at high elevations has been linked to the greater variability in microclimatic factors, such as diurnal temperature changes, radiation (including increased UV-B) and humidity, exerting a mosaic of environmental selection-pressure on upland species (Rapp and Silman 2012, Bahar et al. 2016, Malhi et al. 2016, Asner et al. 2017). This variation is partly driven by the seasonality of the cloud-base, with the highest levels of species turnover occurring at the transition between lower and upland cloud forest (1500-2000 m asl) (Girardin et al. 2013, van de Weg et al. 2014, Fyllas et al. 2017). Despite evidence of elevational shifts in community leaf trait distributions, the acclimatory potential of individual species along the Andes-Amazon gradient is not well understood. This is particularly important considering that 75% of the most common tree genera along the Andes-Amazon gradient have shifted their distributions upslope (Feeley et al. 2011) driven by climate-induced mortality at the lower range edge.

We hypothesise that if mortality is driven by long-term warming in addition to extreme climatic events such as drought, there will be evidence of physiological stress within adult individuals at the lower range edge, as a result of being near their climatic limits (Colwell et al. 2008). In addition we anticipate that seedlings will differ from adults in their stress response, as individuals from early ontogenetic stages are more vulnerable to environmental fluctuations (Ettinger and HilleRisLambers 2013). Understanding the response of seedlings at range edges is critical, as their successful establishment determines whether the population is able to persist at that locale in the future (Grubb 1977, Ishida et al. 2005b, Poorter 2007). It is therefore important to consider leaf trait variation and environmental stress acting upon both seedling and adult ontogenetic stages. In this way, the response of populations as a whole to changing climate can be better predicted.

Physiological stress can be assessed using changes in quantum efficiency as a sensitive and early indicator of declines in plant function (Maxwell and Johnson 2000). This is particularly useful when trying to detect small changes in the overall fitness of a population. Using field-based chlorophyll fluorescence techniques to measure individuals along an elevational transect, we are able to study changes in physiological traits across species' entire elevational range yet within narrow geographic space (Körner 2007). This study is the first to investigate intraspecific changes in physiological stress as well as functional traits along such a large environmental gradient.

Here, we assess the variation in leaf traits along an Andes-Amazon migration corridor for twelve species differing in their elevational ranges. We measured physiological stress and leaf anatomical traits for adults and seedlings of each species at the centre and at the edges of their elevational range. The overall aim was to test if individuals at range edges were able to successfully acclimate to their environment, thus avoiding physiological stress. In particular we asked: (1) Are individuals at the lower range edge more stressed than individuals at the centre and upper range edge? (2) Are there differences in intraspecific leaf traits and physiological stress between adult canopy and understorey seedlings? (3) Does intraspecific variation, specifically in traits related to leaf economics, play a role in determining leaf trait frequency distributions, or are interspecific and community-level shifts more important?

3.2 Methods

3.2.1 Elevational transect

This study was carried out along an Andes-Amazon gradient along the Kosñipata Valley, Manu National Park, to Madre de Dios basin, south-east Peru. Ten sites were selected from a series of 14 1 ha Andean forest inventory plots established in 2003-04, with repeat censuses occurring on average every 4 years since establishment (Feeley et al. 2011). In addition, three 1 ha study plots established by ABERG (Andes Biodiversity and Ecosystem Research Group) (Malhi et al. 2010) were selected to extend the range of this study from tropical montane to lowland forest. The plots are situated at intervals of between 50-500 vertical metres, spanning an elevational range of 400 to 3400 m above sea level (asl), with mean annual temperatures ranging from between approximately 10 to 25 °C. Mean annual precipitation ranges between 1500- 5300 mm yr⁻¹ across the gradient (Malhi et al. 2016), with a higher

frequency of cloud immersion above 1500 m asl, peaking between 2000 to 3500 m asl (Halladay et al. 2012).

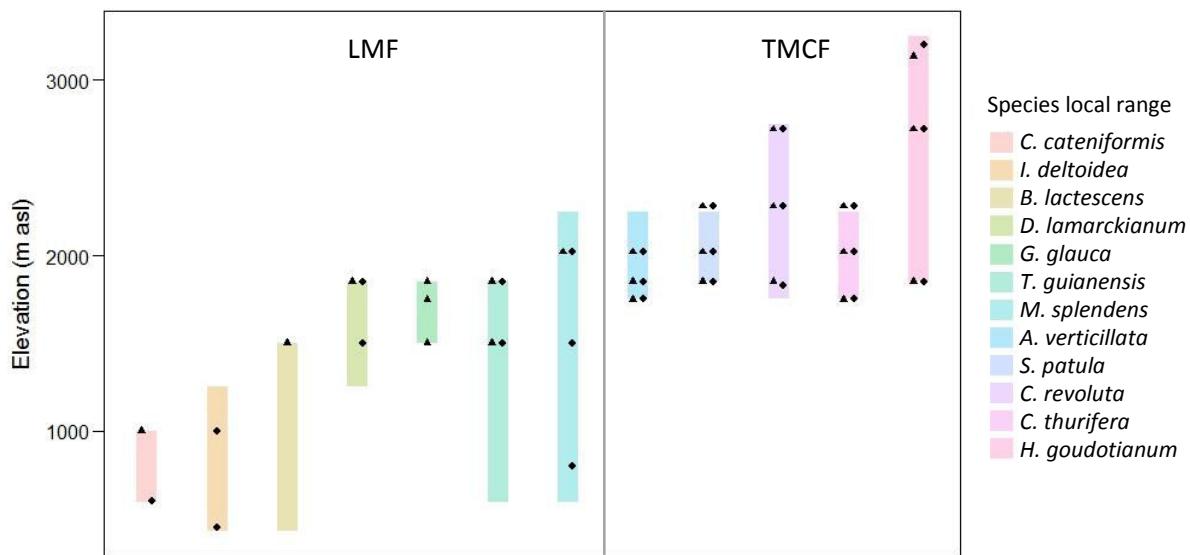


Figure 3-1 Sample location of adult individuals (triangles) and seedlings (circles) of each species relative to local estimated elevational range (based on composition of 1ha permanent plots (Feeley et al. 2011)). LMF, lower and pre-montane forest species; TMCF, tropical montane cloud forest species

3.2.2 Species selection

Native tree species were selected from two broad elevational communities; high tropical montane cloud forest (TMCF) species (mean elevation 2150 m asl +/- 400 m); and lowland and pre-montane forest (LMF) species (mean elevation below 1000 m asl and mean elevation 1500 m asl +/- 150 m, respectively). The species were chosen to be representative of different elevational range widths (Figure 3-1) and locally abundant taxa within each forest type (Table 3-1). The LMF species were; *Cedrelinga cateniformis* (Ducke) Ducke; *Iriartea deltoidea* Ruiz & Pav.; *Brosimum lactescens* (S. Moore) C. C. Berg; *Dictyocaryum lamarckianum* (Mart.) H. Wendl.; *Guatteria glauca* Ruiz & Pav.; and *Tapirira cf. guianensis* Aubl. The selected TMCF species were *Alzatea verticillata* Ruiz & Pav.; *Schefflera patula* (Rusby) Harms; *Clethra revoluta* (Ruiz & Pav.) Spreng.; *Clusia thurifera* Planch. & Triana; and *Hedyosmum goudotianum* Solms.

Table 3-1 Tree species selected for transplant experiment by elevational community. Abundance is within a series of 1 ha plots along the Andes to Amazon gradient (Feeley et al. 2011). *Hemi-epiphytic species (Austin 1993). a (Stimm et al. 2008), b (Lütte 2007), c (Roth 1987), d (Graham 1984), e (Todzia 1988), f (Russo et al. 2005), g (Swamy 2008).

| Species | Common name | Family | Abundance | Dispersal |
|----------------------------------|-------------------|----------------|-----------|--------------------------------------|
| <i>Clethra revoluta</i> | | Clethraceae | 182 | Wind ^a |
| <i>Clusia thurifera*</i> | | Clusiaceae | 172 | Birds ^b |
| <i>Myrcia splendens</i> | | Myrtaceae | 87 | Birds ^c |
| <i>Alzatea verticillata*</i> | | Alzateaceae | 423 | Most likely by wind ^d |
| <i>Hedyosmum goudatianum</i> | | Chloranthaceae | 96 | Birds ^e |
| <i>Schefflera patula*</i> | | Araliaceae | 41 | Birds, bats and mammals ^c |
| <i>Tapirira cf. guianensis</i> | | Anacardiaceae | 37 | Birds and mammals ^c |
| <i>Guatteria glauca</i> | | Anonaceae | 47 | Birds, bats and mammals ^c |
| <i>Dictyocaryum lamarckianum</i> | Andean royal palm | Arecaceae | 5 | Birds and bats ^c |
| <i>Cedrelinga cateniformis</i> | Tornillo | Fabaceae | 32 | Mammals ^f |
| <i>Theobroma cacao</i> | Cocoa tree | Malvaceae | na | Mammals ^g |
| <i>Iriartea deltoidea</i> | Copa palm | Arecaceae | 215 | Mammals ^f |
| <i>Brosimum lactescens</i> | | Moraceae | 7 | Birds and mammals ^{fg} |

TMCF, Tropical montane cloud forest; LMF, lower montane cloud forest.

3.2.3 Sampling strategy

Species were sampled where possible at three elevations; high, centre and low, corresponding to target sites within 250 vertical metres of the estimated minimum, mean and maximum elevation of each species (data from Feeley et al. 2011). The sampling design was limited by the uneven distribution of plots along the gradient, access to sites and identification of a sufficient number of specimens at certain target plots. This resulted in the use of additional plots with greater elevational deviations from the target elevation (up to 375 vertical metres) or the removal of a target elevation from the sample design (Figure 3-1).

At each elevation five adult trees and 15 tree seedlings of each species were measured. Sites were visited over a period of five weeks during the dry season (July and August 2015) for up to four days per site. At each site trees were identified using the inventory records and the expertise of local botanists. One sunlit branch was cut per adult tree and recut underwater to re-establish the water supply (Pérez-Harguindeguy et al. 2013). 5 leaves were chosen at random and marked. The branch was then positioned in a canopy gap prior to measurement. Seedlings were sampled *in situ* and, where high abundances allowed, at least 2 m from

another sampled seedling. The uppermost fully expanded leaf was selected for physiological measurements.

3.2.4 Measurement protocol

Chlorophyll fluorescence techniques were used to detect decreases in the photosynthetic efficiency of PSII (equations 1a-b), giving the first indicators of physiological stress caused by damage to the photosynthetic machinery of the leaf (Maxwell and Johnson 2000). Chlorophyll fluorescence measurements were made of the leaf lamina using cross-calibrated MINI-PAM-I and MINI-PAM-II fluorometer (Walz, Germany) throughout each measurement day (08:00-18:00). The MINI-PAM leaf clip was attached to the centre of the leaf lamina, avoiding veins and maintaining a natural leaf angle. The MINI-PAM-I was used to measure the quantum yield of photosystem II (PSII), which is a measure of the realised quantum efficiency of PSII (ϕ_{PSII}) and the MINI-PAM-II was used to measure the potential quantum efficiency of PSII (F_v/F_m). Each measurement (equations 1a-b) can be made from a single fluorescence trace, where F_m' is maximum fluorescence, F_t is steady state fluorescence under light conditions and F_m and F_o are maximum and minimum fluorescence under dark conditions. As F_v/F_m must be measured under dark conditions, the leaf was dark-adapted using a leaf clip (LI-COR, Lincoln, NE, USA) for at least 20 minutes prior to measurement (Maxwell and Johnson 2000).

$$a) \quad \phi_{PSII} = \frac{(F_m' - F_t)}{F_m'} \quad b) \quad F_v/F_m = \frac{(F_m - F_o)}{F_m}$$

Concurrent with each measurement, leaf temperature (NiCr-Ni thermocouple) and photosynthetically active radiation (PAR, LS-C Mini Quantum Sensor) were measured. In addition, mean soil volumetric water content (ML3 - ThetaProbe Soil Moisture Sensor, Delta-T Devices) and soil temperature were recorded for each individual. These microclimatic measurements were compared to wet season data collected at understorey micrometeorological stations. Each station consisted of a temperature/ relative humidity smart sensor ((HOBO, 12-bit, #S-THB-M002; resolution of 0.02 °C at 25 °C; temperature accuracy of +/- 0.21 °C from 0 to 50 °C; relative humidity accuracy of +/- 2.5 % from 10 % to 90 % humidity, mounted inside a protective enclosure), a soil moisture smart sensor (10HS #S-SMD-M005; resolution of 0.08 %; accuracy of +/- 3.3 % from 0 to 50 °C) and a PAR smart sensor (#S-LIA-M003; resolution of 2.5 μmol m⁻² s⁻¹; accuracy of +/- 5 μmol m⁻² s⁻¹ with an additional temperature-induced error +/- 0.75 μmol m⁻² s⁻¹ above 25 °C; Cosine corrected 0 -

80 °, 360 ° rotation). Data were logged every hour (HOBO U23 Pro v2 External Temperature Logger #U23-004, HOBO USB micro station data logger #H21-USB) during the set-up and throughout the transplant experiment

All leaves measured were collected and rehydrated overnight before processing (Prior et al. 2003). Measurements of relative chlorophyll content on an area basis (*SPAD* value, SPAD 502 Chlorophyll Meter, Spectrum tech. Inc.) (Richardson et al. 2002) and leaf thickness (L_{th} , digital calliper) were made at multiple points across the leaf lamina. The petiole was removed and the fresh mass was ascertained. The leaf was photographed against a white background with an object of known size (Nikon Coolpix A10) and later analysed using image J (version 1.58v, NIH, USA) to determine leaf area. The leaf material was dried to a constant mass using silica gel (van de Weg et al. 2012) and weighed to determine dry leaf matter content (*LDMC*, mg g⁻¹) and specific leaf area (*SLA*, m² g⁻¹) (Pérez-Harguindeguy et al. 2013).

3.2.5 Statistical Analysis

To account for the nested experimental design and differences in sample size at each elevation (due to differing abundance of species), mixed effect models were used to identify factors with the most predictive power for modelling the response of leaf-level quantum efficiency and anatomical traits. Mixed effects modelling was performed following standard methods (Burnham and Anderson 2003, Zuur et al. 2009), using the lme4 package (Bates 2015) in R (Version 3.2.3, R Core Team 2015). Data was transformed to adjust the fit of the model residuals; Fluorescence measurements were transformed using the box-cox transformation (Box and Cox 1964), anatomical traits were log-transformed and random effects were centred using the scale function (Wickham and 2016, Becker et al. (1988)).

The categorical fixed effect factors of intraspecific elevational range location (high, centre, and low), ontogenetic stage (adult or seedling) and community group (LMF or TMCF) were included in the initial full model. The optimum random effect structure was selected to include a combination of random intercept (RI) factors of: species identity, absolute elevation, individual identification number and light levels at the point of measurement. An additional random slope (RS) was tested with different slopes for each species response to range location (Appendix, Figure 3-7). Fixed effect terms were then removed sequentially and the relative fit of each model was assessed using Akaike Information Criterion (AIC and sample-size adjusted AICc). Likelihood ratio test (LRT) statistics were used to compare full models to

each other and to a null model which contained only random effect terms. In this way, the significance of fixed effect terms were ascertained and the final best-fit model was selected.

To describe the spectrum of leaf- trait strategies employed by the study species, principal component analysis (PCA) was used on log-transformed anatomical leaf traits (Horikoshi and Tang 2016, Tang et al. 2016). Traits were included in the analysis if they were important to the first or second principal component axes. Leaf-trait clusters were examined within each species, community group, ontogenetic stage and intraspecific elevational range location.

3.3. Results

3.3.1 Understorey microclimate

Air and soil temperature decreased linearly by approximately $5 \text{ }^{\circ}\text{C km}^{-1}$ (Figure 3-2a, c). The air temperature during the measurement period (dry season) was 2-5 $^{\circ}\text{C}$ greater than that experienced during the preceding wet season at cloud forest sites. Soil temperature was consistent between dry and wet season measurements, but was approximately 2 $^{\circ}\text{C}$ greater in gap plots for the majority of the elevational gradient.

PAR was significantly higher at open than closed sites during the wet season and slightly higher in canopy gaps during the dry season (Figure 3-2b). Dry season PAR peaked at the mid-cloud forest site (2150 m asl) with maximum values of $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ measured in a canopy gap at the ridge-line. Seedlings experienced an average PAR of between 1 and $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at the point of measurement, which was equivalent to the wet season shaded sites, and a maximum of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

Soil moisture was highly variable across all elevations, with a median of between 15- 40 % during the measurement period, which was slightly but not consistently lower than the wet season values (Figure 3-2d). Lowland sites tended to be less variable than montane sites, with the greatest variability observed between 1500 and 2000 m asl.

Measurement days below 1750 m asl were similar to the preceding wet season across all microclimatic measurements. Measurement days between 1750 and 2300 m asl were on average hotter, drier and brighter than the wet season mean. Measurement days above 2300 m asl tended to be hotter than the wet season mean.

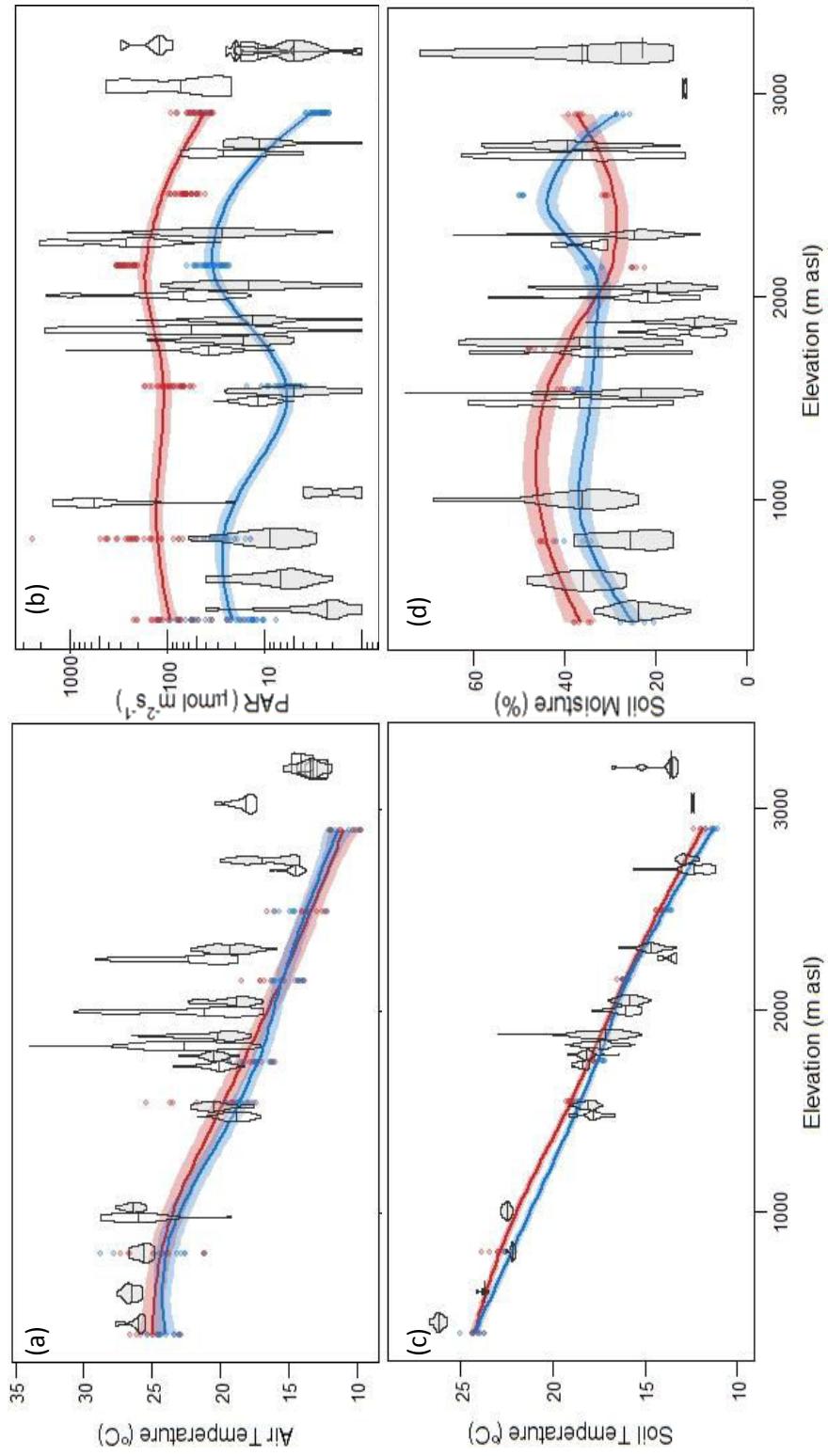


Figure 3-2 Probability density and median of understorey microclimatic measurements made during the dry season (from July-August) at each sampled seedling (grey) and canopy gaps (white). Monthly daytime means and 95% confidence intervals (fitted with loess smoothing) during preceding wet season (November-March) at open (red) and shaded (blue) from understorey data loggers.

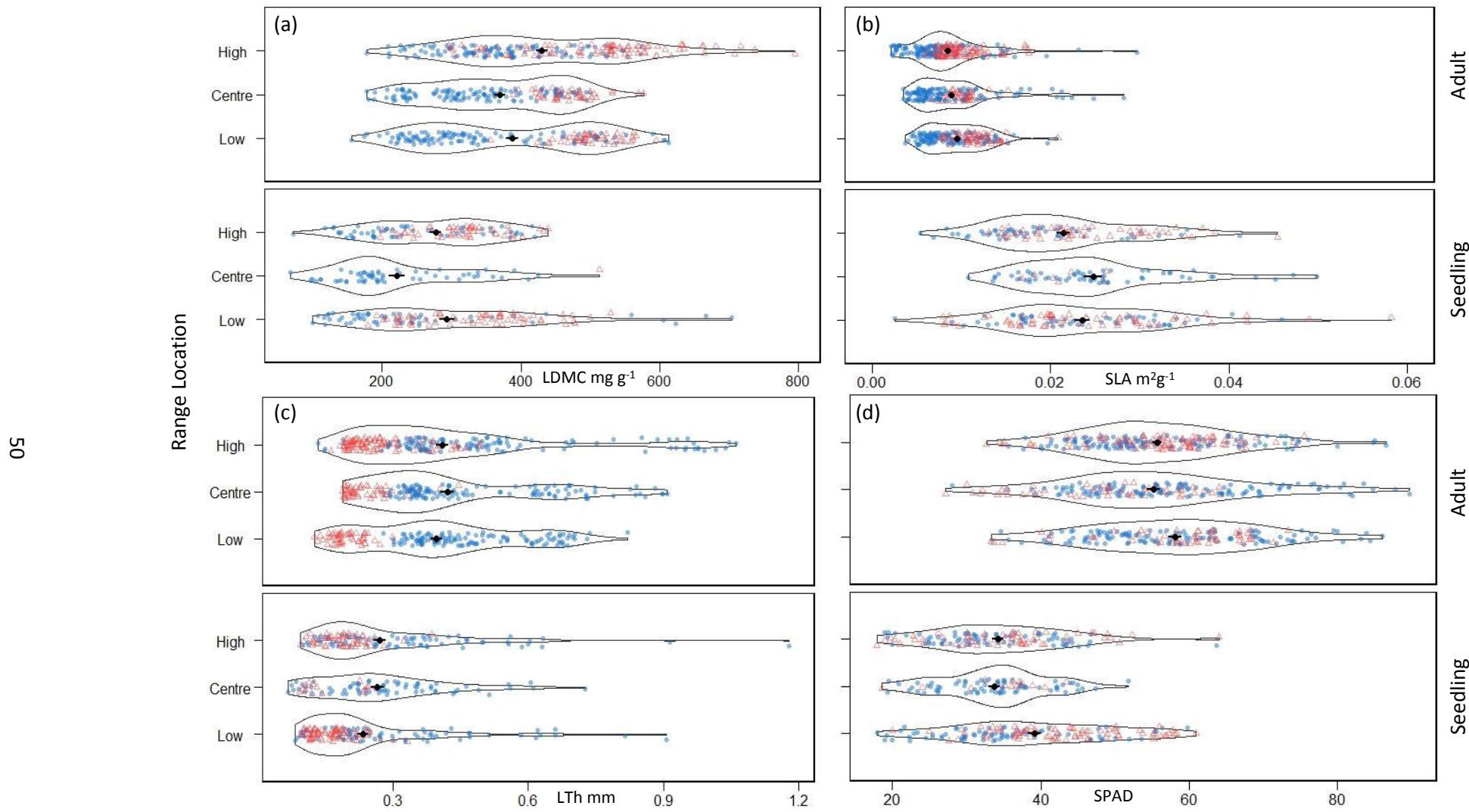


Figure 3-3 Probability density (violin plots with jittered points) and mean \pm SE of leaf anatomical traits of adults (upper) and seedlings (lower) at each intraspecific range location (High, Centre and Low). (a) Leaf dry matter content, (b) Specific leaf area, (c) leaf thickness, (d) relative chlorophyll content. Blue points, tropical montane cloud forest species; red points, low-mid forest species; Low, centre and high range locations are relative to each other within the species' elevational range.

3.3.2 Leaf Anatomical Traits

Variation in leaf anatomical traits were best explained by the random effects of species and individual identity, followed by plot elevation. Microclimatic factors of air temperature and PAR were not strong predictors of leaf traits, so were not included in the models (Appendix Figure 3-7). Variation in *LDMC* and *L_{th}* were explained by the fixed effects of ontogenetic stage in addition to community group (LRT, P <0.05), with TMCF species having significantly thicker leaves but lower *LDMC* than LMF species (Figure 3-3a, c). Differences in *SPAD* values were explained by ontogenetic stage and the interaction with community group (LRT, P <0.001), as the TMCF group had the greatest chlorophyll content for adults, but the lowest for seedlings (Figure 3-3d). *SLA* was best explained by the interaction between ontogenetic stage and intraspecific range location (LRT, P <0.05), as mean intraspecific *SLA* decreased by 11% from low to high range location for adult individuals, but remained constant for seedlings (Figure 3-3b).

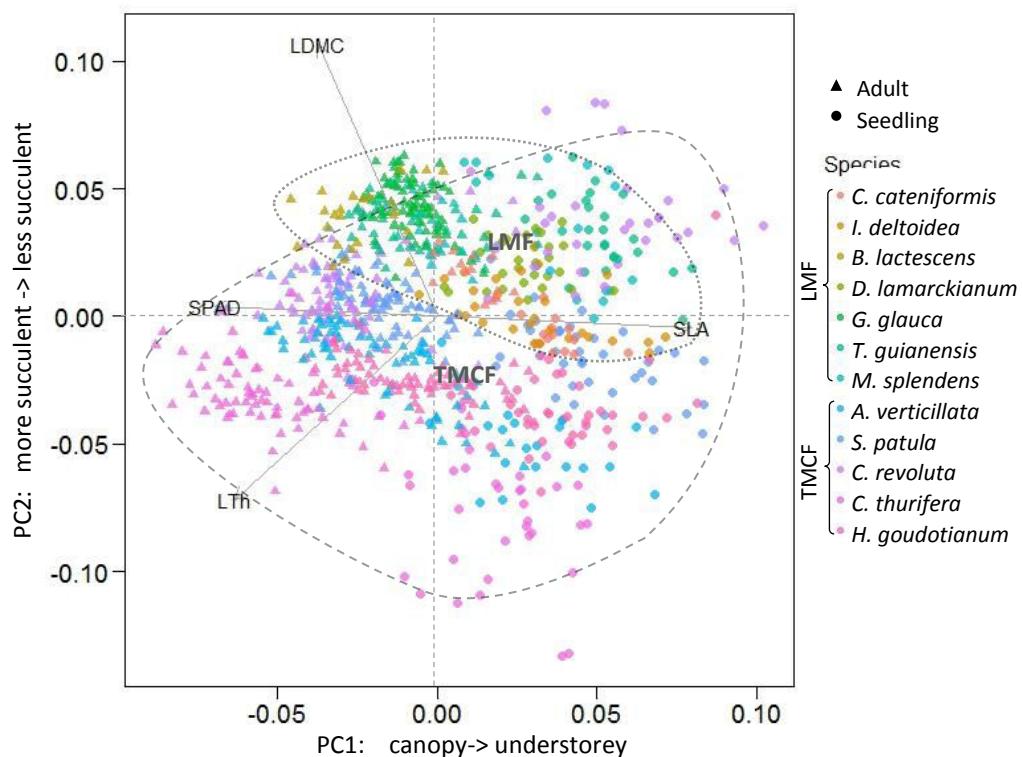


Figure 3-4 Results of the principal component analysis applied to log-transformed seedling leaf traits of all tree species (n =831). LMF (dotted outline), low-mid forest species; TMCF (dashed outline), tropical montane cloud forest species. *LDMC*, leaf dry matter content; *SPAD*, relative chlorophyll content on an area basis; *SLA*, specific leaf area, *LTh*, leaf thickness. 59% of the variance was accounted for by PC1 (canopy-understorey) and 28% by PC2 (more to less succulent).

Species ontogenetic stages were significantly different in terms of anatomical traits (LRT, $P<0.001$); adults had approximately 50% higher values of *LDMC* and *SPAD* and 60% lower *SLA* relative to seedlings. This resulted in adults and seedlings differing in their leaf trait strategies, where adults and seedlings were found at different ends of the axis associated with light environment (Figure 3-4, PC1). Variability in leaf traits was similar across ontogenetic stages except for *SLA*, which was more variable in the seedling population (Figure 3-3b).

The extent of intraspecific variability in *LDMC*, *SLA* and *SPAD* was similar for TMCF and LMF species and was within the same magnitude as community group-level estimates. Within-community variability in L_{th} exceeded intraspecific variability for the majority of species, driven by the much greater thickness of a few TMCF species; leaves of the hemi-epiphytic species *C. thurifera* were distinct from other species, occurring at the most succulent end of principal component axis 2, with low *LDMC* but high L_{th} (Figure 3-4). *A. verticillata* and *H. goudotianum* had similar succulent traits but to a lesser extent than *C. thurifera*. Individuals of *C. revoluta* were more similar to LMF species, which had a more consistent leaf-trait strategy across species, with higher *LDMC* and lower L_{th} than the majority of the TMCF group (Figure 3-4).

3.3.3 Quantum efficiency

Values of F_v/F_m ranged from between 0.31 and 0.96, but less than 5% of individuals experienced potential quantum efficiencies of less than 0.6 (Figure 3-5 dashed line). Variation in F_v/F_m could be accounted for by the following random effects, in order of total variance; species identity (random intercept and slope at each range location), individual identity and plot elevation (Appendix, Figure 3-7). Of the fixed effects, the inclusion of ontogenetic stage significantly improved the model (LRT, $\chi^2= 23.0$, $P=<0.001$); seedlings had significantly lower F_v/F_m than adult trees, with seedlings of *C. revoluta*, *C. cateniformis* and *I. deltoidea* experiencing the lowest mean F_v/F_m of less than 0.75, whereas adults of *A. verticillata*, *B. lactescens*, *D. lamarckianum* and *T. guianensis* experienced the greatest mean values of more than 0.8 (Appendix, Table 3-2). There were significant interactions between ontogenetic stage and both intraspecific range location (LRT, $\chi^2= 58.9$, $P=<0.001$) and community group (LRT, $\chi^2= 11.3$, $P=<0.01$), so ontogenetic stages were analysed individually. Together the fixed effects explained 23% of the total variation in F_v/F_m and together with the random effects, explained 49% of the total variation.

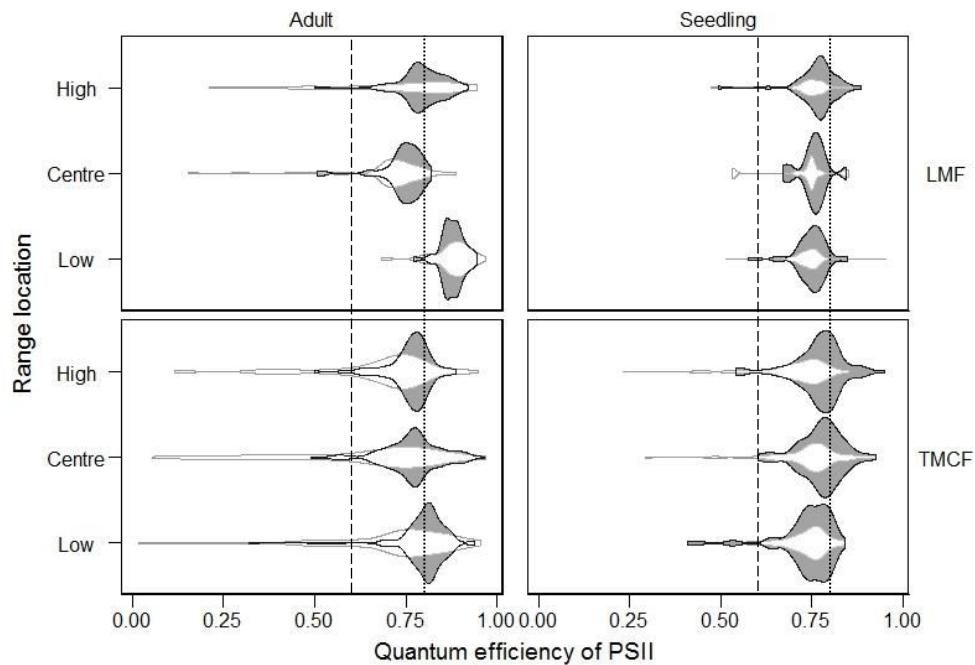


Figure 3-5 Probability density of (dark plots) F_v/F_m (potential quantum efficiency of PSII) and (light plots) Φ_{PSII} (realised quantum yield) for adult and seedling ontogenetic stage and community group. Low, centre and high intraspecific range locations are relative to each other within the species' elevational ranges. LMF; low-mid forest species; TMCF, tropical montane cloud forest species

When F_v/F_m was investigated for adults, a significant amount of variation (12%) was explained by intraspecific range location alone (LRT, $\chi^2= 6.2$, $P=<0.05$), driven by an increase in F_v/F_m at low elevations (Figure 3-5). An additional 16% of the variation was explained by range location plus the interaction with community group (LRT, $\chi^2= 15.9$, $P=<0.01$) (Figure 3-6a). The interaction occurred at low range locations, where LMF species had 10% greater values of F_v/F_m relative to TMCF species (Figure 3-5). With the addition of random effects, the model explained 48% of the variation in adult F_v/F_m . Variation in seedling F_v/F_m was not explained by community group or interactions between fixed effects (Figure 3-6b), but there was a significant effect of intraspecific range location, explaining 4% of the variation in F_v/F_m (LRT, $\chi^2= 15.7$, $P=<0.05$). This was observed as a 3% decrease in mean F_v/F_m at low range locations relative to the central and higher elevations (Figure 3-5). Combined with the random effect of species identity, the model explained only 18% of the total variation in seedling leaf traits.

The realised quantum yield of photosystem II (Φ_{PSII}) was more variable than F_v/F_m , particularly for adult trees (Figure 3-5). The best-fit model included ontogenetic stage and interactions with both community group (LRT, $\chi^2= 19.4$, $P=<0.01$), and intraspecific range location (LRT, $\chi^2= 20.6$, $P=<0.01$), with adults experiencing on average 4% lower Φ_{PSII} than seedlings and TMCF species experiencing on average 6% lower Φ_{PSII} relative to the LMF

group. As with F_v/F_m , the greatest values of ϕ_{PSII} (more than 0.75) occurred in adults at low elevations (Figure 3-5). However, unlike F_v/F_m , the lowest values of ϕ_{PSIII} occurred in the adult TMCF group, with a mean value of 0.67 (SD, ± 0.20), which was 0.1 less than the mean F_v/F_m value. The combination of fixed effects explained only 8% of the variation in ϕ_{PSII} , with an additional 69% of the variation explained by the following random effects in order of explained variance: PAR at the point of measurement, individual code, plot elevation and species.

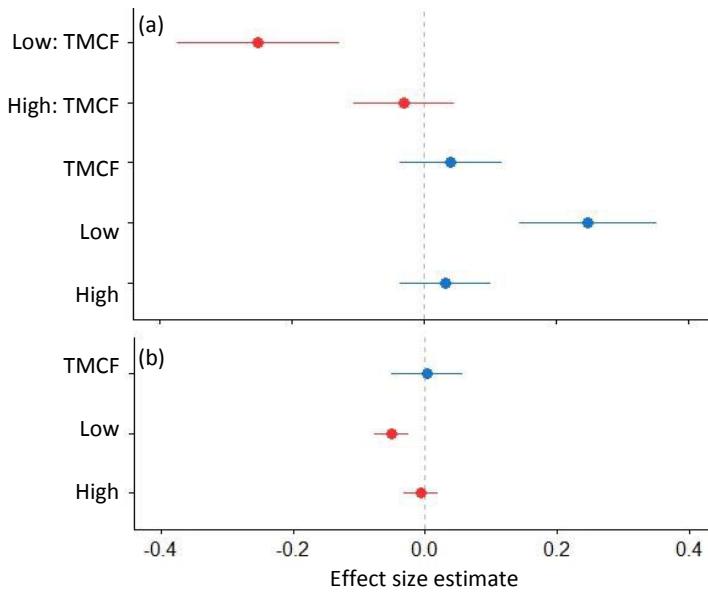


Figure 3-6 Mixed effect model estimates of fixed effects ($\pm SE$) on the response of (F_v/F_m). a) Adult trees b) seedlings. Fixed effects were TMCF community group relative to LMF group and low and high intraspecific range location relative to centre. The interactions (a) are relative to the sum of species range and community group effects. Red line, negative effect; blue line, positive effect; overlap with dashed line, non-significant result; LMF, low-mid forest species; TMCF, tropical montane cloud forest species.

3.4 Discussion

There was no consistent evidence that species along the Andes to Amazon gradient were experiencing greater physiological stress (reduced F_v/F_m) at their lower range edge relative to the rest of their elevational range. Seedling stress was significantly greater at the lower range edge, but the real-world effect of a 3% increase in stress on the seedling population and future upslope migration is unclear. Interspecific variation in leaf traits differed between LMF and TMCF communities, with a greater variability in leaf trait-strategy in higher elevation species. Elevation did not have a strong influence on intraspecific leaf anatomical trait

distribution for most ontogenetic stages and traits, the exception being for *SLA* in adults, which decreased with elevation.

3.4.1 Physiological stress in a changing climate

Overall the minimal levels of physiological stress observed in the population (i.e. there were few F_v/F_m values of less than 0.6) indicates that species are not experiencing long term abiotic stress under current environmental conditions and are therefore able to acclimate to their environment. Although small depressions in F_v/F_m are not believed to directly affect survival, a decrease in F_v/F_m relative to the population as a whole indicates a reduction in photosynthetic performance (Adams and Demmig-Adams 2004). Within the adult population F_v/F_m values were significantly higher at the lower range edge relative to the centre and upper range locations, with the healthiest values (more than 0.8, Figure 3-5, dotted line) occurring at lower sample sites (Cavender-Bares and Bazzaz 2004). This suggests that under normal conditions the adult individuals at low elevations are not experiencing abiotic stress. This supports the theory that at low elevations competition is the dominant selective pressure, whereas with increases in elevation the environment plays a greater role, with stressors such as low temperatures and increasing UV radiation shaping leaf trait strategies and communities (Krause et al. 2003, Read et al. 2014).

In contrast, seedlings at the lower range edges had the lowest mean F_v/F_m (less than 0.75) of all elevational and ontogenetic groups (Figure 3-5). This indicates that these individuals were experiencing the greatest levels of physiological stress and are at the greatest risk of mortality from climatic shifts. In addition where the upper range edge of LMF species and lower range edge of TMCF species overlapped (1750-1800 m asl) there was the greatest disparity in F_v/F_m values. These elevations are characterised by high levels of species turnover (Girardin et al. 2013, Fyllas et al. 2017) and high levels of seasonal climatic variability (Figure 3-2)(Rapp and Silman 2012). This suggests that within this elevational range TMCF seedlings are at a competitive disadvantage relative to LMF seedlings. It may be that TMCF species with highly specialised conservative or succulent trait strategies (e.g. *C. thurifera*) are unable to survive in warmer or more drought-prone conditions, as they are unable to compete with

LMF species with more responsive and acquisitive leaf trait strategies (Valladares and Niinemets 2008, Nicotra et al. 2010).

3.4.2 Ontogenetic differences in physiological stress

Seedling values of F_v/F_m were lower than adult values (Figure 3-5, Appendix, Table 3-2), despite less stressful levels of irradiance (Figure 3-2), lower vapour pressure deficits and lower diurnal temperature gradients observed within the gradient's understorey (Rapp and Silman 2012). However, both adult and seedling values of F_v/F_m were in the same range as previously reported values of tropical leaves (Ishida et al. 2005a, Ishida et al. 2005b), which combined with similar shifts in traits, indicates that seedling photosynthetic efficiency is constrained by the availability and allocation of resources. For example, seedlings may need to invest relatively more in their roots to avoid water stress, due to thin or absent soils in epiphytic species and potential competition from neighbouring trees (Niinemets and Valladares 2004).

In relation to light stress, shade leaves are known to experience greater photoinhibition under high light than sunlit leaves, but this is normally reversed under a short period of dark-adaptation (Murchie and Lawson 2013). However, dark-adapted relaxation can take longer after the influence of bright or long sunflecks, decreasing quantum efficiency for up to two hours post exposure in shade-tolerant understorey plants (Chazdon et al. 1996, Watling et al. 1997, Cavender-Bares and Bazzaz 2004). In comparison, adult leaves demonstrated a high capacity to recover from photoinhibition, with the lowest values of ϕ_{PSII} observed in TMCF trees coinciding with high PAR at the point of measurement (Figure 3-2b), yet with little reduction in F_v/F_m (Figure 3-5, Appendix Table 3-2).

3.4.3 Ontogenetic and community differences in leaf trait strategies

The observed values and variation in adult leaf traits were within the same range as previous studies of montane and lowland forest along the Andes-Amazon transect, with higher L_{th} , lower SLA but little difference in LDMC or investment into photosynthetic pigments with increased elevation (van de Weg et al. 2009, van de Weg et al. 2012, Bahar et al. 2016, Asner et al. 2017). This study partly supports previous global meta-analyses which show shifts towards more conservative trait strategies with increases in elevation, as per predictions based on the leaf economic spectrum, with decreases in temperature and increasing UV

radiation increasing abiotic stress (Tevini 2004, Wright et al. 2004, Körner 2007, Read et al. 2014). However, this trend is confounded by increases in succulence in some TMCF species, changing the relationship in resource investment on an area basis (Poorter et al. 2009, van de Weg et al. 2009) and expanding the variability of leaf trait strategies employed by the TMCF community (Figure 3-4).

Seedling leaf trait values were lower than reported adult trait values, but were comparable to observations made elsewhere on tropical juvenile trees (Houter and Pons 2005, Poorter 2009, Kitajima and Poorter 2010). Variability in the seedling population was not related to intraspecific elevational range location, with the higher variability in *SLA* in particular likely to be due to the range of environmental conditions experienced in the forest understorey (Poorter et al. 2009). The observed seasonal differences in understorey microclimate were similar to trends reported for 2008, with higher mean PAR, temperature and potentially limiting VPD at montane sites in the dry season relative to the wet season (Rapp and Silman 2012). Average seedling-level PAR data indicate that seedlings were experiencing very low light levels, however as these data cover only a short timer-period, they may not be representative of the understorey light environment as a whole, such as capturing sun spot activity ($\text{PAR} > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Way and Pearcy 2012). In addition to direct PAR from canopy gaps, montane seedlings receive diffuse radiation through cloud-immersion and laterally due to the high slope angle. The high topographic variability, increasing frequency of canopy gaps (Asner et al. 2013), and changing cloud frequency (Halladay et al. 2012) could therefore explain the greater diversity of leaf-trait strategies observed in the TMCF relative to the LMF community, reinforcing patterns of elevation-dependent increases in trait variance observed through remote sensing along the transect (Asner et al. 2017).

3.4.4 Trends in intraspecific leaf traits

The lack of consistent elevational trends in intraspecific traits within the seedling community could be characteristic of the plasticity within the population as a result of the high variability of understorey microclimate described above. However, as the adult population also showed limited intraspecific elevational trends in trait frequency distributions, it is more likely that intraspecific variation plays a lesser role than interspecific variation in determining the leaf trait frequency distributions investigated in this study. *SLA* was the only variable that showed intraspecific trends with elevation; significantly declining in the adult population with

elevation (Figure 3-2b). It may be that the other traits investigated here (L_{th} , chlorophyll content and $LDMC$) did not undergo sufficient selective or acclimatory pressure to change directionally in response to climatic shifts with elevation. The lack of persistent ecological stress at the range edge supports this hypothesis.

3.5 Conclusions

As hypothesised we found differences in the response of adults and seedlings to elevational shifts in climate; acclimatory shifts in anatomical traits (SLA) and short-term physiological stress ($\phi PSII$) were observed within the adult population with increased elevation, whereas seedlings experienced greater long-term physiological stress (F_v/F_m) without evidence of an acclimatory response. This indicates that seedlings are less responsive than adults to broad changes in climatic regime, perhaps as a result of the high microclimatic variability within the understorey. There was little evidence of an intra-specific increases in physiological stress at the lower range edge, however there was an increase in TMCF relative to LMF community-level stress at mid-elevations, which may explain the high species turnover observed at these sites (Van de Weg 2011, Asner et al. 2013, Girardin et al. 2014, Bahar et al. 2016). Distinct intraspecific trait strategies and physiological stress responses were evident within LMF and TMCF communities. This highlights the functional similarity of species within these communities (Bastias et al. 2017) and suggests that the response of highly species-diverse forest communities to environmental change can be derived from community rather than species-level shifts in functional leaf traits.

3.6 References

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3.7 Appendix

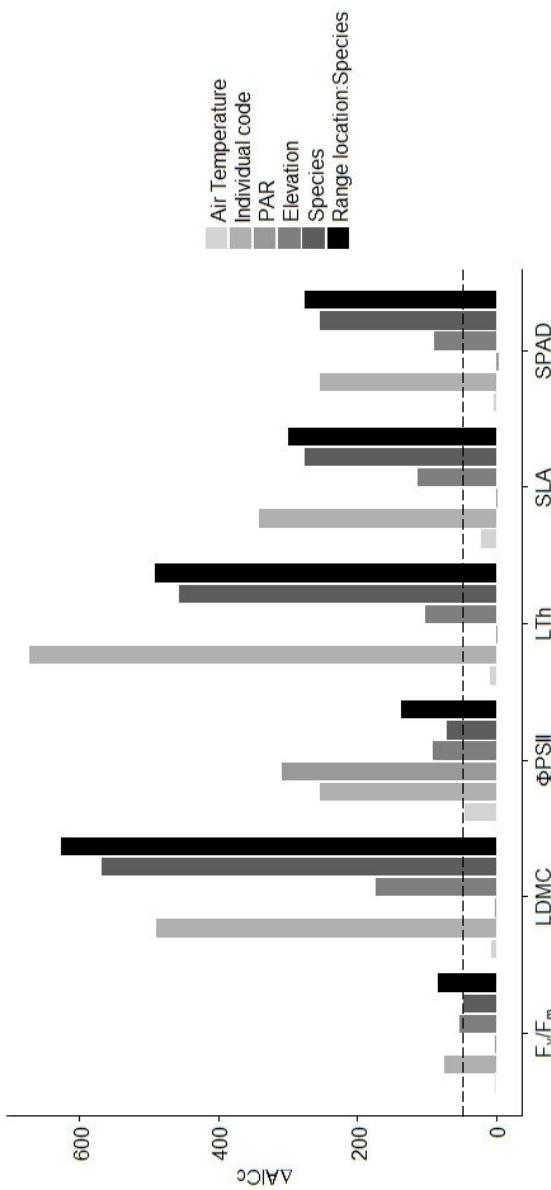


Figure 3-7 Difference in sample-size adjusted Akaike Information Criterion between models for each response variable. Values greater than the dotted line were included in the full model for each response variable. ΔAIC_c : AIC of model without random effects – AIC with each random effect included.

Table 3-2 Mean values and standard deviation of physiological and anatomical leaf-trait for ontogenetic

| | Species | N | $F_{\sqrt{F_m}}$ | Φ_{PSII} | LDMC ($mg\ g^{-1}$) | SLA ($m^2\ g^{-1}$) | LTh (mm) | SPAD |
|----------|------------------------|----|------------------|---------------|-----------------------|-----------------------|-------------|--------------|
| Adult | <i>C. cateniformis</i> | 15 | 0.781 + 0.02 | 0.449 + 0.17 | 347.5 + 41.7 | 0.014 + 0.002 | 0.21 + 0.01 | 49.69 + 4.61 |
| | <i>B. lactescens</i> | 25 | 0.810 + 0.04 | 0.862 + 0.04 | 576.0 + 73.6 | 0.010 + 0.002 | 0.33 + 0.08 | 61.37 + 5.41 |
| | <i>D. lamarkianum</i> | 5 | 0.857 + 0.03 | 0.804 + 0.09 | 681.0 + 66.8 | NA | 0.45 + 0.05 | 69.91 + 3.77 |
| | <i>G. glauca</i> | 75 | 0.800 + 0.09 | 0.756 + 0.17 | 506.4 + 57.0 | 0.011 + 0.002 | 0.22 + 0.04 | 57.74 + 7.59 |
| | <i>T. guianensis</i> | 50 | 0.840 + 0.07 | 0.797 + 0.16 | 482.2 + 52.5 | 0.010 + 0.002 | 0.23 + 0.04 | 52.16 + 9.43 |
| | <i>M. splendens</i> | 25 | 0.745 + 0.04 | 0.718 + 0.02 | 473.8 + 53.4 | 0.009 + 0.002 | 0.27 + 0.06 | 40.27 + 7.42 |
| | <i>A. verticillata</i> | 75 | 0.816 + 0.08 | 0.776 + 0.12 | 310.5 + 70.7 | 0.009 + 0.004 | 0.44 + 0.07 | 58.03 + 10.8 |
| | <i>S. patula</i> | 75 | 0.765 + 0.06 | 0.550 + 0.26 | 380.5 + 62.0 | 0.008 + 0.002 | 0.35 + 0.03 | 52.6 + 7.8 |
| | <i>C. revoluta</i> | 75 | 0.762 + 0.05 | 0.670 + 0.17 | 477.2 + 59.9 | 0.007 + 0.002 | 0.54 + 0.15 | 48.66 + 6.46 |
| | <i>C. thurifera</i> | 75 | 0.759 + 0.07 | 0.692 + 0.13 | 323.7 + 62.8 | 0.005 + 0.002 | 0.75 + 0.16 | 68.12 + 12.4 |
| Seedling | <i>H. goudotianum</i> | 75 | 0.785 + 0.07 | 0.686 + 0.15 | 253.6 + 37.7 | 0.012 + 0.005 | 0.39 + 0.07 | 59.33 + 9.24 |
| | <i>C. cateniformis</i> | 17 | 0.750 + 0.02 | 0.748 + 0.03 | 220.9 + 18.9 | 0.031 + 0.009 | 0.17 + 0.02 | 52.9 + 4.85 |
| | <i>I. deltoidea</i> | 31 | 0.737 + 0.06 | 0.738 + 0.03 | 279.0 + 45.2 | 0.022 + 0.007 | 0.20 + 0.04 | 44.42 + 9.98 |
| | <i>D. lamarkianum</i> | 31 | 0.782 + 0.05 | 0.749 + 0.10 | 369.4 + 36.6 | 0.019 + 0.003 | 0.21 + 0.06 | 39.14 + 6.9 |
| | <i>T. guianensis</i> | 31 | 0.766 + 0.04 | 0.756 + 0.04 | 346.3 + 65.2 | 0.031 + 0.008 | 0.13 + 0.02 | 37.02 + 6.03 |
| | <i>M. splendens</i> | 45 | 0.752 + 0.04 | 0.721 + 0.06 | 393.8 + 86.0 | 0.020 + 0.008 | 0.17 + 0.05 | 32.19 + 7.18 |
| | <i>A. verticillata</i> | 41 | 0.760 + 0.09 | 0.705 + 0.10 | 204.6 + 75.9 | 0.021 + 0.008 | 0.37 + 0.08 | 33.44 + 6.61 |
| | <i>S. patula</i> | 47 | 0.784 + 0.06 | 0.748 + 0.05 | 239.9 + 64.5 | 0.025 + 0.014 | 0.22 + 0.04 | 31.01 + 6.74 |
| | <i>C. revoluta</i> | 43 | 0.712 + 0.07 | 0.666 + 0.12 | 401.4 + 110.9 | 0.028 + 0.010 | 0.14 + 0.05 | 29.72 + 6.47 |
| | <i>C. thurifera</i> | 43 | 0.791 + 0.05 | 0.766 + 0.10 | 151.7 + 57.8 | 0.016 + 0.006 | 0.55 + 0.17 | 42.21 + 8.71 |
| LMF | <i>H. goudotianum</i> | 44 | 0.757 + 0.07 | 0.746 + 0.05 | 195.7 + 33.9 | 0.024 + 0.007 | 0.29 + 0.07 | 33.95 + 8.31 |

LMF; low-mid forest species; TMCF, tropical montane cloud forest species; N, number of surviving seedlings; $F/\sqrt{F_m}$, potential quantum efficiency of PSII; Φ_{PSII} , realised quantum efficiency of PSII; LDMC, leaf dry matter content; SLA, specific leaf area; LTh, leaf thickness; SPAD, relative chlorophyll content on an area basis.

Chapter 4. The response of Andean tree seedlings to a 100 year shift in climate warming

4.1 Introduction

Andean forests are experiencing warming of between 0.1 °C and 0.6 °C per decade (Marengo et al. 2011, Vuille et al. 2015), with predicted increases in air temperature of between +1.7°C to +6.7°C by the end of the century (Magrin et al. 2014). The magnitude of temperature change is expected to increase with elevation, with the greatest impact expected to occur in high elevation communities (Bradley et al. 2006). This warming is likely to be exacerbated by the additional pressures of decreasing precipitation (up to 15% reduction by 2100), the increasing frequency of extreme weather events such as droughts and floods (Lewis et al. 2011, Marengo et al. 2011, Magrin et al. 2014), as well as changes in land-use (Báez et al. 2016).

In response to these shifts in climate, the Andes has been projected to experience species turnover of 90% over the next century, with species gains as a result of the upslope range expansion of lower montane and lowland species (Lawler et al. 2009). Tropical species across a wide range of taxa have been found to shift their population centres upslope, with free-dispersing animals broadly keeping pace with current levels of warming (Pounds et al. 1999, Pounds et al. 2005, Seimon et al. 2007, Colwell et al. 2008, Raxworthy et al. 2008, Chen et al. 2009, Chen et al. 2011a, Chen et al. 2011b). However, migration is limited due to barriers such as habitat fragmentation and dispersal asynchrony between trophic levels (e.g. predator-prey or plant-pollinator) induced by the slower response of immobile or slower reproducing species (Larsen et al. 2011, Rehm 2014, Parmesan and Hanley 2015).

In order to keep pace with predicted warming over the next century, Andean plants need to migrate upslope by 300 to 1300 vertical metres, given a lapse rate of $\sim 5 \text{ }^{\circ}\text{C km}^{-1}$ (Rapp and Silman 2012). Based on current mean migration rates (2.5 m year^{-1} , $\pm 95\% \text{ CI} = +0.6 \text{ to } +4.9 \text{ m year}^{-1}$) and moderate warming ($+4 \text{ }^{\circ}\text{C}$) this is unattainable for the majority of species (Feeley et al. 2011), with populations expected to lag temperature by approximately $5.5 \text{ }^{\circ}\text{C}$ by the end of the century. This mismatch has been predicted to result in population declines of over 45% of Andean tree species, thus increasing the risk of extinction (Feeley and Silman 2010). The extent to which future declines will be driven by mortality at the lower range edge, or

dispersal limitations at the upper range edge, is unclear. Past transplant experiments indicate that range limits often coincide with niche limits (Lee-Yaw et al. 2016), but in shifting populations this is often not the case (Hargreaves et al. 2014). The survival and maintenance of transplanted populations beyond current and predicted future range edges may therefore indicate whether shifting populations will be restricted by the availability of analogous habitats, or by dispersal and establishment limitations.

The rate at which warming and extreme weather events are affecting and will continue to affect tropical populations over their lifetimes demands rapid assessments of the mechanistic causes of these declines. Few studies focus on the response of tropical plants to climate change (Feeley et al. 2017, Stroud and J. Feeley 2017), with few predictions of future range shifts based on experimental manipulations (Hargreaves et al. 2014) or an understanding of the ecophysiological mechanisms behind *in situ* growth and survival of individuals (Ishida et al. 2005, Krause et al. 2006, Krause et al. 2012). Here we assess the response of seedlings to a range of simulated climate change scenarios using a chlorophyll fluorescence ratio, the potential quantum efficiency of PSII (F_v/F_m), as a responsive and non-destructive monitoring tool of seedling physiological stress.

This study aims to simulate upslope migration and climate warming of lowland and montane trees under real-world conditions, addressing the dearth of experimental studies under natural conditions with multiple stressors (Sexton et al. 2009, Sundqvist et al. 2013, Hargreaves et al. 2014, Parmesan and Hanley 2015). Using an elevational transect located along a 3000 m gradient on the eastern slopes of the Peruvian Andes, seedlings were transplanted by the equivalent of ± 2 °C and ± 4 °C (as per low and moderate end-of-century warming scenarios) from their home elevation and were monitored for over a year to assess the seedlings' survival, growth and physiological stress responses. The main objective of this study is to detect if substantial changes in climate and physiological stress impact on seedling survival and growth rates, or whether tropical seedlings show a greater resilience to changing climate than estimated from observational studies of migration rate.

4.2 Methods

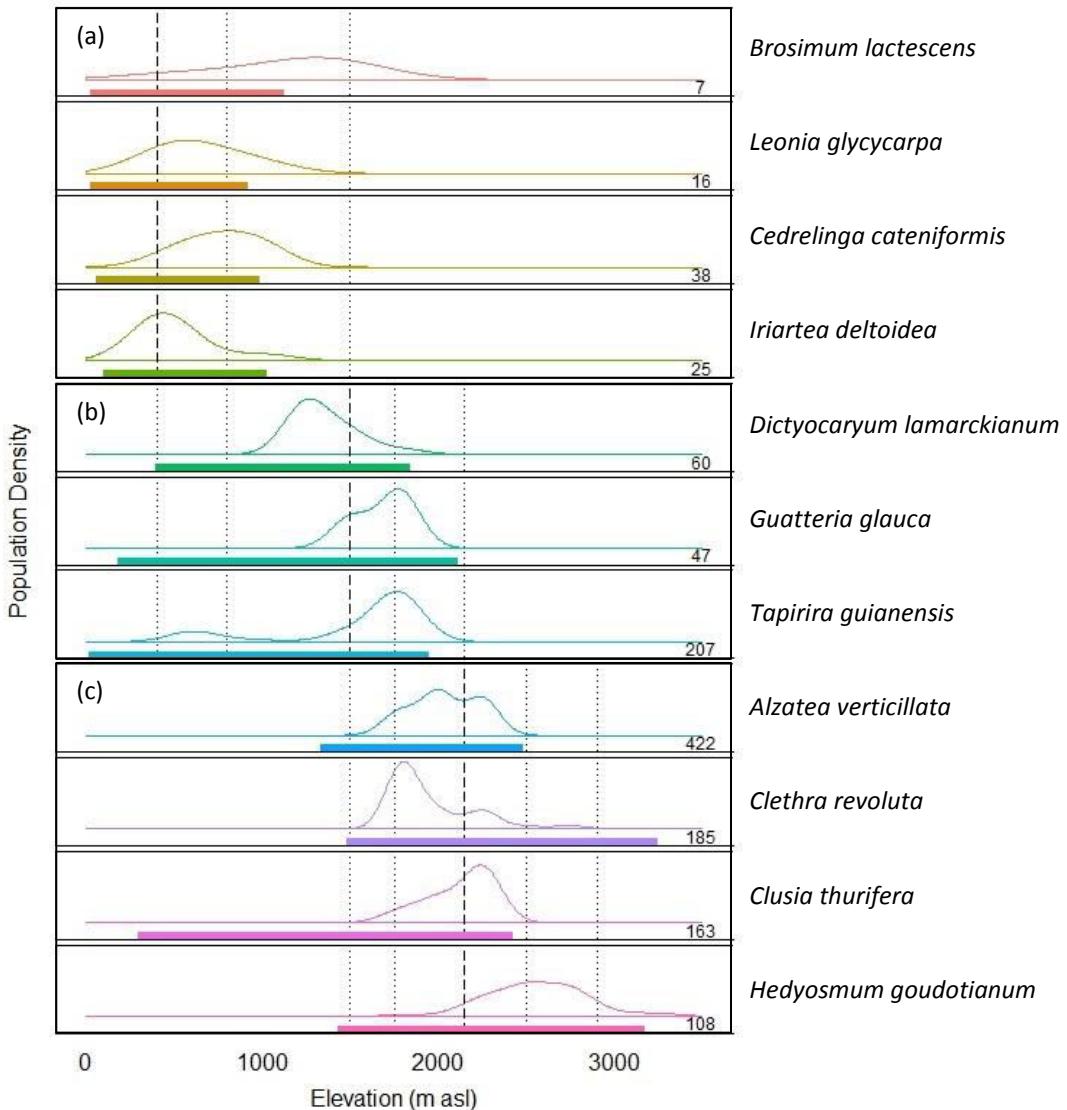


Figure 4-1 Local population density of adult tree species grouped by elevational community (a) lowland forest, (b) lower montane forest, (c) tropical montane cloud forest, estimated from the abundance of species within a series of 1ha permanent plots along the Andes-Amazon gradient (Feeley et al. 2011). Thickened horizontal line; Neotropical elevational range of each species, estimated from the Neo Trop Tree database (Oliveira-Filho 2017), Vertical lines; transplant elevations, dashed lines; home transplant elevation, dotted line; additional transplant elevations, values; number of individuals in plot network.

4.2.1 Species selection and seedling establishment

Species were selected to be representative of three elevational forest communities: lowland forest of less than 1000 m asl (LF), lower montane forests of between 1000 and 2000 m asl (LMF) and tropical montane cloud forest of above 2000 m asl (TMCF). LF seedlings (*Brosimum lactescens* (S. Moore) C. C. Berg, *Leonia glycycarpa* Ruiz & Pav., *Cedrelinga cateniformis* (Ducke) Ducke and *Iriartea deltoidea* Ruiz & Pav.) were grown from local seed sources within a shaded nursery at approximately 400 m asl (Figure 4-1a). LMF seedlings (*Dictyocaryum*

Iamarkianum (Mart.) H. Wendl., *Guatteria glauca* Ruiz & Pav. and *Tapirira cf. guianensis* Aubl) and TMCF seedlings (*Alzatea verticillata* Ruiz & Pav., *Clethra revoluta* (Ruiz & Pav.) Spreng., *Clusia thurifera* Planch. & Triana and *Hedyosmum goudotianum* Solms.) were established from seeds collected from the central elevation of each species' local population and grown within sheltered nurseries at home elevations of approximately 1500 m asl (Figure 4-1b) and 2150 m asl respectively (Figure 4-1c).

4.2.2 Transplant experiment and censuses

Where successful establishment within the nurseries allowed, maximum of 72 seedlings of each species were transplanted to forest sites within their home and alternative transplant elevations (Figure 4-1), equivalent to a change in mean annual temperature of approximately $\pm 2^{\circ}\text{C}$ and $\pm 4^{\circ}\text{C}$. Seedlings were transported in their soil plugs to each transplant elevation, where they were planted into well-draining plastic pots (3.77 l) containing local soil and buried to local soil level. Each species was transplanted to 6 subplots per elevation, with individuals assigned to gap or shade treatment plots within each subplot. Transplantation started at lowland sites in June 2015 and were completed at upper montane sites by August 2015. At the point of installation a unique number was assigned to each seedling, with growth traits recorded for each individual. Seedlings were then censused every 2 weeks from transplantation until September 2015 to assess mortality due to transplant shock. From October 2015 until May 2016, monthly censuses were made to assess seedling stress and survival with a final census of seedling growth traits made in October 2016. Seedling pots were lifted from the soil one year post-transplantation and drainage holes were checked for root outgrowth, of which there was minimal evidence, despite high growth rates (more than 1 m in a year).

4.2.3 Seedling measurements

Traits of seedling height (cm), stem diameter (mm) and number of leaves were recorded over time. Net growth rate was calculated as the increase in height (cm) from installation (June-August 2015) until October 2016, relative to initial height ($\text{cm cm}^{-1} \text{year}^{-1}$). Seedling survival was assumed when a seedling had a measureable leaf area at the point of measurement. Relative survival was calculated at the subplot level as the proportion of surviving individuals relative to those surviving at the first measurement point (i.e. October 2015 or the following

months if leaves were too small to measure). The change in relative survival was calculated as the difference in relative survival from one month to the next, calculated at the subplot level. Due to differences in first measurement point and fluctuations in available leaf area the change in survival varied from -1 to 1.

Seedling physiological stress was assessed by measuring changes in the potential quantum efficiency of PSII (F_v/F_m) for the uppermost leaf of each seedling. F_v/F_m was measured using an OS30p+ chlorophyll fluorometer (Opti-Sciences, Inc., Hudson, NH, USA). To ensure the relaxation of the seedlings' photosynthetic reaction centres, a dark adaptation clip (FL-DC clip) was attached to the leaf lamina whilst avoiding veins and the whole seedling was covered for at least 20 minutes prior to measurements of F_v/F_m (Maxwell and Johnson 2000). Measurements were taken ensuring a natural leaf angle and limiting external light exposure to the leaf lamina.

4.2.4 Microclimatic sensors

Micrometeorological stations were installed in the understorey (0.5 m above ground level) in one gap and one shade plot at each transplant elevation. Each station consisted of a Temperature/ Relative Humidity smart sensor (HOBO, 12-bit, #S-THB-M002), a soil moisture smart sensor (10HS #S-SMD-M005) and a PAR smart sensor (#S-LIA-M003). Data were logged every hour (HOBO U23 Pro v2 External Temperature Logger #U23-004, HOBO USB micro station data logger #H21-USB) during the set-up and throughout the transplant experiment, with the most complete section of the dataset presented here from September 2015 until August 2016.

4.2.5 Analysis

Mean and standard error were calculated for each seedling trait, grouped by species, elevation, subplot and light treatment. Trends in monthly mean relative survival and F_v/F_m at each transplant elevation are reported here from November 2015 until October 2016, whereas growth is presented at the end-point of the experiment. Statistical differences in F_v/F_m and growth between transplant elevations over time were determined using general linear mixed effect models (GLMM). Differences were also calculated between elevational forest communities (LF, LMF and TMCF) and where possible each species. A general linear model (GLM) with a quasipoisson distribution was selected to describe trends in relative

survival, using F_v/F_m and elevation as explanatory factors. All models were selected and tested as per standard methods (Zuur et al. 2009), using the lme4 package (Bates 2015) in R (Version 3.4.1, R Core Team), with variation associated with light treatment, time period and species taken into account during model selection. To summarise and compare the decline in survival between transplant elevations, relative survival was log-transformed and the slope of the new fit was extracted.

The microclimate data were summarised by elevation, month and season; the wet season was considered to be from November until March and the dry season from May until July (Rapp and Silman 2012). Monthly mean, standard error, maximum and minimum values were calculated for all microclimatic variables. Changes in air temperature, soil temperature and soil moisture with elevation were reported over the experimental period. Where there were more than 45 data points per month, these data were presented as continuous smoothed means with 95% confidence intervals, and where data were fewer, mean and standard error were reported. In addition, diurnal trends and hourly maximums were presented for air temperature and PAR.

4.3 Results

4.3.1 Microclimate

Air temperature decreased with elevation with an average lapse rate of approximately $-5.1\text{ }^{\circ}\text{C km}^{-1}$. For TMCF elevations (above 1750 m asl), mean monthly air temperature was approximately 4- 6 $^{\circ}\text{C}$ greater during the wet season (November-March) than the dry season (Figure 4-2a). Whereas air temperature at 1550- 1750 m asl was relatively constant ($\pm 1\text{-}2\text{ }^{\circ}\text{C}$) throughout the measurement period. Seasonal changes in soil temperature were less pronounced across all elevations (an increase of $\sim 2\text{ }^{\circ}\text{C}$ during the wet season), with an average lapse rate of $-4.8\text{ }^{\circ}\text{C km}^{-1}$ (Figure 4-2b).

Soil moisture varied between 10 and 50% across all elevations over the measurement period, with the lowest values experienced at 2150 and 2900 m asl following the dry season and the greatest values at 1500 and 1750 m asl during the wet season. Soil moisture increased during the wet season, peaking between February and April with mean values of between 25 and 45%, but then decreasing by approximately ten percentage points during the dry season (Figure 4-2 c).

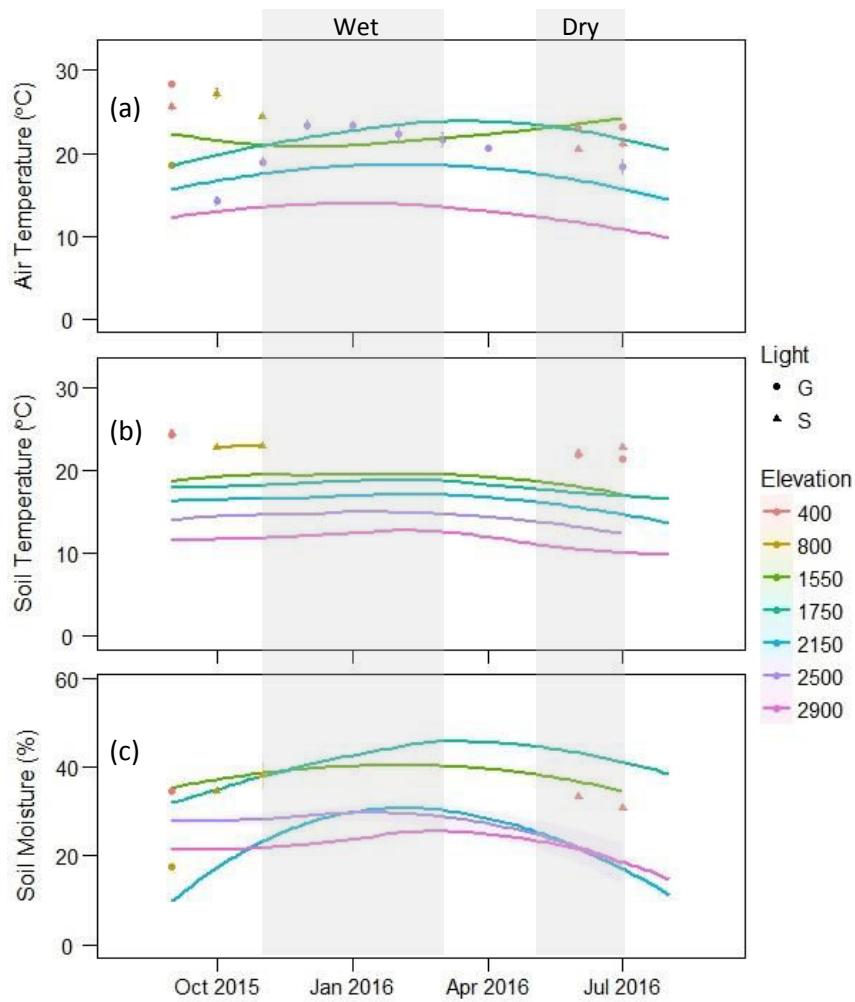


Figure 4-2 Understorey microclimate across seven elevations (m asl) from September 2015 to August 2016; (a) air temperature, (b) soil temperature and (c) soil moisture. Points are monthly mean \pm SE of gap (circles) and shade (triangles) plots for small ($n < 45$) or incomplete time series. Lines are smoothed monthly means with 95% confidence intervals (Wickham 2009a). Nov-March; wet season, May-July; dry season (Rapp and Silman 2012).

Diurnal changes in air temperature during the dry season were more than double those recorded during the wet season, with rapid increases in temperature during the morning, particularly in gaps at TMCF elevations (Figure 4-3a, b). Maximum temperatures and seasonal variability were greatest in gap than shade plots, exceeding 10 °C in difference between day and night mean values during the dry season. Mean values of PAR were approximately ten times higher in gap than shade plots, although maximum values were within the same range (Figure 4-3c, d), reaching 2500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. There were no clear seasonal changes in PAR, although there was some evidence of midday decreases in shade plots during the wet season at 1500 and 2900 m asl.

In summary, during upslope transplantation above 1500 m asl seedlings experienced a cooler and more variable climate, with peaks in soil moisture at mid elevations (1550 and

1750 m asl), but no evidence of drought conditions at any site. Similarly at high elevation sites, seedlings were exposed to greater variability of soil moisture and PAR in gap than shade plots.

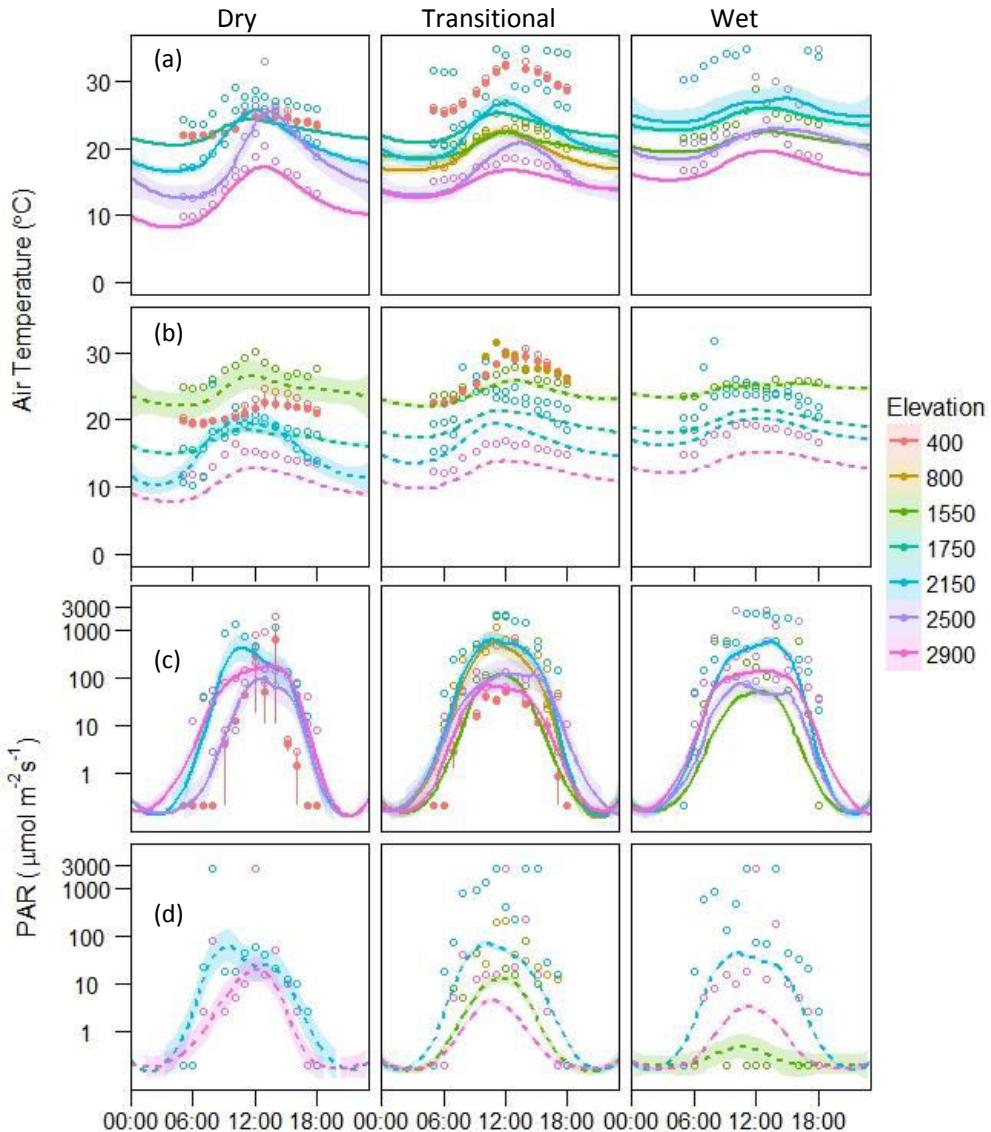


Figure 4-3 Diurnal trends in understory air temperature and photosynthetically active radiation (PAR) during the dry season, wet season and in transition between the two seasons recorded in gap (a, c) and shade (b, d) sites. Closed points are monthly mean $\pm \text{SE}$ for small ($n < 45$) or incomplete time series Lines are smoothed means with 95% confidence intervals (Wickham 2009a). Open points are maximum value recorded during each hour.

4.3.2 Seedling survival response to transplantation

Mean relative survival at the end of the experiment varied between 0.16 and 0.86 for each species (Figure 4.4), with some species maintaining high levels of survival (>0.8) across the transplant experiment (e.g. *L. glycycarpa*), whereas others experienced almost complete eradication (<0.1) of the transplant population at some transplant elevations (e.g. *D.*

lamarckianum, *A. verticillata* and *C. thurifera*). The lowland forest (LF) species (Figure 4.4, top) experienced the highest overall rates of survival amongst elevational groups, with mean values varying between 0.77 and 0.97 across species and elevations. Survival was similar between LF species and transplant elevations, however for *B. lactescens* and *C. cateniformis* survival declined in shade plots at 1500 m asl (the upslope transplant site) resulting in drops in survival rates of 0.23–0.26.

Changes in survival over time was more variable for lower montane forest (LMF) species (Figure 4.4, middle): *D. lamarckianum* experienced almost linear decreases in survival, reaching 0% survival at 1500 masl (home elevation) by October; *T. guinanensis* experienced lesser linear declines at 400, 800 and 2150 m asl, but maintained levels of survival of over 0.5 at 1500, 1750 and 2150 m asl; *G. glauca* maintained survival rates of above 0.5 across elevations, with the largest decline at 400 m asl (downslope transplant) during the dry season. Overall LMF seedlings had the greatest rates of survival at 1750 m asl, with a mixed response at other transplant elevations.

Tropical montane forest (TMCF) species (Figure 4.4, bottom) underwent the most rapid declines in survival of all elevational groups, with greatest mortality occurring during the wet season (November to March). For *A. verticillata* and *H. goudotianum* the decline was greatest at upslope transplant elevations (2500 and 2900 m asl), reducing the transplant population exponentially, with total loss at shade sites within six months. *C. revoluta* and *C. thurifera* experienced similar rates of declines, but at downslope elevations, particularly at 1500 m asl. Where mortality was high, observational evidence suggested that pathogen load was the cause rather than herbivory.

4.3.3 Seedling stress response to transplantation

As with survival response, LF species experienced minimal changes in F_v/F_m relative to other elevational groups (approximately 83% of values were between 0.7 and 0.8), with the only consistent decrease to below values of 0.7 observed in *C. cateniformis* at 1500 m asl (Figure 4.5, top). Trends in F_v/F_m were found to differ significantly (GLMM, $p<0.01$) between the home elevation (400 m asl) and other elevations for *L. glycycarpa* and *C. cateniformis*; seedlings at 800 m asl maintained high F_v/F_m (>0.75), whereas more variable declines in F_v/F_m were observed at 1500 m asl.

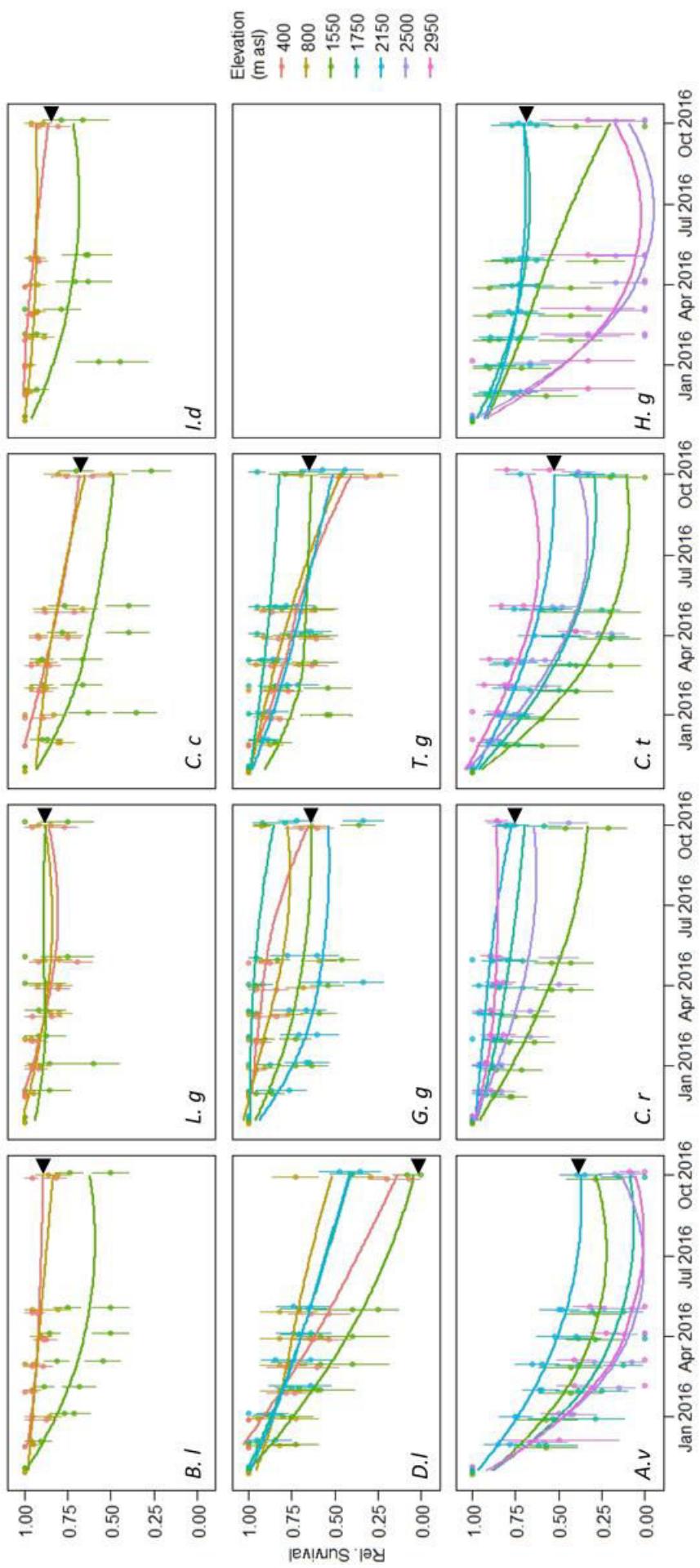


Figure 4.4. Seedling survival for lowland species (LF, top) lower montane species (LMF, middle) and montane cloud forest species (TMCF, bottom). Points are mean (\pm SE) gap and shade plot values. Black triangles indicate ultimate survival at seedling 'home' elevation. Lines are smoothed means with 95% confidence intervals (Wickham 2009a).

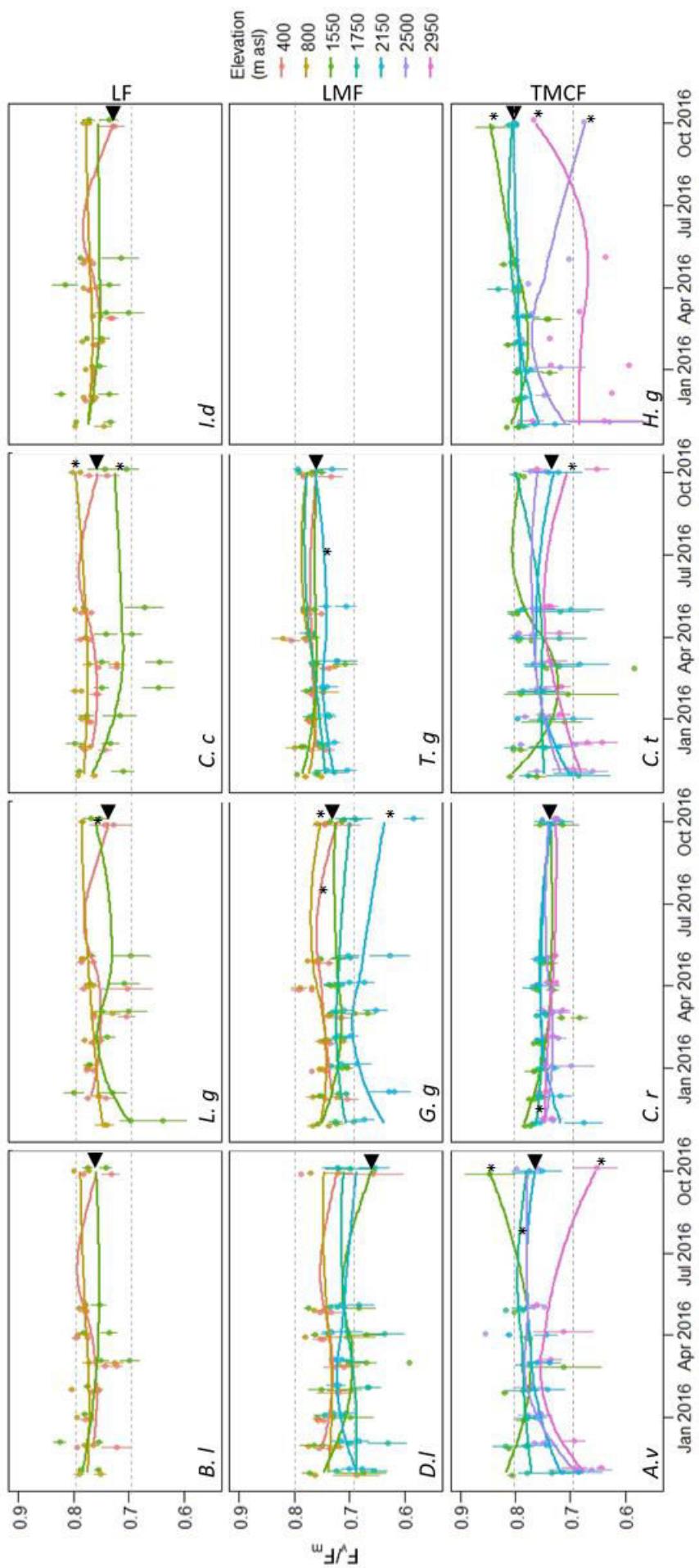


Figure 4.5. Seedling potential quantum efficiency (F_v/F_m) at each transplant elevation for lowland species (LF, top) / lower montane species (LMF, middle) and montane cloud forest species (TMCF, bottom). Points are jittered mean ($\pm SE$) gap and shade plot values. Black triangles indicate ultimate F_v/F_m at seedling 'home' elevation. Lines are smoothed means with 95% confidence intervals (Wickham 2009b). Elevations with significantly different F_v/F_m (GLMM, $p < 0.01$) from the central elevation (triangle) are indicated by *.

20% of LMF seedlings (Figure 4.5, middle) experienced depressions in F_v/F_m of below 0.7 relative to 8-10% in other elevational community groups. However TMCF seedlings (Figure 4.5, bottom) experienced the greatest variability in F_v/F_m (0.3 - 0.95). F_v/F_m tended to decrease with transplant elevation, particularly in gap sites (appendix, Figure 4-9), declining significantly (GLMM, $p<0.01$) for over half of species transplanted to the uppermost sites. A small proportion of seedlings at downslope transplant sites had significantly higher (GLMM, $p<0.01$) and less variable values of F_v/F_m : *G. glauca* at 800 and 400 m asl; *C. revoluta* and *A. verticillata* at 1750 m asl. Overall seedlings at 1500 m asl were found to have the greatest variability in F_v/F_m (0.6 to 0.85), in particular those TMCF species with exponential declines in survival.

4.3.4 Relationship between seedling stress and survival

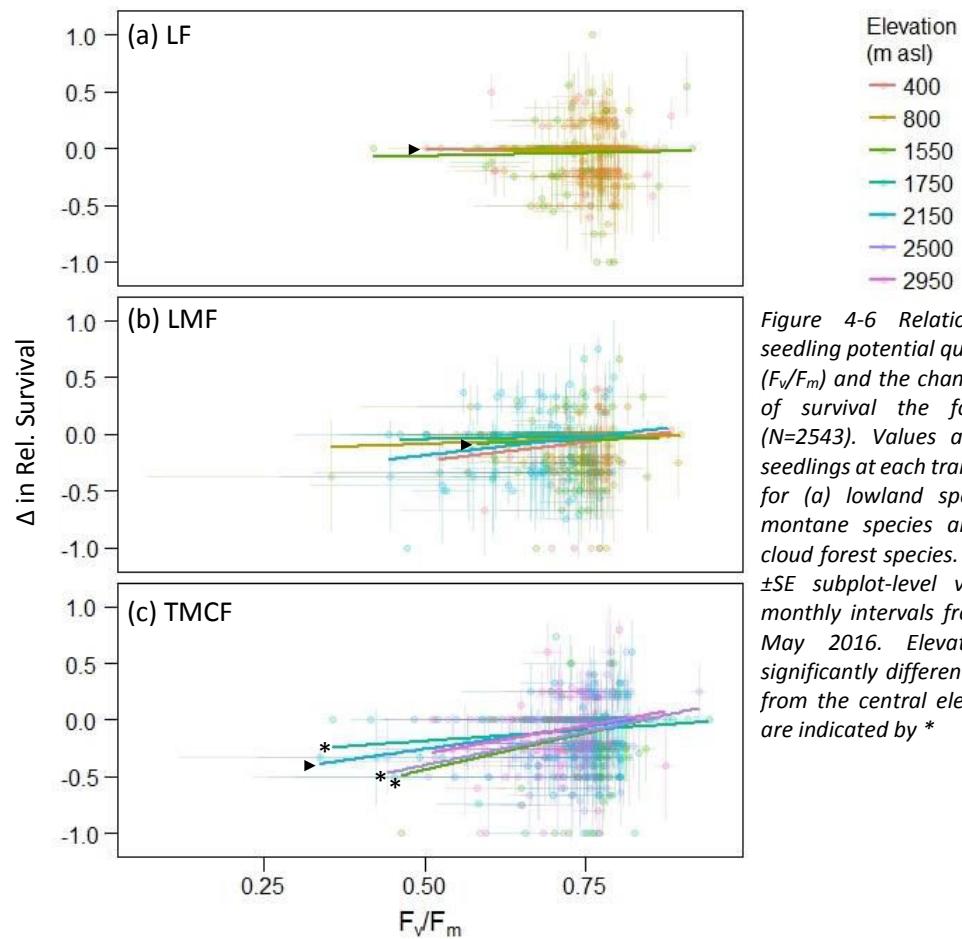


Figure 4-6 Relationship between seedling potential quantum efficiency (F_v/F_m) and the change in probability of survival the following month ($N=2543$). Values are for surviving seedlings at each transplant elevation for (a) lowland species, (b) lower montane species and (c) montane cloud forest species. Points are mean \pm SE subplot-level values taken at monthly intervals from Nov 2015 to May 2016. Elevations that are significantly different (GLM, $p < 0.05$) from the central elevation (triangle) are indicated by *

Changes in the relative survival of seedlings in response to F_v/F_m and transplant elevation were tested for each elevational group, taking into account differences between light treatment, species and time period. For LF species no significant relationship (χ^2 , $p>0.05$) was

found between survival and F_v/F_m or transplant elevation (Figure 4-6, a). For LMF species there was no significant relationship between survival and elevation (χ^2 , $p>0.05$), but there was a positive relationship for F_v/F_m (χ^2 , $p<0.01$) (Figure 4-6, b) with an increase in F_v/F_m from 0.5 to 0.75 increasing survival by 0.08. For TMCF species there was a significant relationship between survival, F_v/F_m (χ^2 , $p<0.001$) and elevation (χ^2 , $p<0.05$); an increase in F_v/F_m from 0.5 to 0.75 conferring an approximate increase in survival of 0.2, whereas transplantation away from central elevation decreased survival by 0.01 to 0.02 (Figure 4-6, c).

4.3.5 Relative Growth of surviving seedlings

Seedlings ranged in their growth response over the transplant year, with changes in height of between -20 and 90 cm and an overall mean increase in height of 9 ± 14 cm. Of the 6% of seedlings that exceeded 50 cm in growth, 75% were of the TMCF species *C. revoluta* and *H. goudotianum*. For those surviving individuals that lost stature, many also lost leaves: leaf loss was most common at 2950 and 1550 m asl for TMCF, 400 m asl for LMF and 1550 m asl for LF species.

Net growth rate was greatest for TMCF species, growing on average 0.5 ± 0.1 cm $\text{cm}^{-1} \text{yr}^{-1}$, relative to 0.26 ± 0.1 and 0.1 ± 0.05 cm $\text{cm}^{-1} \text{yr}^{-1}$, for LF and LMF species respectively. Net growth rate was not significantly related to F_v/F_m for any species group ($\chi^2 < 5$, $p>0.05$) (Appendix, Figure 4-8). For the LF group there was no significant difference in net growth rate between transplant elevations ($\chi^2 = 0.16$, $p>0.05$). Seedlings of *L. glycycarpa* had more than double the growth rate of the other species, peaking at the upper transplant elevation, 1500 m asl (Figure 4-7, top). Where growth occurred, values were similar across the LMF group, however there were seedlings that survived until October 2016 without measureable growth, such as *D. lamarckianum* at 2150 m asl and *T. guianensis* in shade sites at 1500 m asl (Figure 4-7, middle). Transplant elevation had a significant effect on LMF net growth rate ($\chi^2 = 19.6$, $p<0.001$), with an approximate 20% decrease in growth rate at 2150 m asl relative to the home elevation. Similarly net growth rate of TMCF species were affected by elevation ($\chi^2 = 13.1$, $p<0.05$), peaking at 1750 m asl, before declining upslope (Figure 4-7, bottom). As with the LMF species, *C. thurifera* survived at 1500 m asl and 2500 m asl without measureable growth.

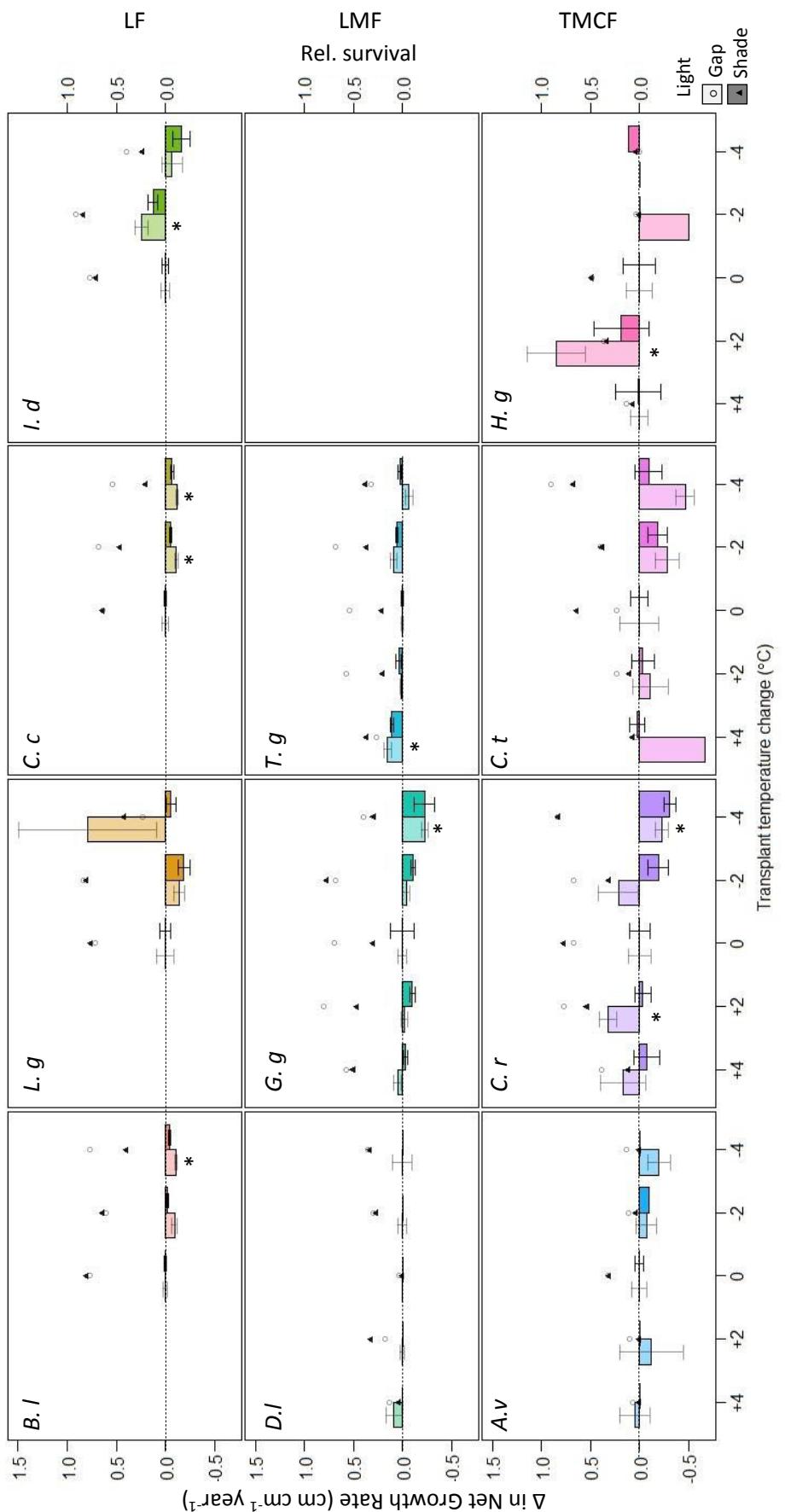


Figure 4-7 Change in net growth rate with transplantation of lowland species (LF, top), lower montane species (LMF, middle) and montane cloud forest species (TMCF, bottom) in each light treatment. Bars are mean ($\pm \text{SE}$) of the increase in height from installation, June-August 2015, until October 2016 relative to initial height for gap (light fill) and shade (dark fill) light treatment plots. Points are mean relative survival at each elevation for gap (circle) and shade (triangle) light treatment plots. Significant changes with transplant elevation (LME, $p < 0.01$) are indicated by *.

4.4 Discussion

Table 4-1 Summary of seedling response to change in temperature with transplantation downslope (+2 °C or +4 °C) and upslope (-2 °C or -4 °C) (a) LF, lowland species, (b) LMF, lower montane species and (c) TMCF, tropical montane cloud forest species. Large symbols, positive or negative transplant effect ($p<0.01$); small symbols, positive or negative transplant effect ($p<0.06$); red symbols, convincing trends, but statistically untested due to low survival; X/✓, indicate if transplant site is within current estimated local range.

(a) LF, 400 m asl home elevation

| Species | <i>B. lactescens</i> | | <i>L. glycyrrapa</i> | | <i>C. cateniformis</i> | | <i>I. deltoidea</i> | |
|-----------------------|----------------------|---------|----------------------|---------|------------------------|---------|---------------------|---------|
| Transplant temp. (°C) | -2 ✓ | -4 X | -2 ✓ | -4 X | -2 ✓ | -4 X | -2 ✓ | -4 X |
| Survival | — | | | | — | | — | |
| Stress | | | — | | + | — | | |
| Growth | - | — | | | — | — | + | - |

(b) LMF, 1550 m asl home elevation

| Species | <i>D. lamarckianum</i> | | | | <i>G. glauca</i> | | | | <i>T. guianensis</i> | | | |
|-----------------------|------------------------|---------|---------|---------|------------------|---------|---------|---------|----------------------|---------|---------|---------|
| Transplant temp. (°C) | +4 X | +2 ✓ | -2 ✓ | -4 X | +4 X | +2 X | -2 ✓ | -4 X | +4 ✓ | +2 ✓ | -2 ✓ | -4 X |
| Survival | + | + | + | | + | + | + | | + | | | |
| Stress | + | + | - | | + | + | | - | | | | |
| Growth | + | + | - | - | | | - | | + | | - | |

(c) TMCF, 2150 m asl home elevation

| Species | <i>A. verticillata</i> | | | | <i>C. revoluta</i> | | | | <i>C. thurifera</i> | | | | <i>H. goudotianum</i> | | | |
|-----------------------|------------------------|---------|---------|---------|--------------------|---------|---------|---------|---------------------|---------|---------|---------|-----------------------|---------|---------|---------|
| Transplant temp. (°C) | +4 X | +2 ✓ | -2 X | -4 X | +4 X | +2 ✓ | -2 X | -4 X | +4 X | +2 ✓ | -2 X | -4 X | +4 X | +2 X | -2 ✓ | -4 ✓ |
| Survival | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Stress | + | + | - | | + | | + | | + | | - | | + | + | - | - |
| Growth | + | + | - | - | + | | - | | - | | - | | + | - | - | - |

The response of seedlings to upslope and downslope transplantation varied between elevational community groups, with changes in survival partially explained by changes in physiological stress and elevation, whereas growth was only related to elevation. In most cases transplantation to the uppermost site, equivalent to -4 °C cooling, resulted in negative responses of survival, stress and growth, whereas transplants downslope (+2 °C and +4 °C warming) resulted in mixed responses at the species level (Table 4-1). Declines upslope were attributed to seedlings' failure to upregulate their metabolism to more variable and cold temperatures, whereas declines downslope were associated with increased herbivory and the observed pathogen load.

4.4.1 Andes-Amazon context

Mean rates of seedling survival from this study (0.16 – 0.86) were more variable than previously reported survivorship from a seedling transplant experiment carried out at the treeline (3150 - 3650 m asl) of the same elevational transect: 0.40 - 0.62 (Rehm and Feeley 2016). This is likely due to the greater range of species and the greater transplant distances used in this study. However, there were parallels between the findings of Rehm and Feeley (2016) and the response of TMCF seedlings, as survival trends were species-specific in relation to elevation: *Weinmannia fagaroides* responded negatively to warming like *C. revoluta* and *C. thurifera*, whereas *Clethra cuneata* responded negatively to cooling like *H. goudotianum* and to a lesser extent *A. verticillata*. Similarly, observed trends in tree growth with elevation along the transect were found to be species specific, but as with TMCF species, growth was found to decrease with elevation above 1750 m asl (Rapp et al. 2012). Decreases in growth with elevation have been observed across the Andes as a whole, with the impacts of cloudiness on radiation plus the a number of direct and indirect effects of low temperature on nutrient cycling (Nottingham et al. 2015) and plant metabolism proposed as the main causes (Báez et al. 2015).

Mean measures of physiological stress (F_v/F_m) were more variable (0.6 – 0.85) than values previously recorded for naturally occurring seedlings of the same species along the Andes to Amazon transect (0.7 – 0.8, Appendix 2.2). Within the natural population seedlings at the lower range edge were more stressed, but there was no evidence of long-term abiotic stress within this sub- population (Chapter 2). Transplantation understandably elicited a greater range of stress responses, considering seedlings were moved outside their realised niche, with the impact of reducing the probability of survival over time for LMF and TMCF species (Figure 4 6).

4.4.2 Upslope transplantation

Consistent with recent syntheses of beyond range transplant experiments (Hargreaves et al. 2014, Lee-Yaw et al. 2016), declines in survival and growth increased in frequency at beyond-range transplant sites particularly towards high elevations (Table 4-1), indicating that for these instances range limits currently overlap with niche boundaries. Declines in relative survival of LMF and TMCF seedlings could be explained by increasing physiological stress

(Figure 4-6, b and c), which correlated with decreasing temperatures and an increase in diurnal and seasonal variability experienced at elevations above 1500 m asl. Declines in survival of LF species at the uppermost transplant site (1500 m asl) were also likely to be due to low temperature and climatic factors, as growth rate simultaneously declined.

Negative seedling responses were also observed within the present range (Table 4-1). Upslope declines in growth for *C. cateniformis*, *D. lamarckianum* were recorded at sites located close to the estimated upper range edge, where the adult populations were small (Figure 4-1, note sample size). This suggests that these edge population may have long experienced low or chance reproduction, establishment and recruitment, perhaps contingent on climatically favourable years. In contrast, the negative response of transplanted *H. goudotianum* seedlings at high elevation is best explained by an alternative process: the increased frequency of clonal reproduction (adventitious root and shoot growth) observed in the natural population. This response is common at high elevations and in stressful environmental conditions as a mechanism to overcome dispersal and seedling establishment barriers (Klimes 2003, Bader et al. 2007, Klimešová and Doležal 2012, Ye et al. 2014) e.g. low growth rates lengthen the seedling establishment period, making seedlings more susceptible to climatic fluctuations.

4.4.3 Downslope transplantation

Previous controlled warming experiments show that an increase in daytime and night-time temperatures of + 2 °C to + 5 °C above current ambient temperatures increased and in some cases doubled tropical seedling growth (Esmail and Oelbermann 2011, Cheesman and Winter 2012, Cheesman and Winter 2013, Krause et al. 2013, Slot and Winter 2016, 2017). Because of this, downslope transplantation was expected to elicit a positive growth response in surviving seedlings. There were significant increases in growth response for *T. guianensis* at the +4°C transplant site and *C. revoluta* and *H. goudotianum* at the +2 °C site, however the growth response was weak or non-existent for the majority of species.

For LMF seedlings, downslope transplantation resulted in neutral or positive survival, stress and growth responses, indicating lessened abiotic pressures and no evidence of a physiological range limit. Neutral responses indicate that although seedlings were able to acclimate to a change in climatic regime, they were unable to exploit the warmer conditions

in order to upregulate their growth, perhaps due to other limiting factors such as light availability or resource competition. Based on the Neotropical distribution of the LMF species (Figure 4-1, horizontal line) it is unsurprising that seedlings are able to survive at these elevations. However, the narrow local elevational ranges of *D. lamarckianum* and *G. glauca* suggests otherwise (Figure 4-1, population density plot), potentially indicating local niche constraints. Equally, the difference may be due to sampling error, as high levels of competition in diverse lowland sites could have reduced species' frequencies below a point that was detectable by the census plot design.

The response of TMCF species to downslope transplantation is yet more complicated, as survival decreased despite neutral or positive responses in stress and growth (*A. verticillata*, *C. revoluta*, *C. thurifera*, Table 4-1). This indicates a lack of physiological barriers to survival, with biotic pressures the most likely cause of seedlings' failure to establish. Biotic factors such as competition, predation and disease are known to play a role in range-limitation across a diversity of taxa (Gaston 2003, Sexton et al. 2009). A recent review of transplant experiments found that biotic factors partially or completely enforced low-elevation range limits in 55% of studies (Hargreaves et al. 2014). Within this study mortality often followed defoliation, but the mechanisms behind this remain unclear. There was little evidence of herbivory on surviving leaf material, however observations of rot within remaining stems indicated that fungal pathogens were present. Previous studies have shown fungal pathogens to cause mortality within two weeks of infection (Bagchi et al. 2010), which could easily have occurred between census intervals. Pathogen virulence has been found to increase with watering frequency (Swinfield et al. 2012), so on transplantation to warmer, wetter sites (1550 and 1750 m asl, Figure 4-2), seedlings were at greater risk of fungal pathogen-induced mortality.

4.4.4 Limitations of transplant experiment

Transplantation along an elevational gradient allowed space for time comparisons simulating low to moderate changes in mean annual temperature under real-world conditions. The replication of the study was limited to one gradient with measurements over the course of one year, making assessments of the long-term effects of transplantation on species' lifetime unadvisable. To put the measurement period in inter-annual context: 2015/16 has been described as a super El Niño year (the maximum Pacific sea surface temperature anomaly

exceeding 3 K in Niño regions) (Rao and Ren 2017). El Niño years are drier and average 0.7–1.3 °C warmer than la Niña years in the tropical Andes (Vuille and Bradley 2000, Vuille et al. 2000) and compared to mean temperatures recorded during the 2007 and 2008 la Niña years (Rapp and Silman 2012), the 2015–2016 period was significantly warmer (approximately +2°C), although with decreasing inter-annual variability at high elevations (above 1750 m asl). This variability makes the measurement period arguably more stressful than an average year, although there was no evidence of drought in the study plots (Figure 4-2c). Declines in seedling survival during the measurement period can be interpreted as impacting on the lifetime fitness of the population, due to impacts on seedling recruitment. However high survival rates must be interpreted with caution, as success at one ontogenetic stage cannot be extrapolated to others (Lee-Yaw et al. 2016).

In terms of spatial replication, the study transect has been found to have levels of heterogeneity; previous studies have observed considerable variability within species and elevational bands (Bahar et al. 2016), as well as high levels of topographic variability (Asner et al. 2013). Indeed, with high levels of intra-specific variability there is little evidence of phenotypic shifts at species' edge populations (Chapter 2). This heterogeneity ensures that by transplanting seedlings from the centre of their range to a variety of sub plots at each elevation, the variability in response will lessen any risk of over-interpreting our results.

The nature of the experiment imposes co-occurring changes in environmental and biotic factors such as soil fertility, cloud cover, pathogen virulence and herbivore abundance that are arguably unrealistic. However, as populations shift their range they will meet an equal variety of limiting factors, which due to the uncertainty in climate change forecasts, are difficult to predict (Huntingford et al. 2013). The inclusion of these complicating factors therefore provides a valuable opportunity to explore the real-world implications of shifting climate (Parmesan and Hanley 2015).

4.4.5 Implications of future climatic shifts

This study supports the asymmetric abiotic stress limitation hypothesis, which predicts that stress plays a greater role at high rather than low elevations in determining range limits, except in the case of drought (Normand et al. 2009). High stress and low survival upslope indicates that the lag in current upslope migration rates relative to warming is less likely to

be a result of dispersal limitations, but rather a climate-driven barrier to seedling establishment and growth. The speed and success of upslope range expansions are therefore dependent upon the trajectory of future climatic shifts, with more favourable adjustments, such as reduced seasonality and warmer temperatures, facilitating seedling establishment. The greatest uncertainty for the future of LMF and TMCF species is associated with shifts in the cloud base, which could dramatically alter levels of rainfall and radiation and thus increase water or light stress (Foster 2001, Halladay et al. 2012). Under these scenarios species that can more readily acclimate to stress, such as *T. guianensis*, will be at an advantage.

Although growth has been found to increase with moderate warming along environmental gradients and within controlled experiments (Báez et al. 2016, Slot and Winter 2016), this was not the case for the majority of species, highlighting the impact on co-occurring natural stressors. Biotic factors associated with lower elevations and high species turnover had a disproportionate effect on seedlings transplanted downslope from TMCF communities, as species acclimated to cold and stressful conditions are unequipped to compete with lowland generalists or to survive defoliation by herbivores or pathogens. If this response is extrapolated across the community it suggests that by the end of the century LF and LMF species will increasingly outcompete TMCF species on the lower Andean slopes.

4.5 Conclusions

Our results suggest that if montane species lag behind their current thermal niche they will experience population declines as a result of mainly biotic factors rather than physiological limitations. However, the lack of a positive growth response in the absence of physiological stress suggests that these species are unable to acclimate to climatic conditions downslope, which in turn could impact on their ability to compete with co-occurring lowland species. With declines downslope, upslope shifts are required to maintain population levels, however our results demonstrate that seedling establishment at high elevations is limited by climatic factors. Increased warming may facilitate upslope movement, but growth rates may not be sufficient to prevent population declines. We therefore predict that TMCF species are at the greatest risk of population declines as a result of climate change, but that LF and LMF species have the acclimatory capacity to maintain population levels.

4.6 References

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4.7 Appendix

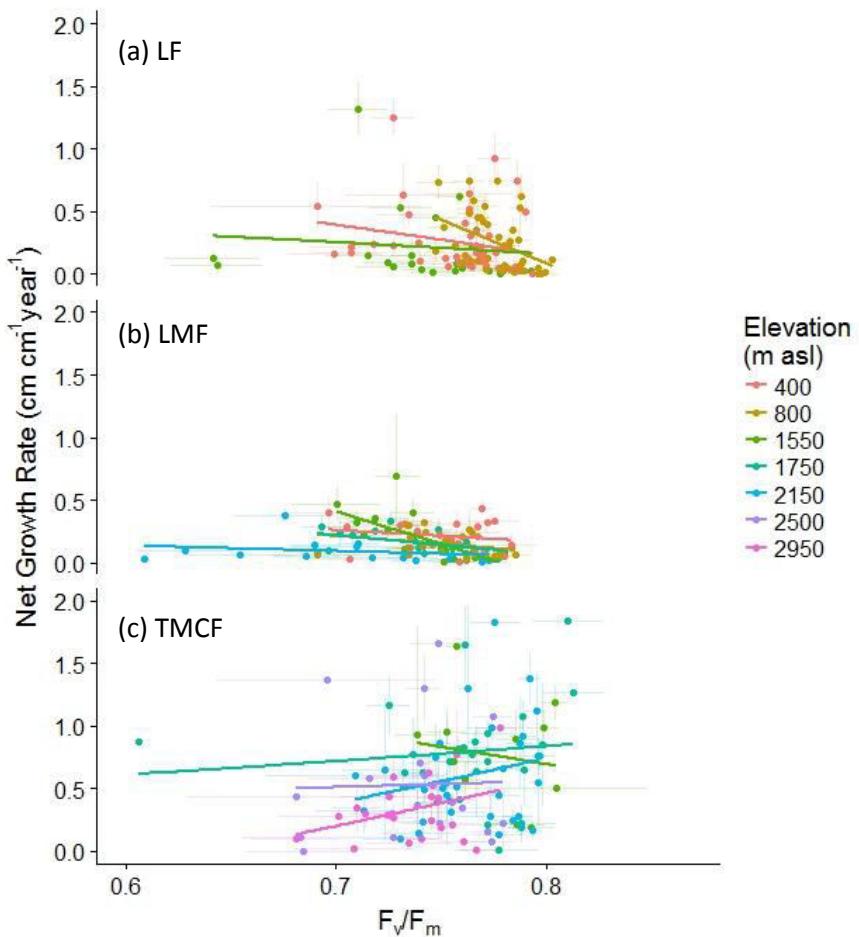


Figure 4-8. Relationship between seedling potential quantum efficiency (F_v/F_m) and net growth rate of surviving censused seedlings ($n=330$) of (a) lowland species, (b) lower montane species and (c) montane cloud forest species. F_v/F_m values are mean $\pm \text{SE}$ of values measured from Nov 2015 to May 2016. Net growth rate is the mean $\pm \text{SE}$ subplot-level values calculated as the increase in height from installation, June-August 2015, until October 2016 relative to the initial height for seedlings

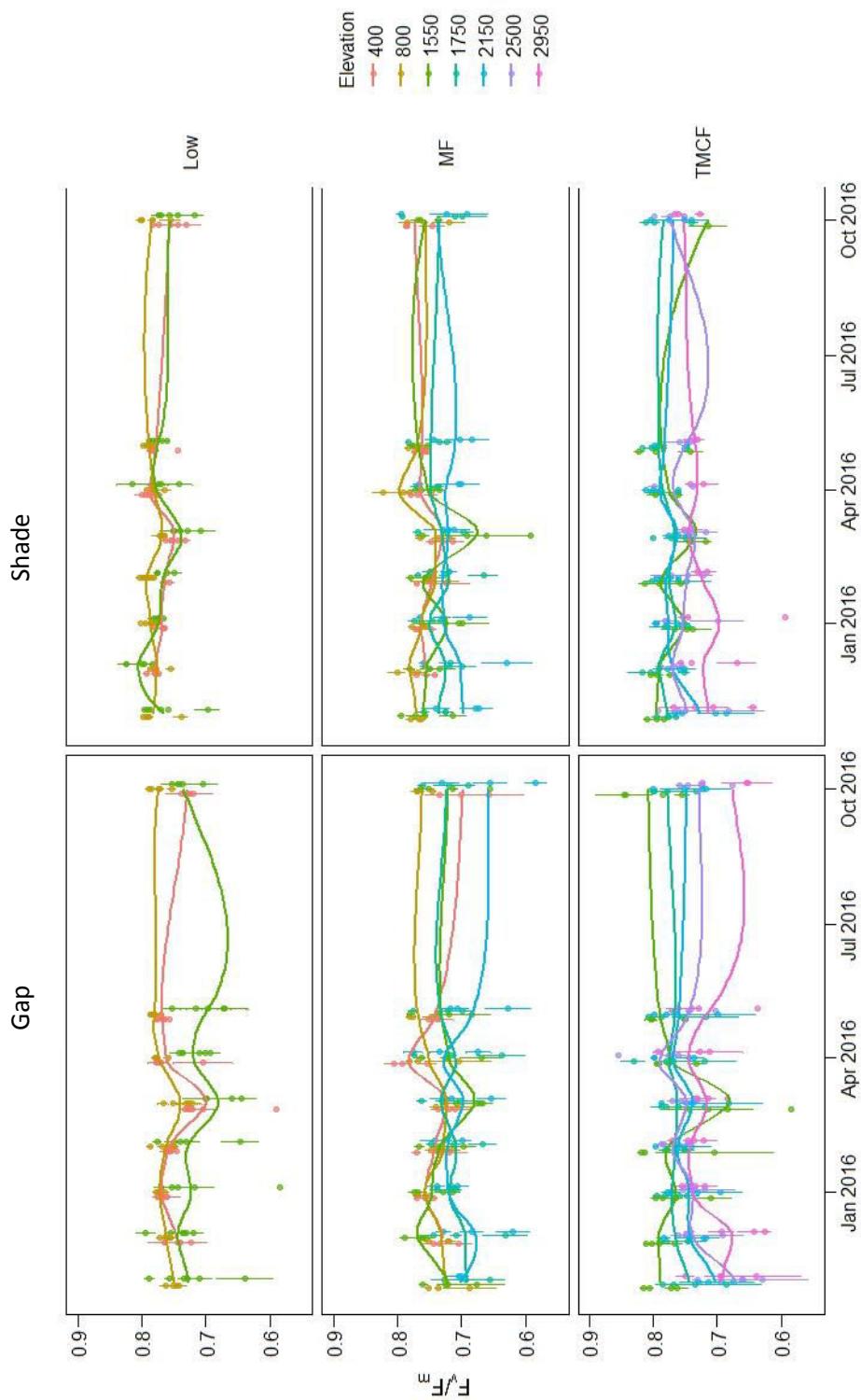


Figure 4-9. Seedling potential quantum efficiency (F_v/F_m) at each transplant elevation for lowland species (LF, top) /lower montane species (LMF, middle) and montane cloud forest species (TMCF, bottom) in gap and shade plots. Points are fitted mean (\pm SE).

Chapter 5. Pushing the limits: using an elevational gradient to experimentally test the acclimation of Neotropical tree seedlings to changing climate

5.1 Introduction

How Neotropical tree species respond to the changing climate is fundamentally important to our understanding of how forests will change in composition and function over the next century. Neotropical forests are of substantial interest because of their high levels of biodiversity, likely susceptibility to climate and land-use change (Báez et al. 2016) and importance in regional and global climate feedbacks (IPCC 2013).

Recent changes in species composition in the Andes of South America have been studied through the use of long-term observational datasets, tracking shifts in species' ranges through repeated census of permanent forest plots along elevational gradients for between 5 and 24 years (Feeley et al. 2011, Feeley et al. 2013, Duque et al. 2015). Studies in Peru, Costa Rica and Colombia show current upslope movements in tree populations of around $1.2\text{-}2.0 \text{ m}\cdot\text{y}^{-1}$, driven by mortality at the lower edge of the range and a lack of growth and recruitment upslope, in combination leading to range retractions and a greater risk of species loss (Feeley et al. 2011, Feeley et al. 2013, Duque et al. 2015). However, these few observational studies are limited in their capacity to predict future trends and to directly attribute the observed upslope migration to alterations in climate, so there is a need for experimentation to investigate the mechanisms behind these range shifts.

The mechanistic response of Neotropical tree species to changing climate can be studied and modelled by assessing the plasticity of functional traits related to productivity and survival. An individual plant's acclimatory response to a long-term alteration in climate may involve changes in leaf anatomy, physiology and biochemistry. When estimating changes in productivity, the acclimatory potential of traits underlying plant physiological responses, such as the maximum rate of Rubisco carboxylation (V_{cmax}), the rate of RuBP (Ribulose-1,5-bisphosphate) regeneration (J_{max}) and the ratio of J_{max} to V_{cmax} (Hikosaka et al. 2006), are important sources of uncertainty in current vegetation models (Huntingford et al. 2013, Rowland et al. 2015a, Seiler et al. 2015, Rogers et al. 2017). Similarly, understanding the acclimatory trends and relationships between photosynthetic capacity (V_{cmax} and J_{max}),

respiratory capacity and their associated leaf traits is essential in describing and estimating the overall carbon balance of ecosystems and consequent vegetation dynamics under climate change scenarios (Lavorel and Garnier 2002, Wright et al. 2005, Galbraith et al. 2010, Atkin et al. 2014, Slot et al. 2014).

Few studies have been carried out into the temperature-response of photosynthesis in tropical trees (Kattge and Knorr 2007, Slot and Winter 2016), however a recent study has shown a consistent *in situ* temperature-response of photosynthesis across 42 ecologically dissimilar species of tropical trees and lianas (Slot and Winter 2017). Under warming scenarios, tropical montane species have been found to decrease their base rates of photosynthetic capacity (J_{max25} and V_{cmax25}) and J_{max} : V_{cmax} ratio (Dusenge et al. 2015). The reciprocal relationship (increases in capacity under cooling scenarios) has been observed in some temperate species (Yamori et al. 2005), but this does not hold across global meta-analyses (Kattge and Knorr 2007). However, the decline in J_{max} : V_{cmax} with measurement temperature is consistent with previous extensive meta-analyses of temperate tree species (Medlyn et al. 2002, Kattge and Knorr 2007), due to the often lower temperature optimum (T_{opt}) of V_{cmax} than J_{max} (Long and Bernacchi 2003). However, the only reported values of the T_{opt} of photosynthetic capacity in the tropics showed that the T_{opt} of J_{max} for montane species was 5-7 °C lower than for V_{cmax} , which could alter the temperature response of the ratio (Vårhammar et al. 2015, Slot and Winter 2017). Because of this changes in V_{cmax} may be a better indication of shifts in temperature dependence than the J_{max} : V_{cmax} ratio (Hikosaka et al. 2006).

Tropical trees are considered to be close to their T_{opt} for photosynthesis, with some evidence of declines in overall productivity with historic climatic warming and drought (Clark 2004, Way and Oren 2010, Doughty 2011, Clark et al. 2013). Tropical montane species are hypothesised to be more sensitive to warming than their lowland counterparts based on evidence of historic elevational range shifts (Bush et al. 2004, Colwell et al. 2008, Morueta-Holme et al. 2015). This is supported by evidence from warming experiments, where high growth temperatures had a greater than expected effect on montane than lowland tree seedling growth and survival, suggesting local epigenetic constraints on the acclimatory potential of montane species (Cheesman and Winter 2013). Similarly, common garden experiments comparing native montane and lowland exotic species in Rwanda found that

montane tree species more often experienced sub-optimal leaf temperatures, with a lower acclimatory potential for the T_{opt} for J_{max} (Vårhammar et al. 2015).

Unlike photosynthesis, respiration has been found to readily acclimate to growth temperature (Atkin and Tjoelker 2003, Atkin et al. 2005). In general, and when compared at a standard temperature, species from cold habitats have higher rates of dark respiration (R_d) relative to V_{cmax25} and N_a (leaf nitrogen on an area basis) than warmer habitat species, indicating a greater allocation of resources to respiratory demands (Atkin et al. 2015). This trend is consistent with elevational studies in the tropics that show strong temperature-driven acclimation of R_d relative to more complex acclimatory patterns for photosynthetic capacity (van de Weg et al. 2012, Wittich et al. 2012). This asynchronous acclimatory potential could be especially significant for the more temperature-sensitive tropical montane cloud forest (TMCF) species, resulting in potential individual plant carbon deficits and mortality under climate warming scenarios (Campbell et al. 2007, Way and Oren 2010, Slot and Winter 2016).

A recent global analysis identified V_{cmax} , foliar mass to area ratios, N_a and P_a (leaf phosphorus on an area basis) as the most important trait predictors for variation in R_d (Atkin et al. 2015), however the relative importance of these traits across the tropics is unclear, especially due to the high climatic, edaphic and taxonomic diversity in these regions (Wright et al. 2005, Quesada et al. 2010, Rowland et al. 2016). Although N_a is considered to have a stronger relationship with photosynthetic and respiratory parameters at the global level (Reich et al. 1997, Reich and Oleksyn 2004, Wright et al. 2004, Atkin et al. 2015), P_a has often been found to be an equal or better predictor of R_d (Meir et al. 2001, Cavalieri et al. 2008, Slot et al. 2014, Rowland et al. 2016) and photosynthesis (Domingues et al. 2010, Cleveland et al. 2011, Domingues et al. 2015) in tropical trees.

Forest canopy and leaf traits have been observed to shift with increased elevation along the Andes-Amazon gradient used in this study. With increases in elevation of the forest, these features have been reported: reduced stature, gaps become more prevalent, NDVI of the canopy declines (Asner et al. 2013); tree growth declines (Robertson et al. 2010, Rapp et al. 2012), GPP, NPP and above-ground biomass decrease (Girardin et al. 2010, Girardin et al. 2014, van de Weg et al. 2014, Malhi et al. 2016); V_{cmax} : N_a (Bahar et al. 2016) and R_d : V_{cmax} ratios increase (van de Weg et al. 2012); and leaf traits become more conservative, increasing

in LMA (leaf mass per unit area) and decreasing in N_{mass} (leaf nitrogen on a mass basis) but not P_{mass} (van de Weg et al. 2009). The underlying drivers of the decline in productivity with elevation in tropical montane forest have been an open question for decades, with much focus on alterations in nutrient availability and cloud cover with elevation (Bruijnzeel and Veneklaas 1998, Tanner et al. 1998). However, the recent intensification of measurements has fed new modelling capacity, enabling for the first time a clear demonstration of the dominant roles of temperature and radiation in determining GPP (van de Weg et al. 2014) followed more recently still by an emphasis on the combination of radiation and leaf trait diversity (Fyllas et al. 2017).

However few ‘real world’ experiments have been carried out to assess the acclimatory potential of tropical trees species to future climate warming in the context of range-shift scenarios. Where experiments have taken place, they tend to focus on highly controlled warming (Cheesman and Winter 2013, Krause et al. 2013) or common garden experiments (Dusenge et al. 2015, Vårhammar et al. 2015), which do not take into account the additional challenges of multiple stressors; the frequency of competitors, altered trophic interactions, and the shifts in the availability of nutrients, light and water. Elevation gradients provide an opportunity to investigate these more complex interactions (Malhi et al. 2010, Sundqvist et al. 2013, Parmesan and Hanley 2015, Slot and Winter 2016).

Here we report on an experimental approach to understand the limits of acclimation to temperature; by transplanting tree seedlings along an elevation gradient away from the centre of each species’ local distribution by the equivalent of ± 4 °C. We used a 3000 m study transect stretching from the Andean treeline to the lowland Amazon in Peru, hereafter referred to as the Kosñipata gradient. The goal was to simulate upslope migration and climate warming for tree seedlings of four lowland and mid elevation forest species (LMF) and four tropical montane cloud forest species (TMCF). We investigated changes amongst species groups in their leaf-level ecophysiological and anatomical traits in response to transplantation. We hypothesised that: 1) TMCF species are more susceptible than LMF species to warming-induced mortality and reductions in photosynthetic capacity; H2) photosynthetic capacity (J_{max} , V_{cmax}) and J_{max} : V_{cmax} ratio will decrease with simulated warming; H3) R_d will readily acclimate to simulated warming and upslope transplantation; H4) the R_d : V_{cmax} ratio will be higher in TMCF species and will increase with upslope transplantation; H5) photosynthetic capacity and R_d will differ in relation to N_a , LMA, LDMC and possibly P_a .

5.2. Methodology

5.2.1 Study sites

Table 5-1 Summary of environmental variables for plots at each transplant elevation at the Kosñipata gradient measured over a week during June-July 2016; where plot data not available, data are provided from nearby 1ha study plots ^a(Malhi et al. 2016), ^b(Nottingham et al. 2015); na, where data are unavailable. Values are mean (\pm SD).

| Site code | Pantiacolla | San Pedro 2 | Trocha Union VII | Trocha Union III |
|--------------------------------------|-------------------|-------------------|--------------------------|--------------------------|
| Elevation (m asl) | 426 (5) | 1487 (80) | 2140 (25) | 2900 (15) |
| Longitude | -12.65250 | -13.04774 | -13.07729 | -13.10825 |
| Latitude | -71.23777 | -71.53608 | -71.56247 | -71.59618 |
| Slope (°) | na | 27.1 ^a | 23.0 (9.4) | 18.8 (7.9) |
| Aspect (°) | na | 125 ^a | 83.2 (68.2) | 117.3 (86.6) |
| Precipitation (mm yr ⁻¹) | 2366 ^a | 5302 ^a | 1827 ^a | 1776 ^a |
| MAT (°C) | 23.5 ^a | 18.8 ^a | 17.4 ^a | 11.8 ^a |
| Total Soil C (%) | 3.42 (1.3) | 11.05 (6.6) | 37 (4.8) ^b | 27.1 (5.5) ^b |
| Total Soil N (%) | 0.16 (0.1) | 0.61 (0.5) | 2.0 (0.24) ^b | 1.57 (0.21) ^b |
| Total Soil P (mg P g ⁻¹) | 0.80 (0.14) | 1.59 (0.4) | 0.71 (0.10) ^b | 0.92 (0.13) ^b |
| <i>T_{leaf}</i> (°C) | 29.7 (1.5) | 23.8 (1.9) | 21.5 (2.9) | 16.9 (1.4) |
| <i>T_{soil}</i> (°C) | 22.52 (0.25) | 17.18 (0.35) | 15.34 (0.57) | 10.59 (0.49) |
| Soil Moisture (%) | 42.4 (8.6) | 43.1 (5.9) | 40.5 (9.6) | 58.7 (11.3) |
| Canopy Openness (%) | | | | |
| Open | 5.3 (2.8) | 13.9 (3.4) | 23.4 (8.2) | 19.8 (8.7) |
| Shade | 4.8 (1.2) | 11.5 (2.1) | 14.2 (2.8) | 14.8 (2.9) |

MAT, mean annual air temperature; *T_{leaf}*, leaf temperature measured by LiCor Li-6400XT Portable Photosynthesis Systems (Li-Cor Inc., Lincoln, NE, USA); *T_{soil}*, daytime soil temperature

The four transplant sites were located along an Andes-Amazon gradient on the eastern slopes of the Peruvian Andes. The montane sites were located along a 10 km stretch of the Kosñipata valley; the top of the cloud immersion zone (2900 m asl); within the cloud immersion zone (2150 m asl); and at the lower edge of the cloud base (1500 m asl) (Girardin et al. 2013). The lowest site (450 m asl) was located in lowland forest at the base of the Pantiacolla range, 50 km along from the Kosñipata valley. Mean annual temperature decreases with increased elevation at a wet adiabatic lapse rate of 4.4 °C km⁻¹, whereas precipitation peaks at the lower edge of the cloud base and solar radiation is reduced in areas of high cloud occurrence (Rapp and Silman 2012, Malhi et al. 2016). Environmental variables recorded during the measurement period show a linear decrease in soil temperature with elevation, high levels of soil moisture across all sites and increasing canopy openness from lowland to montane sites (Table 5-1).

5.2.2 Transplant experiment

The experiment was carried out on locally abundant species of the lowland and sub-montane forest (LMF) and tropical montane cloud forest (TMCF) found along the Kosñipata gradient in southern Peru. The LMF species were the closely related (Bacon et al. 2016) *Iriartea deltoidea* Ruiz & Pav., and *Dictyocaryum lamarckianum* (Mart.) H.Wendl., in addition to *Guatteria glauca* Ruiz & Pav. and *Tapirira cf. guianensis* Aubl. The selected TMCF species were *Clethra revoluta* (Ruiz & Pav.) Spreng., *Clusia thurifera* Planch. & Triana, *Alzatea verticillata* Ruiz & Pav. and *Hedyosmum goudotianum* Solms. These species were selected based on their estimated elevational ranges that centre around three ‘home’ elevations; 450 m asl and 1500 m asl for LMF species and 2150 m asl for TMCF species (Figure 5-1). Species were selected with different local and Neotropical elevational range widths in order to incorporate species with greater (e.g. *T. guianensis*) and lesser (e.g. *A. verticillata*) estimated acclimatory potential.

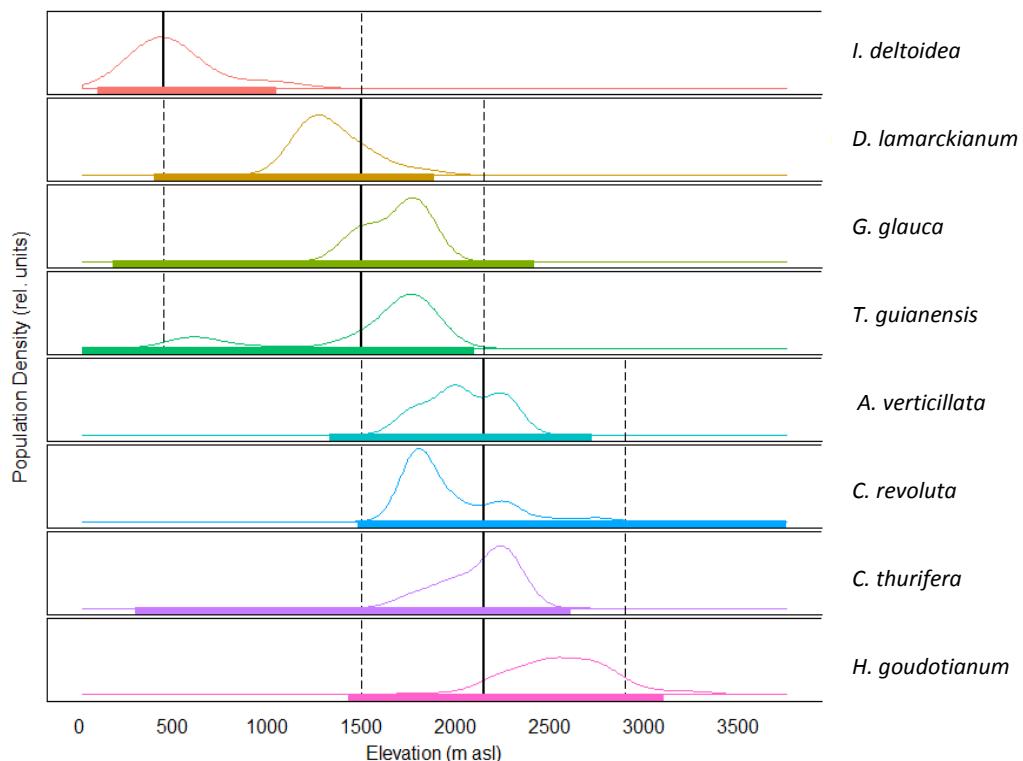


Figure 5-1 Population density plot of adult tree species, based on composition of 1ha permanent plots (Feeley et al. 2011). Thickened horizontal line; species Neotropical elevational range, estimated from the Neo Trop Tree database (Oliveira-Filho 2017), Vertical lines; transplant elevations, Solid lines; ‘home’ transplant elevation.

Seeds and seedlings of the target species were collected and established within a 200 m elevational band of the ‘home’ transplant distribution; seedlings were grown in container plugs within herbivore-exclusion tents for at least two months until the experiment was

installed. Approximately 60 seedlings of each species were selected at random from the collected individuals and transported in ‘home’ soil to each transplant elevation (Figure 5-1). Seedlings were installed from June until August 2015 at each transplant elevation; planted in well-draining plastic pots (3.77 l) containing local soil and buried to local soil level. Seedlings were planted in mixed species groups at six sites at each transplant elevation. Each site contained two plots, one classified as having a closed canopy and the other a more open canopy. The difference in light environment between these open and closed canopy plots was found to be most distinct at the montane sites (summarised in Table 5-1), which is representative of the changing forest canopy structure with increased elevation (Asner et al. 2013, Girardin et al. 2014). Survival rate was calculated as the proportion of transplanted individuals that survived until May 2016.

After one year of transplantation and where survival rates allowed, twelve seedlings per species were selected at each transplant elevation, with six from each light environment. Seedlings were selected from a range of sites at each elevation, with some sites unrepresented due to low survival rates. The seedlings were transported in their pots from the field site to the field station for measurement. The effects of transportation on the health of individuals was assessed to be negligible, as the ecophysiological stress (F_v/F_m) of individuals did not differ significantly before and after transplantation.

5.2.3 Gas-Exchange Measurements

Gas exchange measurements were carried out on fully expanded leaves (selecting leaves with no visible damage), between 09:00 and 14:00, using a LiCor Li-6400XT Portable Photosynthesis Systems (Li-Cor Inc., Lincoln, NE, USA) fitted with an LED light source. Measurements of light-saturated photosynthesis (A_n) were made at 12 intercellular CO₂ concentrations (C_i) and at a pre-determined saturating photon flux density of 1500 μmol m⁻² s⁻¹. Curves were initiated once leaf temperature, humidity and stomatal conductance (g_s) had stabilised for 5 minutes. Measurements were made at ambient leaf temperatures (Table 5-1) and relative humidity between 60- 80%.

A curve fitting protocol described in Domingues et al. (2010) and carried out in the “R” environment , was used to calculate V_{cmax} and J_{max} on an area basis. A_n rates were adjusted to take into account CO₂ diffusion through the leaf-chamber gasket. To allow comparisons of underlying base rates, values were adjusted to a common temperature 25 °C, using

temperature conversion coefficients taken from Sharkey et al. (2007). Data quality was checked visually and measurements were excluded where stomatal conductance dropped below 0.03 mol H₂O m⁻² s⁻¹, thus we retained 181 out of 216 A-C_i curves.

After each A-C_i curve was completed, the leaf was dark-adapted for 20-30 minutes to ensure reliable measurements of leaf dark respiration (R_d) (Rowland et al. 2016). R_d measurements were made at ambient leaf temperature (T_{leaf}) and were adjusted to a common temperature of 25 °C, according to equation 1 (Atkin and Tjoelker 2003, Rowland et al. 2015b) with a Q_{10} of 2.2 (Meir et al. 2001, Atkin et al. 2005).

$$R_{dark,25} = R_{dark} \cdot Q_{10} \left(\frac{25 - T_{leaf}}{10} \right) \quad (\text{Equation 1})$$

5.2.4 Leaf Traits

Immediately after gas-exchange measurements, leaves were detached from the seedling and were rehydrated (Domingues et al. 2010). Leaf weight and leaf thickness were determined for the rehydrated leaf lamina (Pérez-Harguindeguy et al. 2013). A digital image of the flattened leaf surface was taken against a known scale (Nikon Coolpix A10) and analysed using ImageJ (version 1.48v, NIH, USA) to calculate the leaf area. Leaves were subsequently dried to a constant mass using silica gel and weighed (van de Weg et al. 2012). Leaf dry matter content ($LDMC$ mg g⁻²) and leaf mass per unit area (LMA g m⁻²) were then calculated from area and mass measurements. The leaf matter was analysed for nitrogen concentrations using an elemental analyser (Carlo Erba, NA 2500) and phosphorus concentrations were determined by the molybdenum blue method (Allen et al. 1974) using an auto-analyser (Bran and Luebbe, Auto Analyser III).

5.2.5 Statistical Analysis

To account for the nested experimental design and differences in sample size at each transplant elevation (due to differing survival rates between species), mixed effect models were used to identify factors with the most predictive power for modelling the response of seedlings to transplantation. Mixed effects modelling was performed following standard methods (Burnham and Anderson 2003, Zuur et al. 2009), using the lme4 package (Bates et al. 2015) in R (Version 3.2.3, R Core Team 2015).

Seedling survival of LMF and TMCF species in relation to transplant effect was analysed by mixed effects logistic regression, with transplant elevation (downslope, home or upslope) and light environment (open or shade) as fixed effects, plus species and subplot nested within plot elevation as random effects. Odds-ratio (OR) and 95% confidence intervals of treatment effects on seedling survival were calculated from logistic regression model estimates of effect sizes (Lüdecke 2016), where values close to zero indicate lower odds and values above one indicate higher odds of survival relative to the baseline.

Models were also used to test the effect of transplantation on seedling V_{cmax} , J_{max} , R_d , and J_{max} : V_{cmax} , including transplant elevation (downslope, home or upslope), light environment (open or shade) and species type (LMF or TMCF) as fixed effects and species and subplot nested within plot elevation as random effects. Fixed effect terms were removed sequentially and the relative fit of each model was assessed using Akaike Information Criterion (AIC and sample-size adjusted AICc). Likelihood ratio test (LRT) statistics were used to compare full models to each other and to a null model, which contained only random effect terms. In this way, the significance of fixed effect terms were ascertained and the final best-fit model was selected.

Standardised major axis estimation was used to determine the bivariate relationships between log-transformed photosynthetic and anatomical leaf traits ($\alpha=0.05$), using the robust approach of the smatr package (Warton et al. 2006, Warton et al. 2012) in R (Version 3.2.3, R Core Team 2015). The values were log-transformed to ensure normality and heterogeneity of residuals. Differences in the slope and elevation of best fit lines were tested between transplant elevations and species groups. To quantify the spectrum of leaf- trait strategies employed by the study species, principal component analysis (PCA) was used and summarised (Horikoshi and Tang 2016, Tang et al. 2016).

5.3 Results

5.3.1 Survival

After one year, surviving seedlings differed in their visual quality: 6% of seedlings (notably individuals of *C. revoluta* and *H. godotianum* between 1500 and 2150 m asl) grew substantially, reaching 50 cm in height, whereas 59% grew to less than 20 cm in height, with some individuals showing evidence of herbivore and pathogen damage (especially *D. lamarckianum* and *I. deltoidea*) (appendix 1, Figure 5-8).

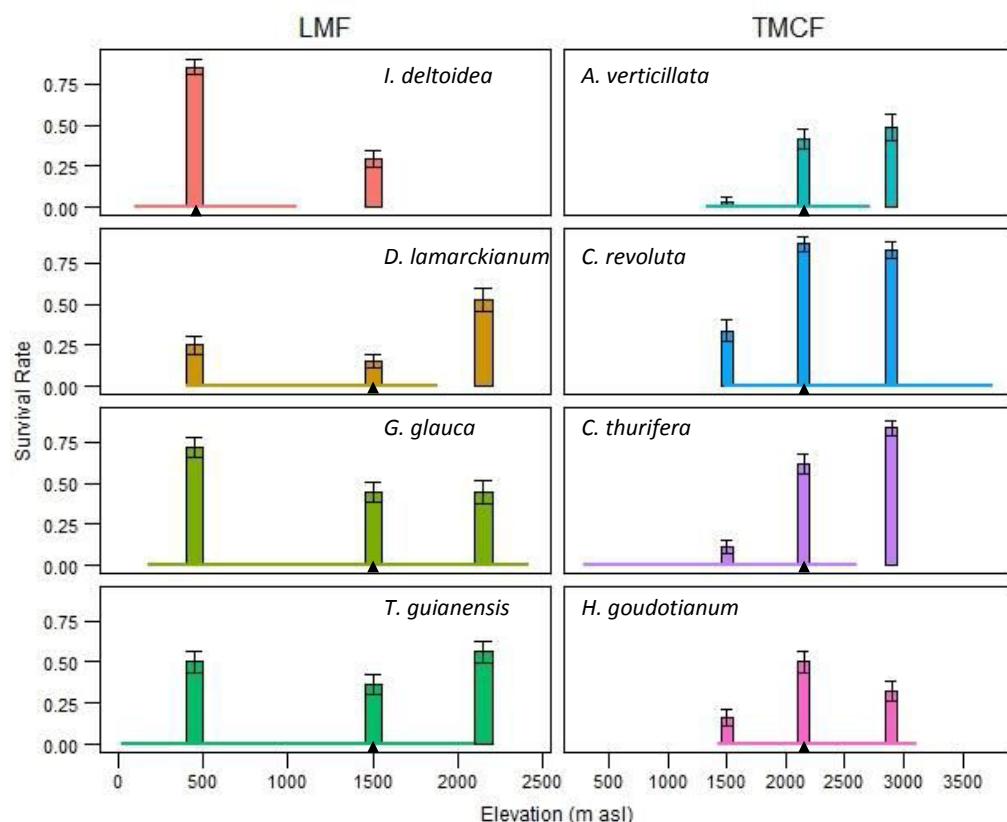


Figure 5-2 Survival rate of seedlings after one year at each transplant elevation for each species \pm SE. LMF, low-mid forest species; TMCF, tropical montane cloud forest species; black triangles, home transplant elevation; Horizontal line; species Neotropical elevational range, estimated from the Neo Trop Tree database (Oliveira-Filho 2017).

Survival rates varied between species from 0.15- 0.87 at 'home' elevations (Figure 5-2) with a mean survival rate of $0.46 \pm SD 0.25$ across all species and transplant sites. Survival rates were lowest at San Pedro 1500 ($0.24 \pm SD 0.14$) and highest at the uppermost montane site, TRU-03, ($0.62 \pm SD 0.26$). Species groups had similar overall survival rates of $0.46 \pm SD 0.20$ for LMF species and $0.46 \pm SD 0.29$ for TMCF species. The largest disparity in survival was seen when transplanting TMCF species downslope to San Pedro 1500; resulting in a reduction

in seedling survival of 73%. Species survival was not constrained to transplant elevations within a species' estimated local and neotropical elevational ranges (Figure 5-2), although the decline in TMCF species survival rates downslope occurred close to the estimated range edge of three species (*A. verticillata*, *C. revoluta* and *H. goudotianum*).

The modelled effect of transplantation on seedling survival differs between LMF and TMCF species; transplanting upslope did not have a significant effect on the odds-ratio (OR) of seedling survival relative to home elevation for either species group, however transplanting downslope was twice as likely to result in mortality for TMCF (OR= 0.09) than LMF species (OR= 0.2) (Figure 5-3). The survival response of TMCF species to transplantation was more consistent than LMF species, which had a larger range of species' responses.

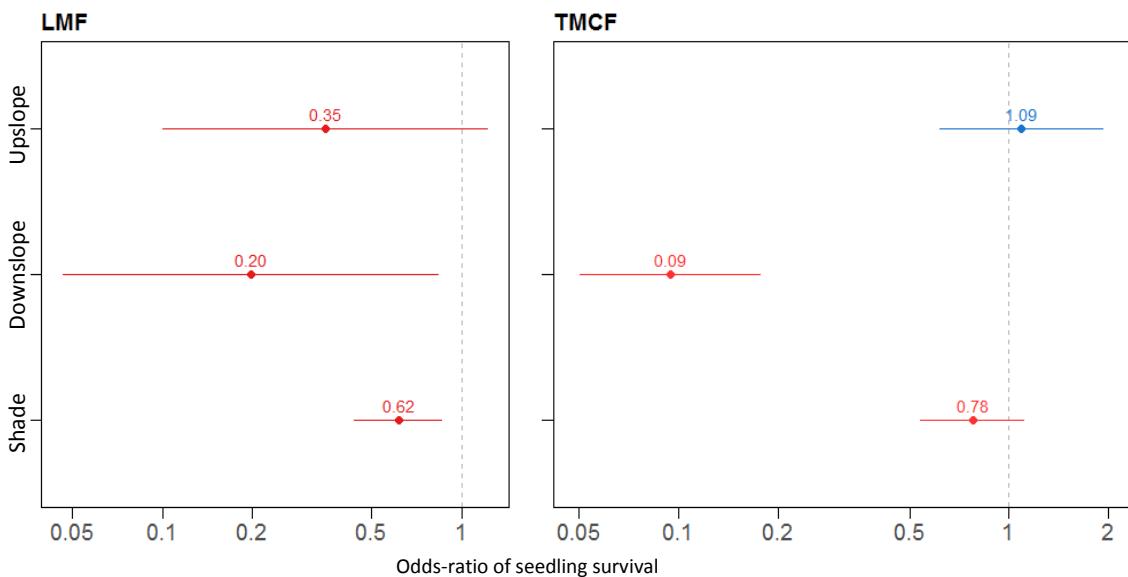


Figure 5-3 Odds-ratio (OR) and 95% confidence interval of treatment effects on seedling survival relative to the treatment baseline (dashed line). Fixed effects were transplant elevation (upslope or downslope) relative to home elevation and light treatment (shade) relative to open plot. Red line, negative effect; blue line, positive effect; overlap with dashed line, non-significant result; LMF, low-mid forest species; TMCF, tropical montane cloud forest species.

The best fit model for TMCF species was not improved by including light treatment as a factor alone (LRT, $\chi^2=1.88$, $p=0.170$), however when included alongside an interaction term (light* transplant) the fit was improved (LRT, $\chi^2=7.21$, $p=0.027$). This is seen as an additional decrease in seedling survival at downslope sites under shaded conditions. The best fit model for LMF species included light treatment (LRT, $\chi^2=7.97$, $p=0.005$) but no interaction term (LRT, $\chi^2=5.82$, $p=0.054$), indicating a significant decrease in survival at shaded sites across all elevations.

5.3.2 Photosynthetic capacity (V_{cmax25} and J_{max25})

Photosynthetic capacity varied between species and transplant sites, ranging from 9.4 to 46.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for mean V_{cmax25} and from 24.1 to 87.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for mean J_{max25} . The lowest mean values were recorded for the two LMF Areceaceae species (*I. deltoidea* and *D. lamarckianum*) and the highest values for the TMCF species (*H. goudotianum* and *C. revoluta*) (Figure 5-4). Photosynthetic capacity increased with site elevation, peaking at 2140 m asl (TRU-07) and then decreased at the highest elevation site (TRU-03).

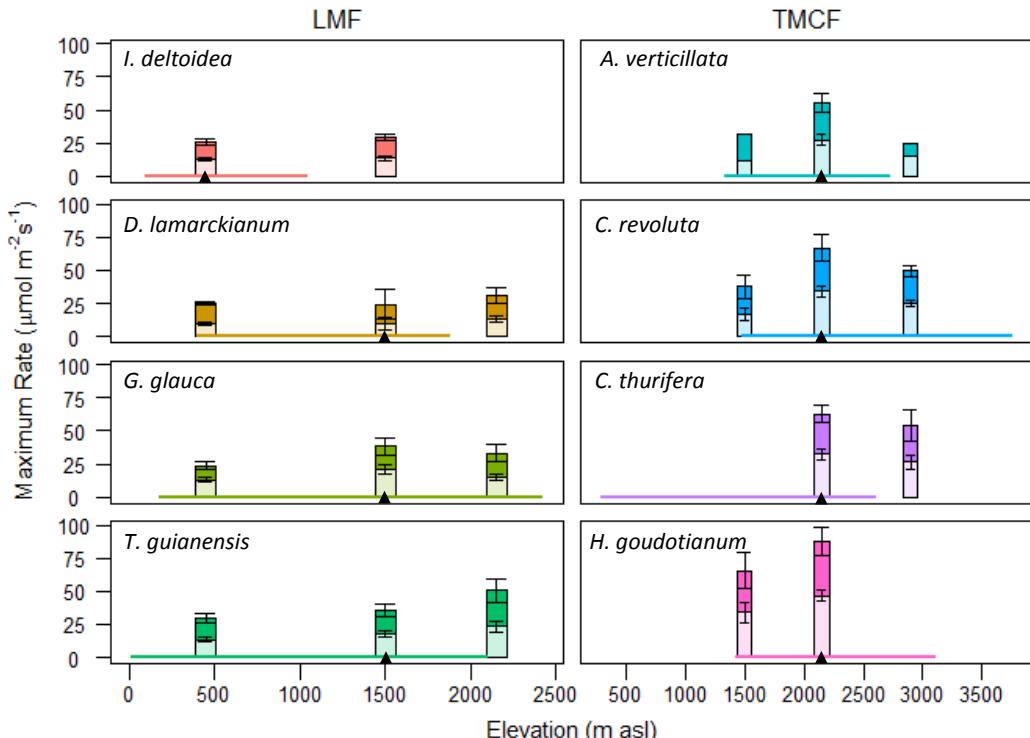


Figure 5-4 Mean and standard error of the maximum rates of RuBP regeneration (J_{max25}) and Rubisco carboxylation (V_{cmax25}) adjusted to 25°C. Dark-coloured wide bars, J_{max25} ; inset light-coloured narrow bars, V_{cmax25} ; LMF, low-mid forest species; TMCF, tropical montane cloud forest species; black triangles, home transplant elevation; Horizontal line; species Neotropical elevational range, estimated from the Neo Trop Tree database(Oliveira-Filho 2017).

Variations in V_{cmax25} and J_{max25} could be accounted for by differences between TMCF and LMF species (LRT; $\log (V_{cmax25})$, $\chi^2=9.93$, $P < 0.01$; $\log (J_{max25})$, $\chi^2=11.55$, $P < 0.001$, Table 5-4), with mean values for TMCF species about twice that of LMF species (Table 5-3). Shade treatments had a significant effect on photosynthetic capacity (LRT; $\log (V_{cmax25})$, $\chi^2=16.27$, $P < 0.001$; $\log (J_{max25})$, $\chi^2=17.21$, $P < 0.001$, Table 5-4), decreasing V_{cmax25} and J_{max25} by approximately 28% relative to open sites. V_{cmax25} and J_{max25} differed between transplant elevations (LRT; $\log (V_{cmax25})$, $\chi^2=9.65$, $P < 0.01$; $\log (J_{max25})$, $\chi^2=6.29$, $P < 0.05$, Table 5-4), but to different extents (Table 5-4, Figure 5-4). Transplanting downslope significantly reduced V_{cmax25} ($P < 0.01$) and

J_{max25} ($P < 0.05$) by approximately 42% and 34% respectively, whereas transplanting upslope reduced values by about 15%, which was not a significant reduction. These changes in baseline photosynthetic capacity resulted in significant reductions in ambient measurements of V_{cmax} at upslope elevations for all species ($P < 0.01$) and reductions downslope for TMCF species only ($P < 0.01$). Ambient J_{max} did not differ between elevations for LMF species, but significantly decreased upslope ($P < 0.01$) and downslope for TMCF species ($P < 0.05$) (Table 5-3).

5.3.3 Respiration (R_{d25})

R_{d25} increased with elevation across all species except for *I. deltoidea*, where no change was observed. TMCF species had double the respiratory capacity of LMF species (Table 5-3), therefore explaining a significant amount of the variation in R_{d25} (LRT; $\log(R_{d25})$, $\chi^2=10.96$, $P < 0.001$). The shade treatment reduced R_{d25} by 27%, consistent with the reduction in V_{cmax25} and J_{max25} (appendix, Table 5-4). Seedling R_{d25} responded significantly to transplantation in both directions (LRT; $\log(R_{d25})$, $\chi^2=7.40$, $P < 0.05$); transplanting downslope decreased R_{d25} by about 39%, whereas transplanting upslope increased R_{d25} by an estimated 40%. This resulted in the maintenance of ambient R_d across all transplant elevations for both species groups (Table 5-3).

5.3.4 Leaf traits

Leaf mass per unit area (LMA) was highly species specific; the LMF species *G. glauca* had the lowest mean LMA ($36.5 \pm 7.6 \text{ g m}^{-2}$) and the TMCF species *C. thurifera* and *A. verticillata* the greatest ($75.0 \pm 25.8 \text{ g m}^{-2}$ and $70.5 \pm 23.3 \text{ g m}^{-2}$). TMCF species had a greater variability in LMA than LMF species (Table 5-3), but there was no significant difference between the groups (LRT; $\log(LMA)$, $\chi^2=0.33$, $P > 0.1$). Shading treatment had a significant effect on LMA (LRT; $\log(LMA)$, $\chi^2=14.36$, $P < 0.001$), reducing values by 10%. Transplantation downslope reduced LMA overall by 5% (Table 5-3), but this was not a significant decrease (LRT; $\log(LMA)$, $\chi^2=2.575$, $P > 0.1$), reflecting the variability in within-species response to transplantation. *C. thurifera* and *A. verticillata* leaves were approximately twice as thick as the LMF species, but with about half the leaf dry matter content ($LDMC$). The TMCF group overall had an estimated 30% lower $LDMC$ than the LMF species (Table 5-3) (LRT; $\log(LDMC)$, $\chi^2=5.8255$, $P < 0.05$). Similarly to LMA , shade treatment reduced $LDMC$ by 5% (LRT; $\log(LDMC)$, $\chi^2=5.82$, $P < 0.01$), but there was no trend with transplantation (LRT; $\log(LDMC)$, $\chi^2=1.66$, $P > 0.1$).

Table 5-3 Mean values and standard deviation of temperature adjusted (left) and ambient (right) physiological leaf-trait for LMF and TMCF species at each transplant elevation

| | <i>N</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | J_{max25} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | $J_{max25}:V_{cmax25}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | R_{d25} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | $V_{cmax25:N_a}$ ($\mu\text{mol gN}^{-1} \text{s}^{-1}$) | A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | | | |
|-------------|--|---|--|---|---|---|--|---|---|--------------------|--------------------|--------------------|
| LMF | 311 | 15.3 ± 8.3 | 32.6 ± 17.9 | 2.17 ± 0.42 | 0.37 ± 0.22 | 0.03 ± 0.02 | 17.3 ± 11.0 | 3.30 ± 1.88 | 8.43 ± 3.30 | 15.2 ± 7.8 | 31.8 ± 15.0 | 0.40 ± 0.22 |
| Home | 118 | 16.6 ± 8.3 | 33.2 ± 16.0 | 2.04 ± 0.36 | 0.33 ± 0.19 | 0.02 ± 0.02 | 18.5 ± 9.0 | 3.70 ± 1.85 | 8.81 ± 3.55 | 17.4 ± 9.1 | 34.4 ± 17.0 | 0.33 ± 0.20 |
| Down | 88 | 12.3 ± 5.3 | 26.3 ± 9.4 | 2.24 ± 0.44 | 0.27 ± 0.14 | 0.02 ± 0.01 | 14.2 ± 6.2 | 3.52 ± 1.47 | 9.11 ± 2.77 | 18.1 ± 7.2 | 34.2 ± 11.1 | 0.40 ± 0.22 |
| Up | 105 | 16.4 ± 9.5 | 36.4 ± 22.3 | 2.21 ± 0.43 | 0.48 ± 0.26 | 0.04 ± 0.03 | 18.3 ± 14.2 | 2.85 ± 2.09 | 7.67 ± 3.37 | 11.7 ± 5.7 | 28.5 ± 15.3 | 0.45 ± 0.23 |
| TMCF | 316 | 30.6 ± 16.1 | 60.1 ± 31.5 | 2.00 ± 0.37 | 0.72 ± 0.39 | 0.03 ± 0.02 | 34.2 ± 15.6 | 4.89 ± 3.13 | 11.46 ± 5.67 | 22.7 ± 14.7 | 48.0 ± 27.4 | 0.54 ± 0.33 |
| Home | 142 | 35.5 ± 15.5 | 68.6 ± 31.9 | 1.95 ± 0.31 | 0.71 ± 0.28 | 0.02 ± 0.02 | 38.4 ± 15.5 | 6.19 ± 2.85 | 13.66 ± 5.30 | 28.0 ± 14.2 | 56.4 ± 26.7 | 0.56 ± 0.36 |
| Down | 39 | 24.3 ± 19.0 | 49.9 ± 33.8 | 2.26 ± 0.48 | 0.46 ± 0.27 | 0.03 ± 0.02 | 25.1 ± 17.6 | 4.28 ± 3.72 | 11.39 ± 6.58 | 21.5 ± 16.5 | 46.4 ± 31.5 | 0.48 ± 0.24 |
| Up | 135 | 25.1 ± 11.1 | 50.0 ± 23.1 | 1.90 ± 0.30 | 0.90 ± 0.52 | 0.04 ± 0.03 | 31.9 ± 10.4 | 2.92 ± 1.80 | 7.61 ± 3.17 | 12.3 ± 6.5 | 30.9 ± 15.1 | 0.52 ± 0.36 |

LMF; low-mid forest species; TMCF, tropical montane cloud forest species; N, number of surviving seedlings; V_{cmax25} , the maximum rate of Rubisco carboxylation adjusted to 25 °C; J_{max25} , the maximum rate of Rubisco regeneration adjusted to 25 °C; $J_{max25}:V_{cmax25}$, the ratio of the maximum rate of Rubisco carboxylation and the maximum rate of Rubisco regeneration, adjusted to 25 °C; R_{d25} ; V_{cmax25} , ratio of dark respiration and the maximum rate of Rubisco carboxylation adjusted to 25 °C per gram of leaf Nitrogen; A_{sat} , area-based net photosynthesis measured in saturating light at ambient temperature, adjusted to a common C_i (315 $\mu\text{mol mol}^{-1}$); A_{max} , area-based net photosynthesis measured in saturating light, CO_2 (1500 $\mu\text{mol mol}^{-1}$) at ambient temperature; V_{cmax} , the maximum rate of Rubisco carboxylation at ambient temperature; J_{max} , the maximum rate of Rubisco regeneration at ambient temperature; R_d , area-based respiration measured in the dark at ambient temperature.

Table 5-3 Mean values and standard deviation of anatomical leaf-trait for LMF and TMCF species at each transplant elevation.

| | <i>LDMC</i> (mg g ⁻¹) | <i>LMA</i> (g m ⁻²) | <i>LTh</i> (mm) | <i>N_a</i> (g m ⁻²) | <i>P_a</i> (g m ⁻²) |
|-------------|-----------------------------------|---------------------------------|--------------------|---|---|
| LMF | 307 ± 60 | 49.5 ± 15.7 | 0.18 ± 0.05 | 0.96 ± 0.29 | 0.077 ± 0.032 |
| Home | 299 ± 67 | 51.5 ± 16.7 | 0.18 ± 0.05 | 0.95 ± 0.23 | 0.083 ± 0.027 |
| Down | 322 ± 66 | 43.4 ± 12 | 0.19 ± 0.04 | 0.87 ± 0.13 | 0.055 ± 0.016 |
| Up | 300 ± 46 | 52.1 ± 16 | 0.18 ± 0.05 | 1.04 ± 0.39 | 0.087 ± 0.038 |
| TMCF | 206 ± 85 | 57.1 ± 23.1 | 0.34 ± 0.17 | 0.94 ± 0.29 | 0.065 ± 0.033 |
| Home | 233 ± 102 | 61.3 ± 23.4 | 0.36 ± 0.17 | 0.99 ± 0.31 | 0.062 ± 0.033 |
| Down | 200 ± 70 | 46.9 ± 15.4 | 0.24 ± 0.08 | 0.96 ± 0.28 | 0.064 ± 0.025 |
| Up | 203 ± 97 | 56.9 ± 25.5 | 0.36 ± 0.2 | 0.86 ± 0.22 | 0.079 ± 0.044 |

LMF; low-mid forest species; TMCF, tropical montane cloud forest species; LDMC, leaf dry matter content; LMA, leaf mass per unit area; LTh, leaf thickness; N_a , leaf nitrogen content on an area basis; P_a , leaf phosphorus content on an area basis.

Leaf phosphorus content (P_a) varied between 0.05- 0.11 g m⁻² across species, and did not change with any of the fixed effect treatments, instead 60% of the variation in P_a was explained by the random components of subplot and species. Leaf nitrogen content (N_a) ranged from 0.3 to 1.4 g m⁻² across species, but did not differ between species groups (LRT; $\log(N_a)$, $\chi^2=0.006$, $P > 0.1$) (Table 5-3). Light treatment had a significant effect on leaf nitrogen content (LRT; $\log(N_a)$, $\chi^2=16.7$, $P < 0.001$), with shade treatment decreasing N_a by 15%. Including an interaction term between species group and transplanting away from home elevation (down and up combined) significantly improved the model (LRT; $\log(N_a)$, $\chi^2=5.3$, $P < 0.05$), where TMCF species had 26% lower N_a away from home elevation, but LMF species had 26% more (Table 5-3).

5.3.5 Bivariate relationships in response to transplant treatment

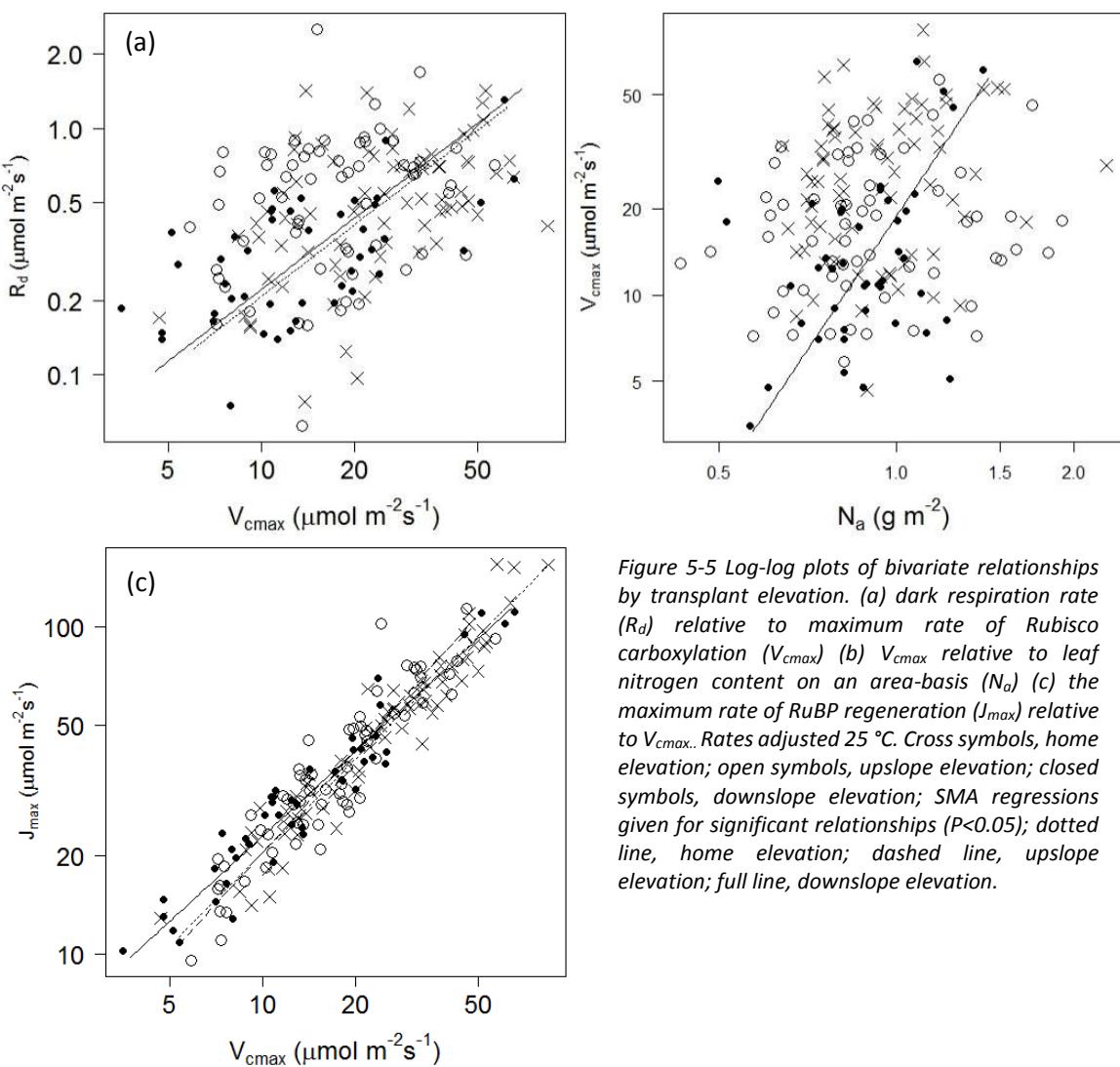


Figure 5-5 Log-log plots of bivariate relationships by transplant elevation. (a) dark respiration rate (R_d) relative to maximum rate of Rubisco carboxylation (V_{cmax}) (b) V_{cmax} relative to leaf nitrogen content on an area-basis (N_a) (c) the maximum rate of RuBP regeneration (J_{max}) relative to V_{cmax} . Rates adjusted 25 °C. Cross symbols, home elevation; open symbols, upslope elevation; closed symbols, downslope elevation; SMA regressions given for significant relationships ($P < 0.05$); dotted line, home elevation; dashed line, upslope elevation; full line, downslope elevation.

Photosynthetic capacity parameters were strongly positively related (SMA, $R^2=0.904$, $P= <0.001$), and changed in proportion with each other at all transplant elevations except for the TMCF downslope site, where there was a lesser reduction in J_{max25} relative to V_{cmax25} (LRT; $\log (J_{max25}: V_{cmax25})$, $\chi^2=7.43$, $P <0.05$), resulting in a 16% increase in the $J_{max25}: V_{cmax25}$ ratio. The slope of the best fit lines differed significantly between transplant sites (LRT; $\chi^2=6.7$, $P<0.05$), with $J_{max25}:V_{cmax25}$ decreasing with photosynthetic capacity at downslope elevations relative to the home elevation, whereas $J_{max25}:V_{cmax25}$ increased with photosynthetic capacity at upslope elevations (Figure 5c).

There was a significant ($P<0.001$) positive relationship between photosynthetic capacity parameters and R_{d25} (SMA, $R^2=0.21$ and 0.18 for V_{cmax25} and J_{max25} respectively). The ratio of $R_{d25}: V_{cmax25}$ did not differ between species groups, but significantly changed with transplantation treatment (LRT; $\log (R_{d25}: V_{cmax25})$, $\chi^2=7.9$, $P <0.05$), increasing with transplantation away from the home elevation by 59% upslope and by a non-significant 10% downslope. There was no significant difference between the best fit slopes between transplant sites, and individual fittings of SMA regression lines were significant only for home and downslope transplant elevations (Figure 5a).

There was a significant ($P<0.01$) but noisy positive relationship between photosynthetic capacity parameters and N_a (SMA, $R^2=0.04$ and $R^2=0.06$ for V_{cmax25} and J_{max25} respectively), but no significant overall relationship with P_a (Figure 6b). However, when analysed individually, the LMF species group was not significantly related to N_a or P_a (Figure 6a and b). There was no significant bivariate relationship between overall R_{d25} and leaf nutrients, but when analysed individually there was a significant positive relationship for TMCF species with N_a ($R^2=0.13$, $P<0.001$, Figure 6e) and P_a ($R^2=0.19$, $P<0.01$, Figure 6f).

Photosynthetic capacity per gram of leaf Nitrogen ($V_{cmax25}: N_a$) was an estimated 75% higher in TMCF than LMF species (LRT; $\log (V_{cmax25}: N_a)$, $\chi^2= 7.0$, $P<0.01$, Table 5-3), and was significantly reduced by the transplantation treatment (LRT; $\log (V_{cmax25}: N_a)$, $\chi^2= 14.4$, $P<0.001$). Transplanting downslope decreased mean $V_{cmax25}: N_a$ by approximately 36 % and significantly increased the slope and fit of the best fit line relative to the home elevation (downslope, $R^2=0.11$, $P<0.05$; home, $R^2=0.04$, $P>0.05$, Figure 5b). Transplanting upslope decreased values by about 10%, which was not significant.

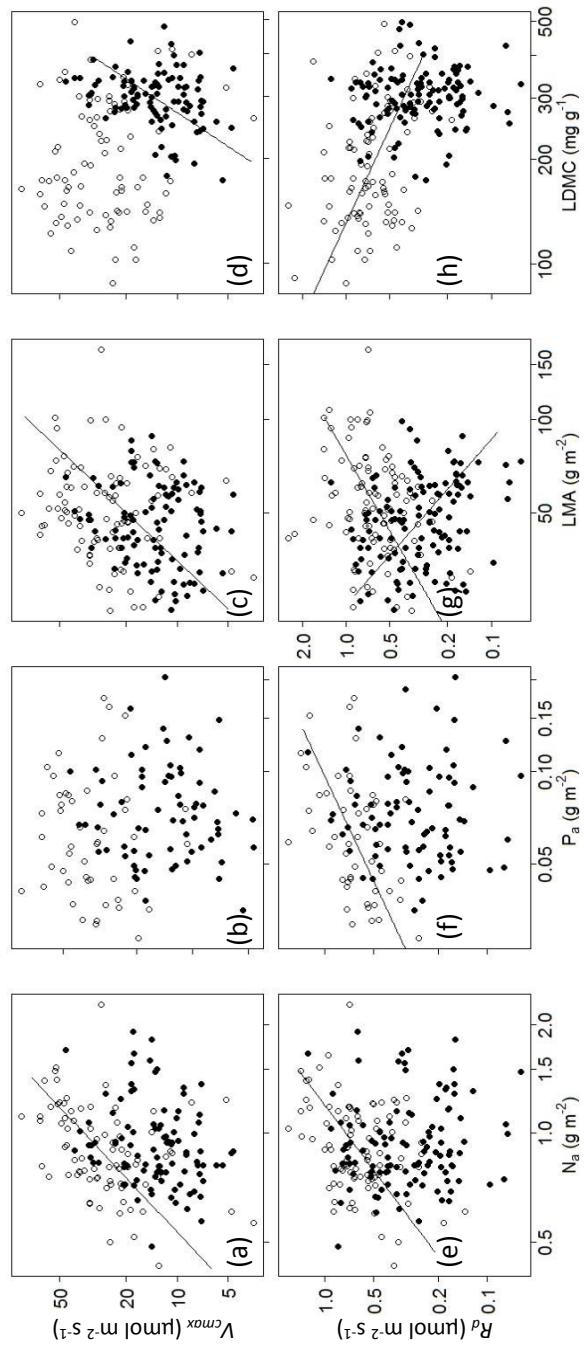


Figure 5-6 Log-log plots of (Upper row) maximum rate of Rubisco carboxylation (V_{max}) and (lower row) dark respiration (R_d) relative to (a, e) leaf nitrogen content on an area-basis (N_a), (b, f) leaf phosphorus content on an area-basis (P_a), (c, g) leaf mass per unit area (LMA) and (d, h) leaf dry matter ($LDMC$). Closed symbols, low-mid forest (LMF) species; open symbols, tropical montane cloud forest (TMCF) species; SMA regressions given for significant relationships ($P < 0.05$); (d, g) significant for TMCF species; (a, e, f, g, h) significant for LMF species; (c) significant for combined data set.

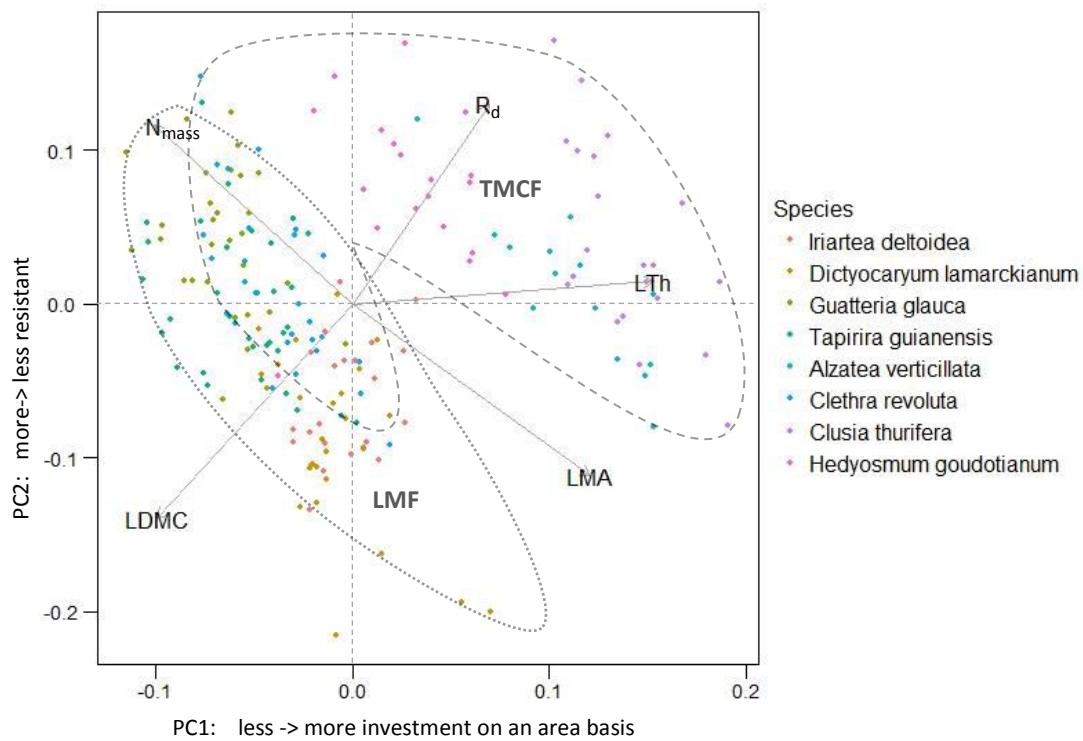


Figure 5-7 Principal component analysis applied to log-transformed seedling leaf traits of all tree species ($n = 193$). LMF (dotted outline), low-mid forest species; TMCF (dashed outline), tropical montane cloud forest species. LDMC, leaf dry matter content; N, leaf nitrogen content on a mass basis; Rd, leaf respiration on an area basis; LMA, leaf mass per unit area; LTh, leaf thickness. 47% of the variance was accounted for by PC1 (less to more investment on an area basis) and 29% by PC2 (more to less resistant).

Photosynthetic capacity parameters had a weak but significant positive relationship with LMA (Figure 5-6c) and a weak but significant negative relationship with LDMC (Table 5-5), neither of which were influenced by transplant treatment. Differences in the strength and direction of relationships between photosynthetic parameters, R_{d25} and anatomical leaf traits ((Figure 5-6, g and h) were due to differences in leaf- trait strategies between species types as demonstrated in the results of the PCA (Figure 5-7). The first PCA axis related to the leaf economic spectrum (Wright et al. 2004), with the greatest investment on an area basis for TMCF species with mid to high LMA and high LTh, but lower LDMC and N_{mass} (*A. verticillata* and *C. thurifera*) (Austin 1993, Wilson et al. 1999). The second PCA axis related to the abiotic and biotic gradient with elevation, with more resistant leaves (high LDMC and LMA) at the lower side for the Arecaceae *I. deltoidea* and *D. lamarckianum* and less resistant leaves for mid and high elevation species.

5.4 Discussion

We aimed to quantify changes in leaf-level physiological and anatomical traits in response to transplantation by ± 4 °C. We found that, as hypothesised (H1), TMCF species were more susceptible to warming-induced mortality and reductions in photosynthetic capacity than LMF species. This was apparent in declines in both V_{cmax} and J_{max} , in response to warming, however there were no concurrent declines in the $J_{max}: V_{cmax}$ ratio, as expected (H2). R_d showed the strongest response to transplantation (H3), acclimating both downslope and upslope across all species, thus maintaining respiratory rates at ambient leaf temperatures. As a result of the mismatch in acclimatory response between photosynthetic and respiratory capacity there was an increase in $R_d: V_{cmax}$ upslope, but no difference between LMF and TMCF species as anticipated (H4). The response of anatomical leaf traits to transplantation tended to be species-specific, with the only clear trend being in the reduction in the $V_{cmax25}: N_a$ ratio downslope (H5).

5.4.1 Tropical context

Values and ranges of photosynthetic capacity (V_{cmax25} and J_{max25}), R_{d25} , N_a , P_a and LMA at ‘home’ elevations were at the lower end or lower than previously reported along the Kosñipata gradient (van de Weg et al. 2012, Bahar et al. 2016), but overlapped with values from the tropical and temperate understory (Meir et al. 2002, Domingues et al. 2007, Weerasinghe et al. 2014) and global trees elsewhere (Atkin et al. 2015). LMF values were most similar to the tropical ground level values of V_{cmax} , J_{max} and R_d made by Carswell et al. (2000). The observed values agree with those in a low light environment (Meir et al. 2002, Weerasinghe et al. 2014), but variations in seedling and adult leaf anatomy could also account for the relatively low values of N_a , P_a and LMA (Ishida et al. 2005).

TMCF species had on average approximately twice the photosynthetic capacity and photosynthetic N-use efficiency of LMF species. This trend has been reported to a lesser extent along the Kosñipata gradient for dominant species, with increases of 36 %, 45 % and 19 % for V_{cmax25} , J_{max25} and $V_{cmax25}: N_a$ (Bahar et al. 2016) from lowland to upland sites. Values of $V_{cmax25}: N_a$ for LMF species were in the same range as previous tropical studies, but values for TMCF species were higher than previously reported along the Kosñipata gradient (van de Weg et al. 2012, Bahar et al. 2016), and higher than values estimated for the tropical plant functional type (Kattge et al. 2009). This may be due to physiological differences between

adult and seedlings, as the TMCF values were within the same range as those reported in the tropical seedling study of Vårhammar et al. (2015).

The observed $J_{max25}: V_{cmax25}$ ratios at home elevations were higher than the average reported values (Medlyn et al. 2002, Kattge and Knorr 2007), but within the same range as values for tropical montane species (van de Weg et al. 2012, Vårhammar et al. 2015, Bahar et al. 2016), shaded saplings (Coste et al. 2005) and crop species (Medlyn et al. 2002). This higher relative investment in J_{max25} compared to V_{cmax25} could be an acclimatory response to lower light levels in the understorey and areas of frequent cloud immersion (Niinemets et al. 1998, van de Weg et al. 2012, Malhi et al. 2016). Equally, the lower relative investment in V_{cmax25} and concurrent low values of $V_{cmax25}: N_a$ may be as a result of species-specific differences in N allocation between photosynthetic and structural proteins (Harrison et al. 2009, Scafaro et al. 2017).

5.4.2 Transplantation effect

The effect of transplantation was catastrophic for over half of the transplanted seedlings, with the lowest odds of survival in shaded plots and at downslope elevations (Figure 5-3). TMCF species were particularly affected when transplanted downslope to the lower edge of the cloud base; a natural zone of transition between sub-montane and montane cloud forest species (Malhi et al. 2016) (Figure 5-1). Survival and physiological stress at 1500 m asl was lower across all species (Appendix, Figure 5-9), which could be due to the combination of warmer temperatures and high rainfall (Table 5-1) facilitating fungal pathogen infection (Swinfield et al. 2012), the presence of which at lowland sites was highlighted upon visual inspection of Arecaceae seedlings (Appendix, Figure 5-8). The frequency of disease has been found to increase in low light environments (McCarthy-Neumann and Ibáñez 2013, DeWalt et al. 2015), potentially contributing to the decreased rates of seedling survival across shaded treatments.

Rates of seedling survival were generally higher than expected when transplanted to the upper edge or above their estimated current Neotropical distribution (Figure 5-2). This increase in seedling survival at the leading range edge may be as a result of enemy-release from pathogen attack and herbivory pressure, which has been found to decrease away from species' central distributions (Hillyer and Silman 2010, Urli et al. 2016), with the diversity of invertebrates often peaking at mid elevations (1600-2000 m asl) along tropical elevational

gradients (Brehm et al. 2003, Brehm et al. 2007, Rodríguez - Castañeda et al. 2010, Maveety et al. 2013).

Transplanting upslope did not cause a significant change in the baseline photosynthetic capacity (J_{max25} and V_{cmax25}) of the seedlings, however as hypothesised (H2) downslope transplantation resulted in a significant reduction. These changes indicate an acclimatory response, which could be considered constructive if resulting in the maintenance of ambient photosynthetic rates with reduced investment, or detractive if photosynthetic and growth rates were not maintained (Way and Yamori 2014). For TMCF species the acclimatory response was detractive, reducing mean ambient values of V_{cmax} and J_{max} by approximately 23 % downslope and 50 % upslope. However, LMF species differed in their acclimatory response, maintaining ambient J_{max} across all transplant elevations and maintaining V_{cmax} downslope, but with a detractive reduction of approximately 33% occurring upslope for V_{cmax} .

Based on the current range (Figure 5-1) and recent upslope shifts of TMCF species' distributions (Feeley et al. 2011), reductions in seedling performance were expected with simulated warming at transplant elevations close to or below the species' trailing edge. This detractive adjustment downslope is likely to be as a result of individual's leaf temperatures exceeding the optimum temperature range of J_{max} and V_{cmax} , as reported in previous tropical warming experiments (Cunningham and Read 2003, Cheesman and Winter 2013, Vårhammar et al. 2015).

Previous studies along the Kosñipata gradient have predicted and observed decreases in productivity with elevation, with sharp declines in NPP at the zone of transition between submontane and montane cloud forest (1500-1700 m), driven by low photosynthetically active radiation (PAR) and the effects of suboptimal temperatures (van de Weg et al. 2014, Malhi et al. 2016). These community-level studies reported that, there was no observed decreases in ambient photosynthetic capacity with elevation, due to the increased capacity of TMCF species relative to LMF species (Bahar et al. 2016) and the high turnover of tree species at the zone of transition. Despite the high capacity of TMCF species in this study, seedlings were not observed to upregulate their capacity further in response to upslope transplantation. In particular, the greater reduction in V_{cmax25} relative to J_{max25} upslope suggest that these species are more limited by carboxylation capacity than RuBP regeneration, being unable to upregulate their investment to maintain enzyme activity in response to cooler temperatures

(Yamori et al. 2005). This may be due to low temperatures constraining the development of new tissues (Cheesman and Winter 2013) or alternatively, these species may already be operating at their highest capacity, with additional low-temperature effects resulting in reductions in productivity.

The response of seedling J_{max25} relative to V_{cmax25} differed between transplant treatments with the $J_{max25}: V_{cmax25}$ ratio increasing downslope for TMCF species, driven by higher J_{max25} relative to V_{cmax25} at low photosynthetic capacities (Figure 5-5c). This differs to previous studies, where $J_{max25}: V_{cmax25}$ have been found to decrease with growth temperature (Medlyn et al. 2002, Kattge and Knorr 2007, Dusenge et al. 2015), although of these studies only one reported measurements of tropical tree species (Dusenge et al. 2015). The observed increase in slope of the $J_{max25}: V_{cmax25}$ ratio from warmer to cooler sites has been reported in previous studies, and has been associated with inter-specific variations in the allocation of resources to photosynthetic carboxylation and RuBP regeneration (Dusenge et al. 2015, Bahar et al. 2016). The greater reductions in V_{cmax25} and $V_{cmax25}: N_a$ at downslope elevations, could be due to a reduction in leaf N allocation to Rubisco (Scafaro et al. 2017), with N preferentially allocated to structural proteins (Harrison et al. 2009) or in the maintenance of efficient light-harvesting under low-light conditions at lowland sites (Table 5-1) (van de Weg et al. 2012, Dusenge et al. 2015). In addition the T_{opt} of J_{max25} has been found to be lower than V_{cmax25} in three tropical montane species (Vårhammar et al. 2015), which could result in increased investment in J_{max25} in order to maximise photosynthetic rates at higher temperatures (Hikosaka et al. 2006).

As hypothesised (H3), R_{d25} readily acclimated with transplantation, increasing consistently with transplant elevation across LMF and TMCF species. This increase in respiratory capacity with decreasing temperatures allows seedlings to maintain ambient rates of R_d at the same level across all transplant elevations, minimising impacts of temperature on leaf maintenance processes (Atkin et al. 2005). Seedling acclimatory potential of R_{d25} was greater than that of photosynthetic capacity, resulting in a significant increase in the $R_{d25}: V_{cmax25}$ ratio at upslope elevations and a non-significant increases at downslope elevations. It was predicted that TMCF species would have a greater $R_{d25}: V_{cmax25}$ ratio than lowland forests (H4), due to their colder habitat (Atkin et al. 2015), but no difference was found between the

species groups, although TMCF species did experience an increase in R_{d25} : V_{cmax25} at downslope elevations relative to the home elevation (Table 5-3).

5.4.3 Leaf trait relationships

Leaf nutrient traits were not strongly related to photosynthetic capacity ($R_2 < 0.1$) or R_{d25} ($R_2 < 0.2$). The strongest relationships were between V_{cmax25} : N_a and R_{d25} : N_a and R_{d25} : P_a , but only for TMCF species, with little or no bivariate relationship for LMF species. As with baseline photosynthetic capacity, the V_{cmax25} : N_a ratio was significantly reduced by downslope transplantation, indicating a possible detractive acclimatory response in seedling N-use efficiency at low elevations. No other consistent acclimatory trends were detected, because of the species-specific leaf-trait strategies employed. 76% of the variance in leaf traits were accounted for by two principal component axes, which combine to form a gradient of increasing succulence and investment on an area-basis for TMCF relative to LMF species. The trend in succulence was driven by the hemi-epiphytic adaptations of the TMCF species *A. verticillata* and *C. thurifera* (Holbrook and Putz 1996), minimising overheating and water loss in the canopy (Petter et al. 2016). LMF species spanned those with thick but resistant leaves (*I. deltoidea* and *D. lamarckianum*) and those with thin, less resistant leaves (*G. glauca* and *T. guianensis* and the TMCF species *C. revoluta*). These indicate distinctive strategies as a response to biotic stress; high investment and damage resistant slow growing leaves (Lohbeck et al. 2015), versus low investment, easily replaceable leaves.

5.4.4 Vulnerability of TMCF species

Taking all the evidence together, there is support for the hypothesis (1) that TMCF species are more susceptible to climate warming than LMF species. TMCF species odds of survival were twice as low as LMF species when transplanted downslope (Figure 5-3) and had significantly lower levels of N_a when transplanted away from their home elevation. Although there was no significant difference in the response of baseline photosynthetic capacity or respiratory capacity to transplantation between the species groups, transplanting TMCF species downslope resulted in significant detractive responses in ambient V_{cmax} and J_{max} , and the greatest detractive changes in V_{cmax25} : N_a and R_{d25} : V_{cmax25} . This suggests that TMCF species were surviving near the lower limit of their acclimatory capacity. The drivers behind this are likely a combination of biotic and abiotic factors, as temperature alone was not a better predictor of survival than transplant treatment and only 25% of the variance in survival

assigned to the random effect of subplot was captured by differences in light and transplant treatment.

5.4.5 Concluding remarks

Neotropical trees are able to survive a change in climate equivalent to ± 4 °C temperature change, but the extent of their acclimatory response differed between species groups and photosynthetic capacity parameters. The response of V_{cmax} was greater than that of J_{max} to transplantation treatment, although the mechanisms behind this difference remains unclear. More mechanistic studies are therefore needed to ascertain if this trend hold across a broader range of tropical species and environments, investigating the underlying temperature-response of these parameters and alterations in within-leaf allocation of nutrients under warming scenarios. LMF species had a greater capacity to constructively acclimate to warming than TMCF species, but neither group were fully able to upregulate their photosynthetic capacity with upslope migration. This has implications for future upslope shifts, with low seedling survival expected at the trailing edge of TMCF species distributions and a slowing in recruitment at the leading edge for all species, due to the temperature and light limitations of upland cloud forest.

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Table 5-4 Summary of the best fit linear mixed effect models, selected using AIC, for the response factors of seedling survival, V_{cmax25} (the maximum rate of Rubisco carboxylation adjusted to 25 °C), J_{max25} (the maximum rate of RuBP regeneration adjusted to 25 °C) and R_{d25} (area-based respiration measured in the dark and adjusted to 25 °C). Fixed effects are relative to open light treatment, LMF species group and home transplant elevation.

| Response variable | Survival LMF | | | | Survival TMCF | | | | $\log(V_{cmax25})$ | | | | $\log(J_{max25})$ | | | | $\log(R_{d25})$ | | | | |
|---------------------|-------------------------------|-------|-------|-------|-------------------------------|-------|------|-------|--------------------|-------|-------|--------|-------------------|-------|-------|--------|-----------------|-------|-------|-------|--------|
| Model type | glmer (binomial) ^a | | | | glmer (binomial) ^a | | | | lmer | | | | lmer | | | | lmer | | | | |
| Fixed effects | Est. | SE | z | p | Est. | SE | z | p | Est. | SE | t | p | Est. | SE | t | p | Est. | SE | t | p | |
| Intercept | 0.98 | 0.69 | | | 0.44 | 0.48 | | | 2.86 | 0.15 | | | 3.59 | 0.13 | | | -1.15 | 0.16 | | | |
| Light shade | -0.49 | 0.17 | -2.82 | 0.005 | 0.08 | 0.29 | 0.26 | 0.792 | -0.28 | 0.07 | -4.25 | <0.001 | -0.30 | 0.07 | -4.43 | <0.001 | -0.15 | 0.06 | -2.55 | 0.011 | |
| Species group | | | | | | | | | | | | | 0.68 | 0.19 | 3.66 | 0.005 | 0.62 | 0.15 | 4.11 | 0.002 | |
| Transplant | (Downslope) | -1.62 | 0.74 | -2.19 | 0.029 | -1.83 | 0.38 | -4.80 | <0.001 | -0.47 | 0.14 | -3.30 | 0.002 | -0.35 | 0.13 | -2.59 | 0.014 | -0.38 | 0.10 | -3.74 | <0.001 |
| | (Upslope) | -1.05 | 0.64 | -1.64 | 0.100 | 0.15 | 0.35 | 0.44 | 0.660 | -0.15 | 0.13 | -1.21 | 0.233 | -0.13 | 0.12 | -1.10 | 0.280 | 0.28 | 0.09 | 3.13 | 0.003 |
| | (TMCF: Downslope) | | | | | -1.22 | 0.49 | -2.50 | 0.012 | | | | | | | | | | | | |
| | (TMCF: Upslope) | | | | | -0.11 | 0.43 | -0.25 | 0.804 | | | | | | | | | | | | |
| Random Effects | VC | SD | | | VC | SD | | | VC | SD | | | VC | SD | | | VC | SD | | | |
| Subplot number | 0.65 | 0.80 | | | 0.13 | 0.36 | | | 0.08 | 0.28 | | | 0.06 | 0.25 | | | 0.02 | 0.15 | | | |
| Species | 0.22 | 0.47 | | | 0.68 | 0.82 | | | 0.04 | 0.21 | | | 0.02 | 0.15 | | | 0.06 | 0.24 | | | |
| Plot elevation | 0.88 | 0.94 | | | | | | | | | | | | | | | | | | | |
| Residual | | | | | | | | | 0.16 | 0.40 | | | 0.18 | 0.42 | | | 0.17 | 0.42 | | | |
| R^2 marginal | 8.95% | | | | 27.8% | | | | 39.5% | | | | 39.5% | | | | 45.6% | | | | |
| R^2 conditional | 36.4% | | | | 40.4% | | | | 66.0% | | | | 53.9% | | | | 63.2% | | | | |
| N_{obs} | 685 | | | | 691 | | | | 184 | | | | 184 | | | | 214 | | | | |
| AIC ^a | 467.9 | | | | 489.3 | | | | 258.4 | | | | 263.8 | | | | 289.5 | | | | |
| AICc ^a | 468.8 | | | | 490.7 | | | | 259.4 | | | | 264.8 | | | | 290.4 | | | | |
| ΔAICc | 13.5 | | | | 9.7 | | | | 26.3 | | | | 26.7 | | | | 11.7 | | | | |

LMF; low-mid forest species; TMCF, tropical montane cloud forest species; Est., Fixed effect estimate; SE, standard error; z, Wald test statistic ; t, REML t-test statistic; p, p-value using approximate degrees of freedom; VC, random variance components; SD, standard deviation; R^2 Marginal and conditional are pseudo R^2 explained by fixed effects and by fixed and random effects; AIC, Akaike Information Criterion; AICc, AIC with correction for small sample size; N_{obs} , Number of observations; ΔAICc , the difference in AICc between the best model and the null model. ^a fitted by Maximum Likelihood, ^b fitted by Restricted Maximum likelihood.

Table 5-5 Significant bivariate relationships between log-transformed leaf traits across all species and transplant elevations

| Log/log | V_{cmax25} | J_{max25} | R_{d25} | N_a | P_a | LMA | LDMC |
|--------------|---------------------|---------------------|----------------------|-------------------|------------------|-------|-------|
| V_{cmax25} | | 0.904 | 0.207 | 0.040 | NS | 0.046 | 0.104 |
| J_{max25} | 1.08 (1.03, 1.13) | | 0.176 | 0.056 | NS | 0.079 | 0.064 |
| R_{d25} | 1.02 (0.90, 1.16) | 0.95 (0.83, 1.09) | | NS | NS | NS | 0.214 |
| N_a | 2.54 (2.18, 2.96) | 2.37 (2.03, 2.76) | NS | | 0.412 | 0.439 | NS |
| P_a | NS | NS | NS | 9.76(8.47, 11.26) | | 0.184 | NS |
| | | | | | 0.0019 | | |
| LMA | 1.93 (1.67, 2.22) | 1.86 (1.61, 2.15) | NS | 1.23 (1.09, 1.38) | (0.0016, 0.0022) | | NS |
| LDMC | -1.78(-2.06, -1.55) | -1.68(-1.95, -1.44) | -1.77 (-2.01, -1.56) | NS | NS | NS | |

Robust standardised major axis (SMA) slopes and 95% confidence intervals given in left section of table (x-axis variable in row and y-axis variable in column). R² values for significant (P<0.05) bivariate relationships in upper right section of table. NS, non-significant bivariate relationship.

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Figure 5-8 (a) Large individuals of *C. revoluta* and *H. goudotianum*, (b) fungal pathogen presence on individual of *D. lamarckianum*

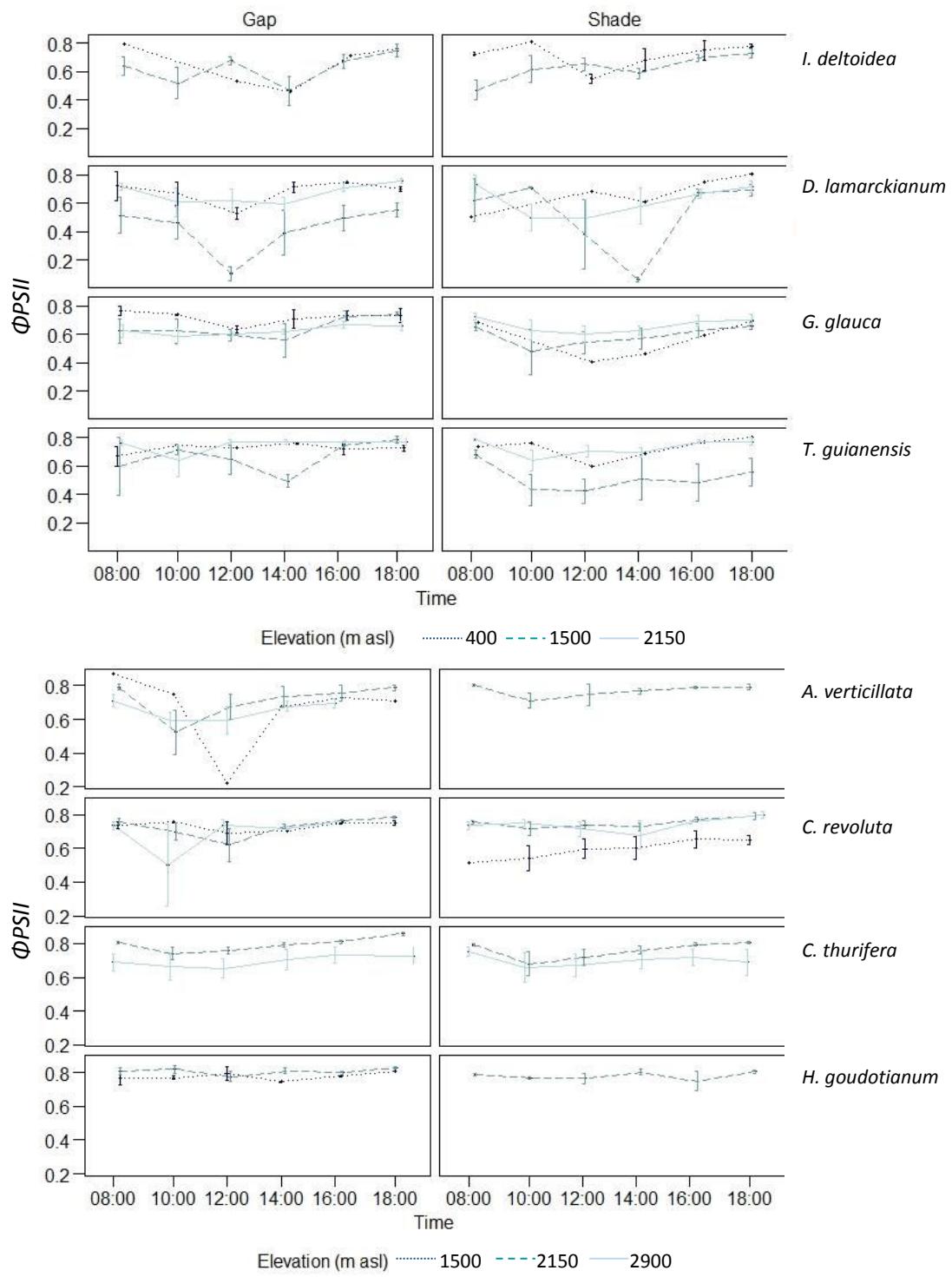


Figure 5-9 Diurnal quantum yield of photosystem II for (top) LMF and bottom (TMCF) transplanted seedlings grown in the gap and shade. Declines show evidence of short-term physiological stress.

Chapter 6. General discussion and conclusions

The overall aim of the thesis was to explore the survival and growth response of Neotropical tree species to an experimental change in climatic regime and to assess species' acclimatory abilities through the study of anatomical and physiological leaf traits. This aim was addressed via an *in situ* seedling transplant experiment along an Andes-Amazon elevational gradient, in which seedlings were transplanted by the equivalent of ± 2 °C and ± 4 °C and their performance followed over a year (Chapter 4). The acclimatory response of a subset of these seedlings was measured and interpreted (Chapter 5) in the context of an observational study of anatomical and physiological leaf traits carried out on the local populations (Chapter 3). The main findings are summarised here in relation to the following key questions:

Q1. Can we use leaf traits to predict how species will respond to climate change?

Q2. How do tree seedlings perform with a ± 2 °C and ± 4 °C change in climatic regime?

Q3. Do tree species differ in their response to climate change?

Q4. Is there evidence that tree seedlings can acclimate to new transplant conditions?

6.1 Key findings

6.1.1 Using leaf traits to predict how species will respond to climatic change

- Adults have greater directional shifts in leaf traits and a greater ability to recover from physiological stress than seedlings (Chapter 3).
- Intraspecific leaf trait variation was not a useful indicator of response to a change in climate (Chapters 3 and 5).
- An increase in the frequency of specialist trait strategies in TMCF species may indicate a reduced capacity to respond to warming (Chapters 3 and 5).
- There was little evidence of physiological stress (as quantified by F_v/F_m) in response to climatic change within species' local populations (Chapter 3).

- Chlorophyll fluorescence (F_v/F_m) could be used to detect early declines in seedling performance in response to abiotic stress (Chapter 4).

As mentioned in chapter 5 the increasing global coverage of leaf trait variation studies make them a valuable resource for understanding and predicting ecosystem processes in the face of rapid climatic change (Lavorel and Garnier 2002, Wright et al. 2005, Galbraith et al. 2010, Atkin et al. 2014, Slot et al. 2014). Although intraspecific variation in traits associated with the leaf economic spectrum, such as *LMA* and N_a , have previously been found to be important in determining community shifts in leaf traits along elevational gradients globally (Read et al. 2014), this was less important along the Kosñipata gradient (chapter 3). The single gradient of conservative to acquisitive leaf trait strategies as described in the leaf economic spectrum (Wright et al. 2004) (from high to low *LMA*, *LDMC* and leaf nutrients on an area basis), was only partly supported by evidence along the Andes to Amazon gradient. The trend was confounded by an increase in succulence (high *LMA* and low *LDMC*) of hemi-epiphytic cloud forest species (chapter 3) and an increase in resilience (low *LTh* but high *LDMC*) of Arecaceae leaves (chapter 5). With more complex community-level trait strategies, the response of species to climate warming was better described by the trade-off between abiotic and biotic specialisations i.e. where TMCF species invested in abiotic leaf-trait specialisations (high *LMA* and nutrients on an area basis) were disproportionately affected by biotic factors when transplanted downslope (chapter 4).

Along the Kosñipata gradient functional leaf traits (*LMA*, N_a , P_a , V_{cmax} and J_{max}) have been incorporated into models explaining the decline in forest productivity with elevation (van de Weg et al. 2014, Fyllas et al. 2017). In Fyllas et al. (2017) photosynthetic rate was expressed as a function of anatomical leaf traits and radiation intensity, based on evidence that there was no difference in A_{sat} between lowland and upland sites at the measurement temperatures used ($>20^{\circ}\text{C}$) (Bahar et al. 2016). However for the species in this study the relationships between anatomical and physiological leaf traits were weak and A_{sat} was found to decrease with upslope transplantation (chapter 5). Although there were differences in methodologies between these studies, e.g. seedlings versus adults and different measurement T_{leaf} , this discrepancy highlights the need for further study of the acclimatory limitations of these communities.

In addition to anatomical traits we used leaf-level chlorophyll fluorescence parameters to assess the physiological stress in response to climatic change. Although little difference in stress was detected within the natural population in response to elevational changes in climate (Chapter 2), stress was a good predictor of the abiotic limitations of transplanted seedlings: F_v/F_m correlated with the probability of survival the following month (chapter 4, Figure 4-6) and coincided with acclimatory limitations to photosynthesis (chapter 5, see summary Figure 6-1). Future work should take advantage of this technique to quickly and efficiently monitor tropical plants, adding to current canopy-level remote-sensing studies (section 1.4.2) by assessing a greater number of species at multiple ontogenetic stages within the forest understorey. In this way abiotic limitations to range-edge recruitment may be detected prior to declines in the performance of canopy trees.

Q1. In this study anatomical leaf traits alone were not useful in understanding the response of species to climate change, instead leaf-trait strategies and measures of physiological stress aided the interpretation of species' response.

6.1.2 Tree seedling performance with a ± 2 °C and ± 4 °C change in climatic regime

The following seedling performance responses were predicted based on previous observational studies and warming experiments (section 2.9.1, Figure 2-5): 1) decreases in survival and growth in response to upslope transplantation as a result of the increased abiotic stress at higher elevation; 2) decreases in survival and growth downslope in response to drought or extreme warming events; 3) increases in growth downslope in response to moderate warming; 4) decreases in survival due to biotic pressure downslope.

- LMF species were twice as likely to survive as TMCF species when transplanted downslope to warmer conditions (Chapters 4 and 5).
- Seedlings survived better than expected based on their elevational range i.e. the fundamental niche appeared to be larger than the realised niche (Chapter 4).
- Growth performance did not increase downslope as expected based on controlled warming experiments (Chapter 4).
- All seedlings were negatively affected by transplantation upslope as a result of abiotic stressors (e.g. low temperature and UV-B) (Chapter 4 and 5).

As per the prediction (1) upslope transplantation decreased seedlings' survival and growth rates (chapter 4) but not to the same extent for all species. Upslope trends for LMF species were weaker than for LF and TMCF species, potentially because upslope transplant sites (1750 and 2150 m asl) did not experience dramatic changes in cloud cover or variability relative to the home elevation (1500 m asl). However where the most dramatic changes in temperature, cloud cover and diurnal variability occurred (400 to 1500 m asl and 2150 to 2900 m asl) seedling stress and mortality increased.

In contrast, there was no evidence of direct abiotic limitations to survival and growth downslope as predicted (2), probably due to the absence of intense drought or warming during the measurement period (chapter 4). Because of this, the survival of LMF species downslope was much greater than anticipated, although there was no concurrent increase in growth response as anticipated (3) from warming experiments (Cheesman and Winter 2012, Cheesman and Winter 2013, Slot and Winter 2017), suggesting that factors other than temperature were limiting productivity, such as light or nutrient availability (see chapter 5).

The prediction that biotic pressures would increase downslope (4) was supported by observations of leaf damage downslope (presence of fungal pathogens), however this only adversely affected the survival of TMCF species. Despite convincing evidence of pathogen-induced seedling mortality elsewhere in the literature (Bell et al. 2006, Swinfield et al. 2012, Bagchi et al. 2014), without the experimental manipulation of biotic factors we are unable to directly quantify this effect.

Q2. In summary, this seedling transplant experiment supports previous findings of the survival and growth performance of tropical trees to climate change: seedling performance declined away from the home elevation for the majority of species, with upslope declines as a result of abiotic limitations, and downslope declines due to biotic limitations.

6.1.3 Differences in the response of tree species to climate change

- TMCF species were more specialised than LMF species in their adaptations to abiotic stress (Chapters 2 and 5).

- The response of leaf-trait to simulated climate change was species-specific (Chapters 2 and 5).
- LMF seedlings performed better than TMCF seedlings to transplantation (Chapters 4 and 5).
- LMF species had a greater acclimatory capacity for photosynthesis than TMCF species (Chapter 5).

Tropical species are considered to be potentially more vulnerable to climate change relative to temperate species (section 1.5.1), but their vulnerability relative to one another is less clear (section 1.5.2). Although TMCF and LMF species are less likely to experience lowland extremes of temperature, their distributions span a greater range of environmental conditions than LF species, so they are considered likely to be less vulnerable to fluctuations in climate (Colwell et al. 2008, Doughty and Goulden 2008, Corlett 2011). However, LMF species have been found to be less responsive than LF species when warmed, with a lesser acclimatory capacity of photosynthesis and lower rates of survival (Cheesman and Winter 2013, Vårhammar et al. 2015, Slot and Winter 2017).

Within the natural population LF/LMF species had trait strategies related to tolerance of biotic stress, whereas TMCF species were found to be more adapted to abiotic stress (chapter 3). It was anticipated that at the extremes of species ranges (chapter 3) and in response to transplantation (chapter 5) species traits would show evidence of acclimation to elevational changes in climate. There was evidence of acclimatory trends at the species-level (Appendix-Figure 6-3, Figure 6-4, Figure 6-5, Figure 6-6), but few coherent trends across species or community groups as a result of the range of leaf trait strategies in evidence. The lack of evidence of physiological stress within the natural population suggests that despite the limited amount of intra-specific variation observed in the populations, the performance of the studied seedlings was not limited. However that does not mean to say that they were perfectly adapted to their environment.

By following the performance of transplanted seedlings over a year, distinct differences in the physiological stress response of LF/LMF and TMCF communities were observed. As with the increase in climatic variability with elevation, the variability in stress response of seedlings also increased with the elevation of transplant sites. There was a step-up in stress response from lowland to transition zone sites for LF species, a gradual increase in stress with

elevation for LMF species and a much greater variability in stress response for TMCF species (chapter 4). Similarly when investigating the performance limitations of each group there were distinct differences between LF/LMF and TMCF communities. Whereas LF and LMF species experienced only abiotic limitations to their physiology upslope, TMCF species experienced multiple direct and indirect limitations to survival and growth (Figure 6-1). Direct limitations were hypothesised to be as a result of TMCF species facing unprecedented levels of biotic pressure at warm and wet sites downslope (chapter 4) and extreme low temperature and light limitations to growth upslope. All species experienced imperfect acclimation in their photosynthetic capacity, but only TMCF species experienced this at both upslope and downslope sites (chapter 5).

Q3. As a whole this suggests that TMCF species are more vulnerable than LF and LMF species to climate change, due to their reduced ability to acclimate or respond to changes in biotic and abiotic conditions.

6.1.4 Evidence of tree seedling acclimation to new transplant conditions

We expected that (1) seedling respiration would most readily adapt to moderate changes in climate so that (2) the ratio of R_{d25} : V_{cmax25} would increase upslope consistent with local (van de Weg et al. 2012) and global trends in temperature response (Atkin et al. 2000, O'Sullivan et al. 2013, Heskell et al. 2016) (section 1.4.1). Predictions of the response of photosynthetic capacity to transplantation were tentative, as the photosynthetic temperature-response of tropical trees is not well-studied (Vårhammar et al. 2015, Slot and Winter 2016). Consistent with the only previous tropical study, we anticipated that (3) V_{cmax25} and J_{max25} would decrease downslope (Dusenge et al. 2015) and as found in previous global meta-analyses (4) the J_{max25} : V_{cmax25} ratio would decrease downslope with warming (Medlyn et al. 2002, Kattge and Knorr 2007, Dusenge et al. 2015).

- TMCF and LMF species were able to adjust their respiratory capacity in order to maintain rates of R_d at ambient measurement temperatures (Chapter 5).
- TMCF species were unable to acclimate their photosynthetic capacity in response to transplantation.
- LMF species were able to adjust J_{max} in order to maintain rates at ambient temperatures, but were not able to upregulate V_{cmax} upslope (Chapter 5).

- The $R_{d25}: V_{cmax25}$ ratio increased by approximately 59% upslope for TMCF and LMF seedlings (Chapter 5).
- The $V_{cmax25}: N_a$ ratio decreased by approximately 35% downslope for TMCF and LMF seedlings (Chapter 5).
- The $J_{max25}: V_{cmax25}$ ratio of TMCF seedlings increased by 16% downslope (Chapter 5).

In the absence of significant drought or nutrient-limitation gradients, seedling respiratory capacity increased in response to warming, maintaining ambient respiratory rates across all transplant sites. Thus, as predicted (1) seedlings' respiration acclimated more or less perfectly to changes in climate. In contrast photosynthesis did not upregulate upslope to the same extent, resulting in (2) decreases in $R_{d25}: V_{cmax25}$. As predicted (3) we found a significant decrease in V_{cmax25} and J_{max25} with downslope transplantation, but also a smaller decrease upslope. The impact of this on ambient rates was detractive for both TMCF and LMF species at upslope sites, but constructive for LMF species at downslope sites. This constructive acclimation enables ambient rates to be maintained, yet with a lower investment in capacity.

The ratio of $J_{max25}: V_{cmax25}$ did not decline with warming as predicted (4), but was maintained in LMF species, as observed in a previous local study (Bahar et al. 2016), and increased downslope in TMCF seedlings. This increase may have been as a result of differences in the temperature optima of V_{cmax25} and J_{max25} in tropical trees (Dusenge et al. 2015), or combined with a decrease in $V_{cmax25}: N_a$ at downslope elevations, a result of changing N allocation within the plants (Scafaro et al. 2017).

Q4. Based on this transplant experiment we conclude that the acclimatory response of TMCF species has not been under-estimated by observational and modelling studies and that there is a real risk that these species will continue to decline at their lower range edge. LMF species are able to acclimate to moderate warming scenarios, so their future distribution will instead be limited by the availability of favourable habitat upslope.

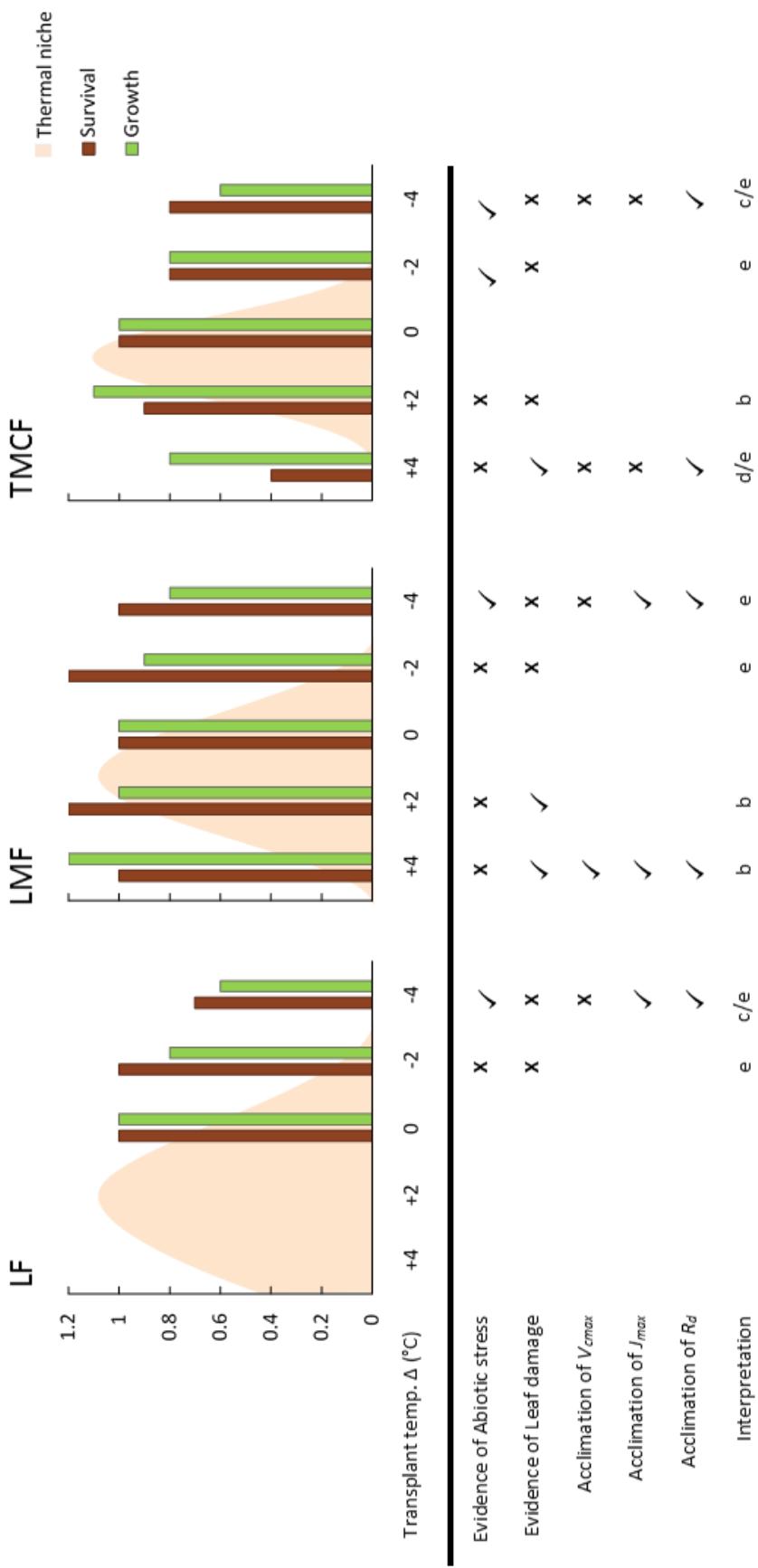


Figure 6-1 Overall growth and survival response of transplanted seedlings relative to centre of species' thermal niche. LF, lowland species; LMF, lower montane species; TMCF, montane cloud forest species. Interpretation of limitation: (a) full limitation (abiotic and biotic); (b) no additional limitation (abiotic or biotic); (c) full abiotic limitation to physiology; (d) direct biotic limitation (herbivory or pathogens); (e) acclimatory limitation to physiology potentially resulting in indirect biotic limitation (competition).

6.2 Interpretation of transplant experiment

As visualised in Figure 6-1 we interpreted the response of each elevational community group to transplantation, based on the framework described in Chapter 2 and utilising the following sources of evidence: elevational range (approximate thermal niche), survival, growth, physiological stress, biotic leaf damage, acclimation of photosynthetic capacity, acclimation of respiratory capacity and overall leaf trait strategy.

6.2.1 Upslope

Based on the evidence for LMF species, we observed no additional limitation to survival upslope, but an increasing limitation to growth with distance away from the home elevation. This was interpreted as an acclimatory limitation to seedling physiology (Figure 6-1 e), as seedlings were unable to upregulate their V_{cmax} in response to greater abiotic stress (reductions in seedling F_v/F_m) at the -4 °C transplant site. LF species acclimated in the same way as LMF species, but were also observed to experience greater declines in survival at the -4 °C transplant site. This was interpreted as an additional direct abiotic effect (Figure 6-1 c, e), in response to transplantation into the lowland-montane transition zone (1500 m asl).

The survival and growth of TMCF seedlings declined in response to upslope transplantation, to the extent that abiotic stress was considered to play a direct as well as an indirect role in seedling performance (Figure 6-1 c, e). Within the -4 °C site (2950 m asl), specifically the gap plots, seedlings were the most physiologically stressed (Chapter 4, Figure 4-9), being the most exposed to variability in light and temperature, as well as receiving the highest levels of damaging UV-B. Despite this, shade plots experienced the greatest declines in survival, growth and photosynthetic capacity (V_{cmax} and J_{max}), suggesting that light as well as temperature is limiting at high elevations.

6.2.2 Downslope

LMF species that were transplanted downslope acclimated perfectly to +2 and +4 °C conditions, maintaining ambient rates of V_{cmax} , J_{max} and R_d across these sites (Figure 6-1 e). There was no evidence of abiotic stress (no declines in seedling physiological stress), but some evidence of leaf damage due to biotic stress at these sites, although this did not adversely affect seedling survival or growth. In contrast, TMCF seedlings survived poorly with

transplantation away from the home elevation, with low survival and declines in growth at +4 °C. There was no evidence of direct abiotic stress at this elevation and similar levels of leaf damage as observed in LMF seedlings, however in the case of TMCF species survival was severely impeded (Figure 6-1 d). Declines in survival due to biotic factors may have been facilitated by acclimatory limitations, as TMCF seedlings were unable to acclimate their V_{cmax} or J_{max} , increasing their $J_{max25}: V_{cmax25}$ ratio (Figure 6-1 d/e).

6.2.3 Caveats of interpretation

Where we describe transplantation as a change in temperature, and indeed this is the overwhelming environmental gradient, there are other abiotic factors that also change with elevation along the Andes-Amazon transect, such as: UV-B exposure, rainfall, nutrient availability, geology and slope (section 2.6.2). This abiotic stress gradient is mostly captured within the seedling physiological stress measurements and the transplant light treatment, however we are still limited when interpreting multiple drivers of seedling performance. Likewise, the biotic gradient adds an extra layer of complexity to the experiment (section 2.6.3), so interpretations are limited in this respect.

6.3 Future research areas

In order to fully address the mechanisms behind the findings in this thesis, the temperature-response of underlying photosynthetic parameters in tropical trees must be further investigated. For example, to-date no studies have been carried out to assess the impact of increasing growth temperatures on the *in situ* photosynthetic temperature-response of tropical tree seedlings i.e. it is not known how climate change will affect the ability of tropical trees to respond to high temperature, droughts, floods or changes in seasonality. This could be addressed by further manipulating transplanted seedlings (Figure 6-2, Climatic).

Although differences in response between gap and shade plots were examined in this thesis, the underlying limitations related to light regime have not been explored. Damage due to UV-B exposure at high elevations could be experimentally alleviated by the use of UV-filters, without influencing light levels. In addition low PAR limitation within the cloud forest could be explored by the addition of lighting (practicalities aside) or alternatively, simulated under more controlled conditions (Figure 6-2, Light).

Soil processes were not fully explored during the experiment (seedlings were transplanted with uncleaned roots into new soils), so there may have been unquantified stressors acting upon the seedlings. To investigate the hypothesised low temperature limitations to nutrient availability, seedlings transplanted upslope could be warmed *in situ* (practicalities allowing) concurrently with manipulations of soil abiotic or biotic properties, in theory removing low temperature effects and stimulating growth (Figure 6-2, Soil).

| Climatic | | |
|--|---|--|
| <p><i>Drought/flood events</i> Transplant with drought (Rowland et al. 2015) or flood events (Jentsch et al. 2007)</p> | <p><i>Temperature extremes</i> <i>In situ</i> testing of thermal limits (Krause et al. 2010)</p> | <p><i>Temperature variability</i> <i>In situ</i> variable/intermittent warming (Kreyling and Beier 2013)</p> |
| Light | | |
| <p><i>UV-B</i> Transplant with UV-B filter manipulation (Krause et al. 2003)</p> | <p><i>Low PAR</i> Transplant under additive light regime e.g. spotlights or cloud manipulation (A.R. Griffiths 2017 personal communication)</p> | |
| Soil | | |
| <p><i>Low temperature</i> <i>In situ</i> moderate warming of upslope transplanted seedlings (soil and air) (Clark et al. 2014)</p> | <p><i>Nutrients</i> Transplantation with home soil or addition of fertiliser (Alvarez-Clare et al. 2013)</p> | <p><i>Biota</i> Transplantation of seedlings germinated in sterilised or unsterilised soil (Gundale et al. 2014)</p> |
| Biotic | | |
| <p><i>Herbivores</i> Herbivore exclusion and/or monitoring of leaf damage on transplanted seedlings (Garibaldi et al. 2011)</p> | <p><i>Pathogens</i> Application of fungicide on transplanted seedling (Swinfield et al. 2012)</p> | <p><i>Competition</i> Germinate and monitor seedlings in mixed planting groups (LMF vs. TMCF) (Hovick et al. 2012)</p> |

Figure 6-2 Additional transplant treatments to investigate underlying abiotic and biotic limitations.

To unravel the biotic limitations to seedling performance factorial transplant experiments could be designed, incorporating direct biotic effects via the exclusion of natural enemies and indirect effects such as inter-seedling competition. In this way the effect of warming-induced increases in biotic pressure on the competitive advantage of seedlings (LMF versus TMCF) could be quantified (Figure 6-2, Biotic). Apart from studying seedling establishment and growth, little is known about the effects of climate warming on equally important biotic

barriers to population growth, such as reproductive success and germination rates. In particular, how asynchrony in migration between plants and other trophic levels will affect tree population dynamics, i.e. if pollinators or vectors of dispersal migrate at a faster rate than their tree hosts. To understand this complexity a more holistic approach is required, investigating multiple ontogenetic stages and their positive and negative inter-trophic relationships.

6.4 Conclusions

- Leaf-trait strategies and measures of physiological stress can provide insight into the response of tropical tree species to climate change (Chapters 3 to 5)
- There are significant differences in the response of ontogenetic stages to physiological stress (Chapter 3)
- LMF species are more responsive and acclimate more readily than TMCF species to changes in climatic regime (Chapters 4 and 5)
- The lack of acclimatory capacity of photosynthesis, particularly V_{cmax} , is limiting the response of species to climate change.

The research presented in this thesis furthers our understanding of the mechanisms behind the upslope migration of tropical trees, assessing current variation in intra-specific traits and physiological stress responses along an elevational migration corridor, and experimentally testing the performance and acclimation of seedlings beyond their natural range. Through transplantation this research simulates upslope migration and climate change equivalent to moderate end-of-century warming scenarios, allowing the response of seedlings from multiple elevational communities to be quantified and compared. This research demonstrates the importance of leaf-level functional traits in monitoring and predicting the response of species to climate warming, as well as highlighting ontogenetic differences in trait-response that may impact upon future population dynamics. In response to end-of-century climate change, these results suggest that: (i) upslope migration will be limited by abiotic stressors (low temperature, PAR); (ii) moderate warming may not impact on the performance of LF and LMF species; (iii) moderate warming will impact on the performance of TMCF seedlings; (iv) warming-related increases in biotic stress will directly and indirectly impact on the persistence of TMCF species downslope.

Under this warming scenario, the impact on community dynamics will be a gradual replacement of TMCF species by LMF species at mid-elevations and minimal upslope shifts at the upper range edge, resulting in range retractions or extinctions of TMCF species. These findings are in keeping with previous estimates of population declines based on current migration rates (Feeley and Silman 2010). There is a great amount of uncertainty arising from projected climate change scenarios, as warming may facilitate the establishment and growth of individuals upslope, or equally result in declines at any elevation in combination with drought or declining cloud frequency. Finally, it is important to recognise that although this research breaks new ground in our understanding of the *in situ* responses of tropical tree species to climate change, particularly at the crucial seedling stage, many of the underlying mechanisms behind these responses remain unexplored in these communities.

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6.6 Appendix

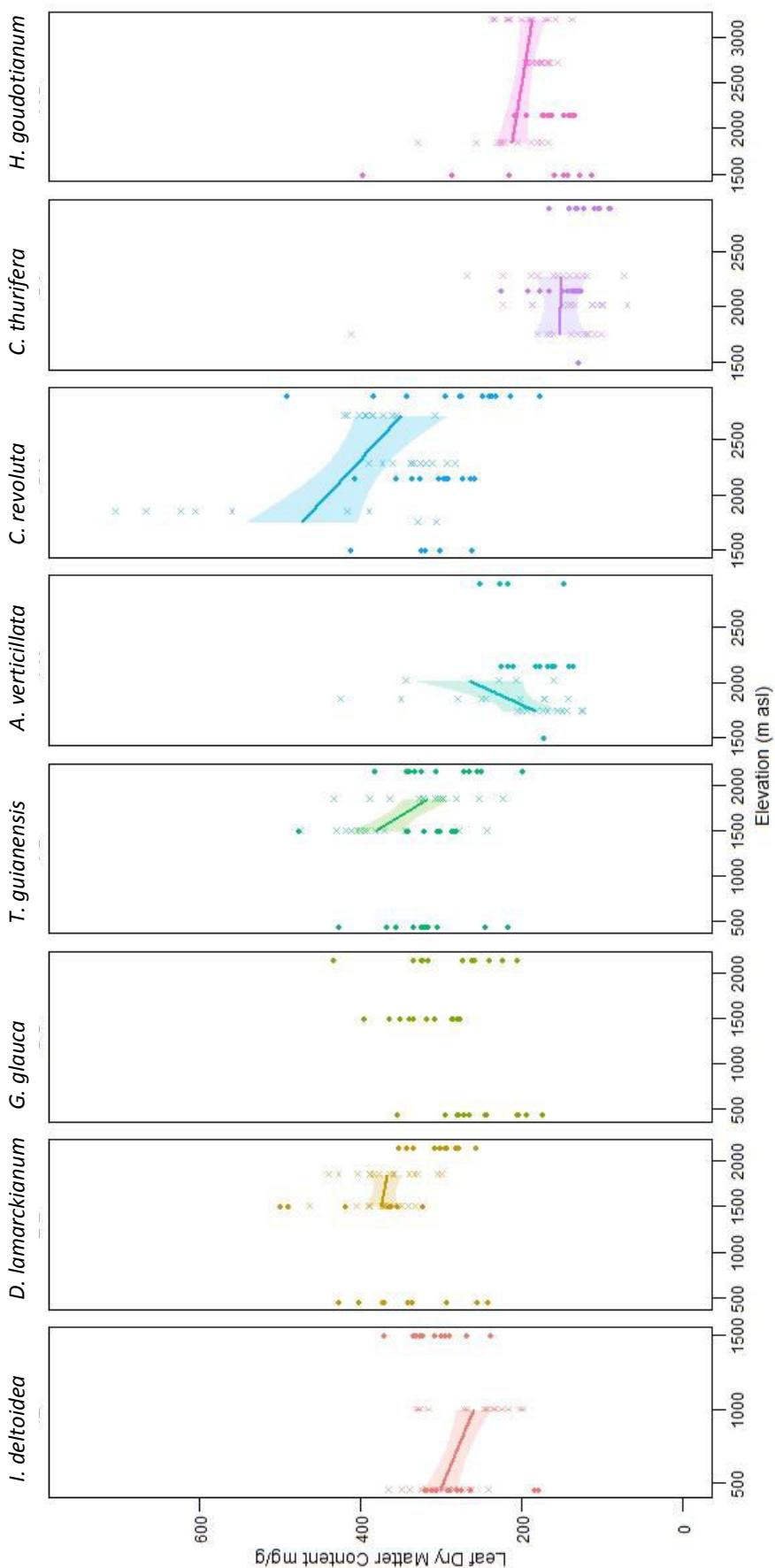


Figure 6-3 The species-level response of leaf dry matter content to elevation. Crosses and trend line; natural seedling variation (chapter 3) and points; transplanted seedling variation (chapter 5)

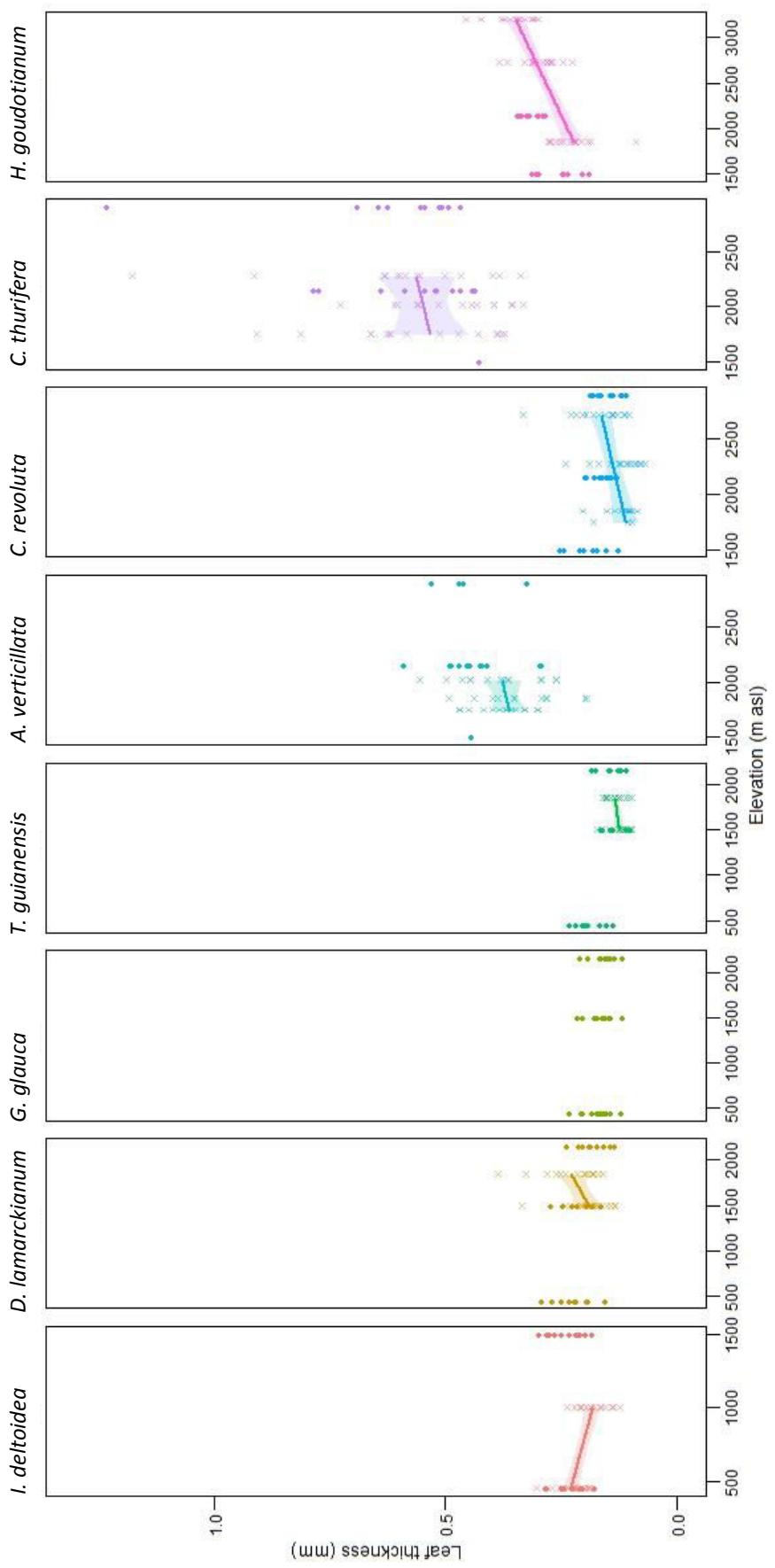


Figure 6-4 The species-level response of leaf thickness to elevation. Crosses and trend line; natural seedling variation (chapter 3) and points; transplanted seedling variation (chapter 5)

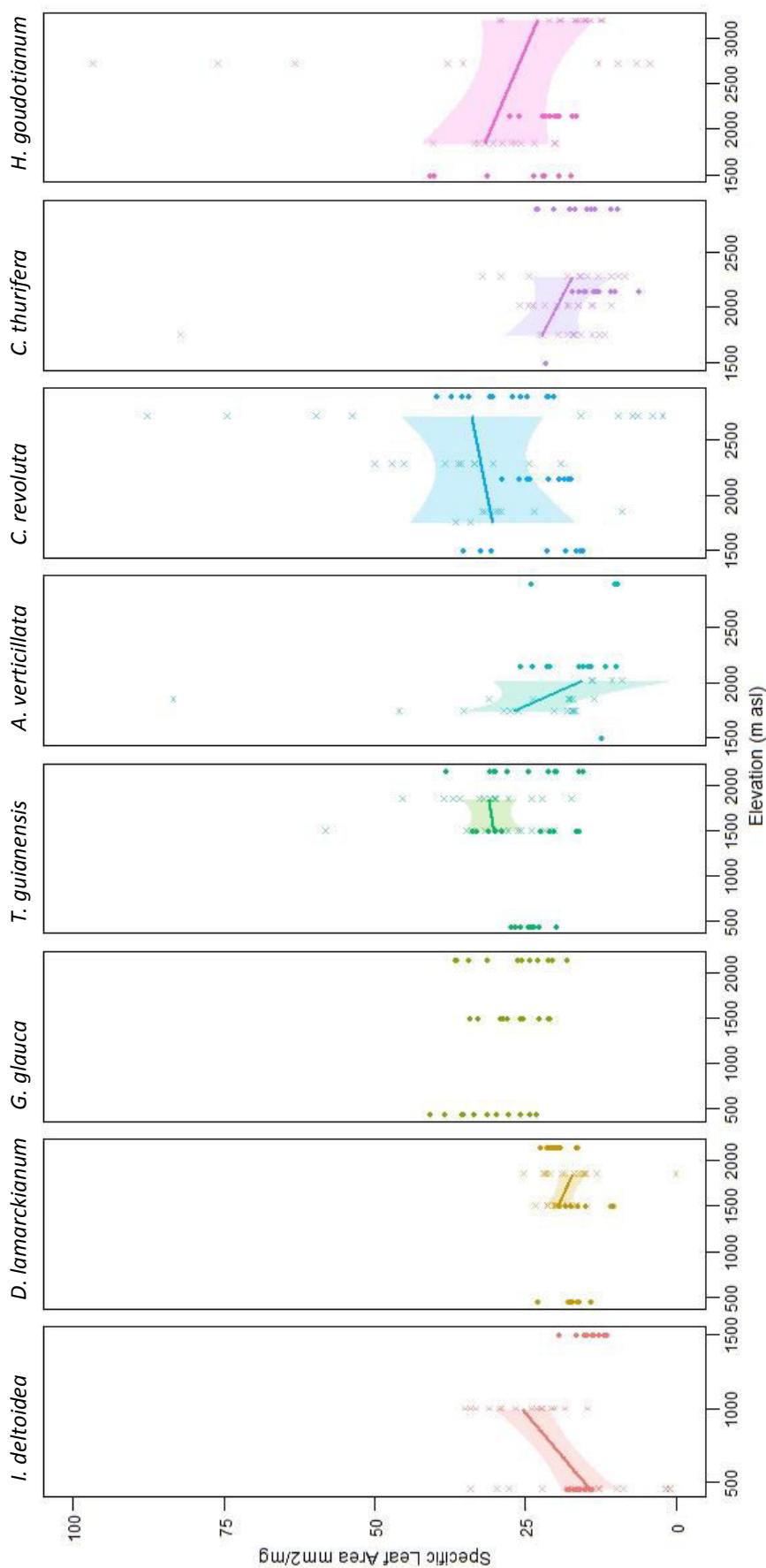


Figure 6-5 The species-level response of specific leaf area to elevation. Crosses and trend line; natural seedling variation (chapter 3) and points; transplanted seedling variation (chapter 5)

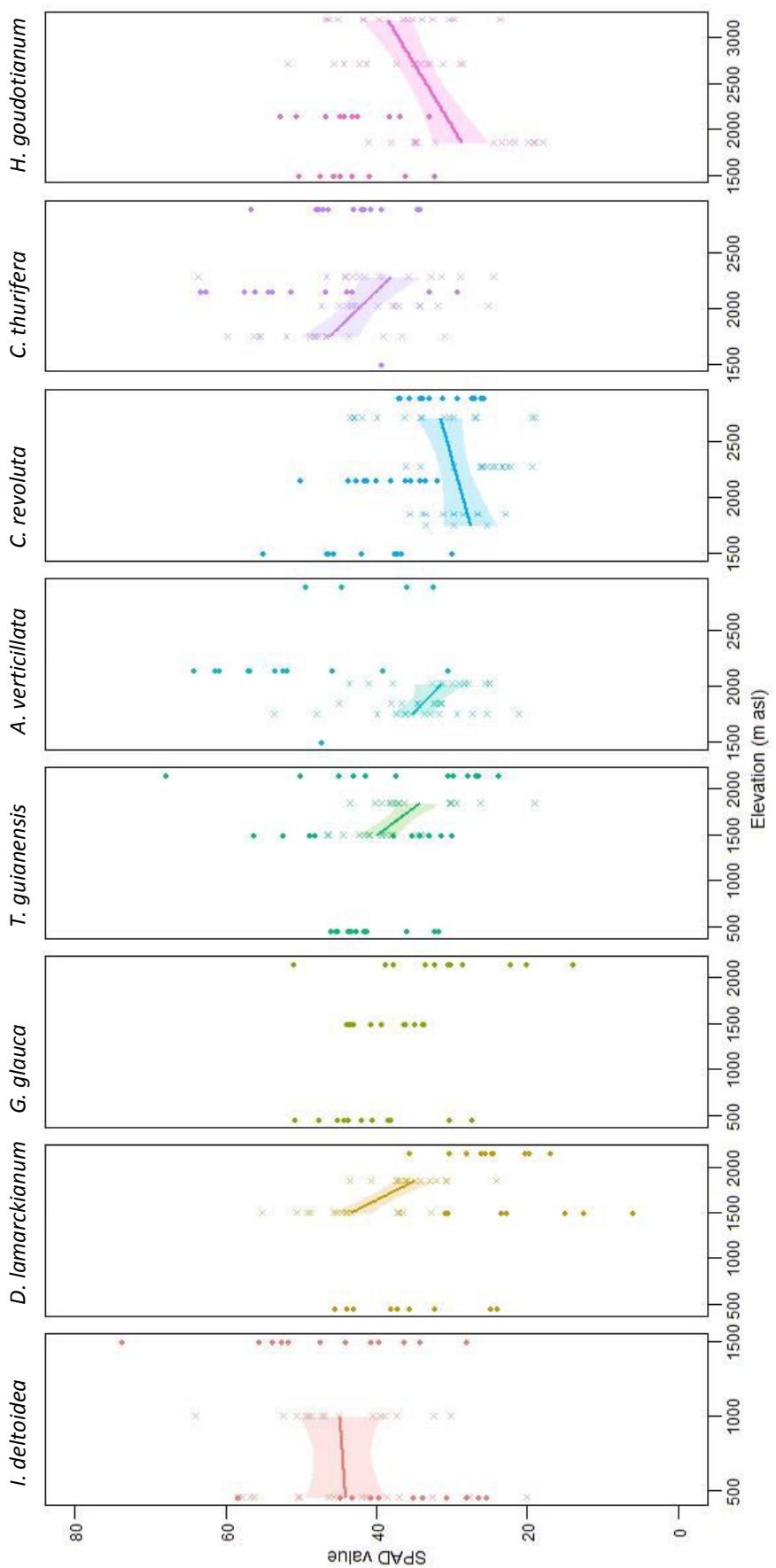


Figure 6-6 The species-level response of chlorophyll content (SPAD value) to elevation. Crosses and trend line; natural seedling variation (chapter 3) and points; transplanted seedling variation (chapter 5)