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Factors Affecting Current and Future Treeline Locations and Dynamics in the Peruvian Andes

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

FACTORS AFFECTING CURRENT AND FUTURE TREELINE LOCATIONS AND
DYNAMICS IN THE PERUVIAN ANDES

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Evan Mitchell Rehm

2015

To: Interim Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Evan Mitchell Rehm, and entitled Factors Affecting Current and Future Treeline Locations and Dynamics in the Peruvian Andes, having been approved in respect to style and intellectual content, is referred to you for judgment.

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ABSTRACT OF THE DISSERTATION
FACTORS AFFECTING CURRENT AND FUTURE TREELINE LOCATIONS AND
DYNAMICS IN THE PERUVIAN ANDES

by

Evan Mitchell Rehm

Florida International University, 2015

Miami, Florida

Professor Kenneth J. Feeley, Major Professor

The elevational distributions of tropical treelines are thought to be determined by temperature, and are predicted to shift upslope in response to global warming. In contrast to this hypothesis, global-scale studies have shown that only half of all studied treelines are shifting upslope. Understanding how treelines will respond to climate change has important implications for global biodiversity, especially in the tropics, because tropical treelines generally represent the upper-elevation distribution limit of the hyper-diverse cloudforest ecosystem. In Chapter 1, I introduce the idea that grasslands found above tropical treelines may represent a potential grass ceiling which forest species cannot cross or invade. I use an extensive literature review to outline potential mechanisms which may be acting to stabilize treeline and prevent forest expansion into high-elevation grasslands. In Chapters 2-4, I begin to explore these potential mechanisms through the use of observational and experimental methods. In Chapter 2, I show that there are significant numbers of seedlings occurring just outside of the treeline in the open grasslands and that seed rain is unlikely to limit seedling recruitment above treeline. I also show that microclimates outside of the closed-canopy cloudforest are highly variable and that mean

temperatures are likely a poor explanation of tropical treeline elevations. In Chapter 3, I show that juvenile trees maintain freezing resistances similar to adults, but nighttime radiative cooling near the ground in the open grassland results in lower cold temperatures relative to the free atmosphere, exposing seedlings of some species growing above treeline to lethal frost events. In Chapter 4, I use a large-scale seedling transplant experiment to test the effects of mean temperature, absolute low temperature and shade on transplanted seedling survival. I find that increasing mean temperature negatively affects seedling survival of two treeline species while benefiting another. In addition, low temperature extremes and the presence of shade also appear to be important factors affecting seedling survival above tropical treelines. This work demonstrates that mean temperature is a poor predictor of tropical treelines and that temperature extremes, especially low temperatures, and non-climatic variables should be included in predictions of current and future tropical treeline dynamics.

TABLE OF CONTENTS

CHAPTER	PAGE
I	THE INABILITY OF TROPICAL CLOUD FOREST SPECIES TO INVADE GRASSLANDS ABOVE TREELINE DURING CLIMATE CHANGE: POTENTIAL EXPLANATIONS AND CONSEQUENCES 1 ABSTRACT 2 INTRODUCTION 3 WHAT DETERMINES THE LOCATION OF TREELINE IN THE TROPICS ..5 PAST AND PRESENT TROPICAL TREELINE SHIFTS AND CONDITIONS 14 TROPICAL TREELINES IN THE FUTURE 18 STATIONARY TROPICAL TREELINES AND CLOUD FOREST BIODIVERSITY 20 ACKNOWLEDGMENTS 24 REFERENCES 28
II	FOREST PATCHES AND THE UPWARD MIGRATION OF TIMBERLINE IN THE SOUTHERN PERUVIAN ANDES 35 ABSTRACT 36 INTRODUCTION 38 MATERIALS AND METHODS 41 RESULTS 46 DISCUSSION 48 CONCLUSION 53 ACKNOWLEDGMENTS 54 REFERENCES 60
III	FREEZING TEMPERATURES AS A LIMIT TO FOREST RECRUITMENT ABOVE TROPICAL ANDEAN TREELINES 68 ABSTRACT 69 INTRODUCTION 71 METHODS 75 RESULTS 82 DISCUSSION 84 ACKNOWLEDGMENTS 89 REFERENCES 93
IV	EXPERIMENTAL SEEDLING TRANSPLANTS SHOW THAT FACTORS OTHER THAN MEAN TEMPERATURE MAY DETERMINE TROPICAL TREELINE DYNAMICS 98 ABSTRACT 99 INTRODUCTION 101 METHODS 104 RESULTS 110

DISCUSSION.....	112
ACKNOWLEDGMENTS	118
REFERENCES	125
V CONCLUSIONS AND FUTURE DIRECTIONS.....	135
VITA.....	143

LIST OF TABLES

TABLE		PAGE
CHAPTER II		
2.1	Descriptions of 14 study transects, extending from forest below timberline to above-timberline forest patches in Manu National Park, Cusco, Peru.	55
2.2	Daily mean, mean high and mean low temperature, overall minimum temperature and total number of days below freezing as measured at five habitats along three study transects over the course of one year (May 2011 – Apr 2012). Letters represent significant differences ($p < 0.05$) in temperature between habitats within a transect according to label permutation tests.	56
CHAPTER III		
3.1	Estimated freezing resistances (LT_{50} ; $^{\circ}C \pm (1 SE)$) of four dominant treeline species measured during two different seasons (cold and warm) at two life stages (adult and juvenile). $n = 6$ for each species, life stage, and season combination. Significant differences (posthoc Tukey honestly significant difference tests, $p < 0.05$) are shown in bold and represent difference in freezing resistance within the same season and species but between life stage. ...	90
CHAPTER IV		
4.1	Results of generalized linear mixed-effects models (GLMMs) and generalized linear models (GLMs) testing which predictor variables best explain survival of transplanted seedlings. GLMMs were used for <i>W. fagaroides</i> as there were sufficient seedlings available to create full replication across all treatments and study sites. GLMMs included fixed effects of mean temperature since transplant, absolute low temperature since transplant, transplant season, shade, and the interactions between shade and mean temperature and shade and low temperature and transplant site entered as a random effect. GLMs were used for <i>G. nitida</i> , <i>C. cuneata</i> , and <i>S. baehinii</i> because seedlings were available for transplant at only two of four transplant sites. GLMs included the same fixed effects used in the GLMMs except for transplant season because all seedlings were transplanted during the same season. For both GLMMs and GLMs variable selection followed a backward selection procedure using the Likelihood Ratio Test and $p = 0.05$	119
S4.1	Mean and absolute low temperatures of each treatment for each transplant site from 1 May 2012 – 24 July 2014. Temperatures are given to provide a reference for the efficacy of the experimental treatments. Note that	

temperature measurements given here are from the entire experimental period but actual temperature measurements for each species, treatment, and transplant site combination may vary because microclimatic measurements were taken from the day individuals were transplanted which was often shorter than the full measurement period.....129

S4.2 Summary data for seedlings transplants of all species under all treatments and transplant sites. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.....130

LIST OF FIGURES

FIGURE		PAGE
CHAPTER I		
1.1	The area of land in the tropics and above 1000m a.s.l. (a) per 100 meter elevational bands under different habitat types in (b) the neotropics, (c) tropical Africa, Madagascar and southwest Asia, and (d) Tropical southeast Asia, Australia, and India. Elevations are based on the SRTM Digital Elevation Model (Rabus et al. 2003) with a resolution of 30 arc seconds and habitat types are based on WWF biome classifications (Olson et al. 2001). In the neotropics, the extent of land area increases at high elevations due to the shift from steep, forested slopes to a relatively-flat hill and valley topography dominated by montane grasslands (locally referred to as puna and páramo) above ~3600m a.s.l. Shading is consistent between all panels. For clarity we grouped the high elevation grasslands of South America (e.g. puna and páramo), Africa (e.g. Ethiopian Highlands and moorlands), and southeast Asia, Australia and India (e.g. subalpine heathlands) into one category of montane grasslands and shrublands.....	25
1.2	(a,b) Gradual and (c,d) abrupt treeline ecotones between tropical montane cloud forests and the alpine grassland habitats (puna) in the Peruvian Andes. Treeline (sometimes called timberline) is defined as the transition between the upper elevational limit of closed canopy forest with trees at least 3 m in height and the overlying grasslands (Körner 2012).....	27
CHAPTER II		
2.1	The mean number of seeds collected and seed diversity (Shannon-Weiner index) per square meter of seed trap at different distances from the timberline (light gray) and patch (dark gray) as measured over a period of 12 months along 14 study transects. Letters indicate significant differences ($p < 0.05$) according to label permutation tests and vertical bars indicate 95% CIs.	57
2.2	Average number of seedlings per m ² along timberline and patch transects (n = 14). Letters refer to significant differences ($p < 0.05$) according to label permutation tests and vertical bars indicate 1 s.d..	58
2.3	Daily mean, daily mean high, and daily mean low temperatures within 5 different habitats (within forest, at forest-puna ecotone [timberline], in open puna, at puna-patch ecotone, within patch) along three arbitrarily chosen study transects. White lines represent mean temperatures.	59
S2.1	Schematic diagram of sample transect showing locations of above-ground forest	

patches, timberline, the puna matrix, and associated habitat ecotones and edges. Distance between forest patch and timberline varied between transects. Forest-puna ecotone refers to the edge between forest and puna at both timberline and patches while forest-patch edge refers to only the forest patch and puna edge.....66

S2.2 Schematic diagram of seed trap transects at timberline and above-timberline forest patches. Traps were deployed in an unbalanced design with 16 traps at patches and 12 at treeline at each of the 14 study transects. Seed traps 10 m into the puna were only present at patches.67

CHAPTER III

3.1 Variation of freezing resistance (LT_{50} ; $^{\circ}C \pm (1 SE)$) for each species at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Asterisks represent significant differences (posthoc Tukey honestly significant difference tests, $p < 0.05$) in freezing resistance within the same species and life stage but during different seasons.91

3.2 Variation of freezing resistance (LT_{50} ; $^{\circ}C \pm (1 SE)$) among species at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Letters represent significant differences (posthoc Tukey honestly significant difference tests, $p < 0.05$) in freezing resistance within a season and life stage but among different species. For adults, there were no significant differences among species for either season.92

CHAPTER IV

4.1 Mean (solid line) and 95% confidence intervals (shaded area) for the modeled relationship between *W. fagaroides* seedling survival and mean temperature across all transplant sites and treatments according to generalized linear mixed-effects models. Points indicate observed results of experiments. Different symbols distinguish results from different transplant sites while shading distinguishes different experimental treatments. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.121

4.2 Modeled relationships between *S. baehinii* seedling survival and a) mean temperature and b) absolute low temperature. Solid lines represents the modeled mean and the shaded area is the 95% confidence intervals as determined by generalized linear models. Squares are results from the high-elevation site A while diamonds are results from the low-elevation site. Different shades of symbols represent different treatments; black = treeline,

dark gray = 60% shade with nighttime warming, light gray = 60% shade with minimal nighttime warming, and white = open grassland.122

4.3 *C. cuneata* seedling survival and a) mean temperature, b) absolute low temperature and c) shade. Solid lines in a) and b) represent the modeled mean and the shaded area is the 95% confidence intervals as determined by generalized linear models. Squares are results from the high-elevation site A while diamonds area results from the low-elevation site. Different shades of symbols represent different treatments; black = treeline, dark gray = 60% shade with nighttime warming, light gray = 60% shade with minimal nighttime warming, and white = open grassland. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.123

4.4 *G. nitida* seedling survival under various treatments. Seedlings planted into the treeline, and two shade treatments were classified as being Shaded and seedlings transplanted into the open grassland were classified as Not Shaded ...124

CHAPTER I

THE INABILITY OF TROPICAL CLOUD FOREST SPECIES TO INVADE GRASSLANDS ABOVE TREELINE DURING CLIMATE CHANGE: POTENTIAL EXPLANATIONS AND CONSEQUENCES

Rehm, E.M. and K.J. Feeley. *in Press*. The inability of tropical cloud forest species to invade grasslands above treeline during climate change: potential explanations and consequences. *Ecography*.

ABSTRACT

The upper elevational range edges of most tropical cloud forest tree species and hence the “treeline” are thought to be determined primarily by temperatures. For this reason, the treeline ecotone between cloud forests and the overlying grasslands is generally predicted to shift upslope as species migrate to higher elevations in response to global warming. Here, we propose that, despite rising mean temperatures, other factors are preventing tropical trees from shifting or expanding their ranges to include high elevation areas currently under grassland, resulting in stationary treelines. The inability of cloud forest species to invade the grasslands, a phenomenon which we refer to as the ‘grass ceiling’ effect, poses a major threat to tropical biodiversity as it will greatly increase risk of extinctions and biotic attrition in diverse tropical cloud forests. In this review, we discuss some of the natural factors, as well as anthropogenic influences, that may prevent cloud forest tree species from expanding their ranges to higher elevations. In the absence of human disturbances, tropical treelines have historically shifted up- and down-slope with changes in temperature. Over the past few millennia, increased human activity has limited forests to lower elevations (i.e., has depressed treelines), and often broken the equilibrium between species range limits and climate. Yet even in areas where anthropogenic influences are halted, cloud forests have not expanded to higher elevations. Despite the critical importance of understanding the distributional responses of tropical species to climate change, few studies have addressed the factors that influence treeline location and dynamics, severely hindering our ability to predict the fate of these diverse and important ecosystems.

INTRODUCTION

Tropical montane cloud forests (hereafter referred to as cloud forests) are considered to be some of the world's most diverse ecosystems because of the large number of endemic and endangered species that they support (Myers et al. 2000). Many cloud forest species at low and middle elevations have already begun responding to contemporary increases in temperatures by shifting their distributions upslope (Freeman and Class Freeman 2014, Rehm 2014). To sustain biodiversity in the face of climate change, cloud forest species must be continue to shift their distributions upslope and expand their ranges into the grassland areas found above the current upper elevational distribution limit of the cloud forest ecosystem. The distributional limit of forest, or ecotone, is known as the treeline and in the tropics is formed by the convergence of dozens of cloud forest species' upper range edges and grassland lower range edges (Gentry 1988).

Interest in treelines as indicators of climate change has been growing because treeline locations are thought to be controlled by low temperatures (Körner 1998, Jobbágy and Jackson 2000, Körner and Paulsen 2004) and therefore are expected to shift upslope with warming. An upward shift in treeline elevation would provide a clear and visible indication that cloud forests are responding to climate change by expanding or shifting their ranges into the grasslands and alpine areas above treeline (Harsch et al. 2009). In contrast to this expectation, global-scale studies have shown that only half of all studied treelines are shifting upslope and that in some cases high elevation forests and associated treelines are actually retreating downslope (Harsch et al. 2009).

Our ability to predict the future persistence and distribution of cloud forests under climate change is highly dependent on understanding what controls treeline location and dynamics. In a recent global meta-analysis, Harsch *et al.* (2009) found that only 52% of the 166 treeline study sites worldwide have reported upslope treeline movement with modern climate change. As a consequence of geographic research biases, the included studies focused primarily on European and North American locations, with only seven treeline sites (4.2%) from the tropics - all from the Andes. Not a single one of these seven tropical sites reported an upslope movement of treeline forest in response to the recent rise in global temperatures (Harsch *et al.* 2009).

In this review, we examine the possible mechanisms that limit the upper elevational distributions of cloud forest tree species (and hence determine treeline elevation) and the potential movements of tropical treelines during climate change. Most information related to tropical treelines comes from the Andes, especially within the context of climate change. In addition, in the Andes very large expanses of land area exist above tropical treelines (Fig. 1). In other tropical regions, such as East Africa and the Malay archipelago, mountains generally do not reach sufficient heights to form a climatic treeline or, when present, treelines are restricted to isolated mountains that are separated from each other by large non-forested habitats. This review will therefore focus primarily on the tropical Andes, but we will draw on available examples from other tropical montane systems and temperate treelines when appropriate. While we draw on some general information from temperate treeline studies (reviewed in Holtmeier 2009, Körner 2012), one goal of this review is to focus explicitly on tropical systems and to explore how factors controlling treeline location and dynamics differ between tropical and

temperate systems. By focusing on cloud forest biodiversity and the upslope shift of tropical treelines we do not discount the value of the biodiverse grasslands found above tropical treelines and the ecosystem services they provide (Luteyn 1999). However, plant diversity and endemism tends to peak at mid-elevations (1500 – 3500 m.a.s.l) within the cloud forest belt (Kessler 2002), hence we emphasize the importance of tropical treeline shifts for the maintenance of cloud forest tree biodiversity.

WHAT DETERMINES THE LOCATION OF TREELINE IN THE TROPICS

Climate

The locations of natural treelines worldwide are believed to be largely determined by temperature, with most treelines occurring at elevations associated with the 5-8 °C soil root zone mean growing season temperature (Körner and Paulsen 2004). Tree growth is severely reduced below the 5 °C tissue formation threshold of higher plants and hence the 5 °C isocline may represent the thermal limit for woody plant growth ("growth limitation hypothesis" - Körner 1998, 2003; but see Wiley and Helliker 2012). Low soil temperature is also linked to slowed soil nutrient cycling and mineralization rates in cloud forests below treeline (Tanner et al. 1998). To our knowledge, however, there has been no attempt to experimentally test for possible controls on tropical treeline position due to edaphic conditions. Therefore soil limitations will not be discussed further.

Tropical treelines mostly occur between 3,000 and 4,000 metres above sea level (m a.s.l.), although some woody species (*Polylepis*) may extend their ranges up to 5,000 m a.s.l but not forming continuous forests (Körner 1998). At high elevations, where mean annual temperatures are below 5°C, upright woody species are commonly replaced by

open grasslands. However, many tropical treelines occur at elevations below the 5°C thermal isocline. We do not contend that cold temperature growth limitations are not applicable to some tropical treeline forests. Rather, we suggest that the presence of tropical treelines at elevations lower than predicted derived from the “growth limitation hypothesis” indicates that additional factors beyond mean temperature are needed to explain treeline growth form and location in many areas.

One striking difference in climate at temperate *versus* tropical treelines is the degree of seasonality. At temperate treelines, there are often large seasonal fluctuations in temperature, with winters being relatively harsh and plants entering into a state of dormancy during the coldest months of the year (Körner 2012). In addition, there is often an insulating layer of snow cover during the winter at temperate treelines that protects young seedlings from extreme cold temperatures. In contrast, at tropical treelines there is little or no seasonality in temperature and daily temperature fluctuations exceed mean annual temperature variation.

The lack of seasonality in the tropics means that plants occurring in treeline forests maintain actively growing tissue, which is known to be more sensitive than dormant tissue, throughout the year even during mild freezing events (Sakai and Larcher 1987). In addition, microclimate is known to be ‘harsher’ in the grasslands above treeline than in the forests below treeline. For example, frost events are typically more frequent and more severe in these grasslands relative to nearby closed-canopy forests, especially at ground level where seedlings maintain most of their leaves (Rada et al. 2009, Rehm and Feeley 2013, Rehm and Feeley *in Press*). Therefore, extreme low temperature events may be more important than mean temperatures in determining the upper elevational limits of

some tropical treeline-forming species and the overall treeline dynamics (Wesche et al. 2008, Harsch and Bader 2011). Wesche et al. (2008) demonstrated that the frequency and severity of frost events, coupled with water stress, limit recruitment above Andean and African treelines. It has also been suggested that freezing temperatures directly limit elevational distributions of some Venezuelan treeline species while not affecting others (Cavieres et al. 2000). Similarly, Rehm and Feeley (*in Press*) found that juveniles of several dominant treeline forming species in Peru were vulnerable to tissue damage due to low temperatures common in the grasslands above treeline forests. Extreme cold temperature events may prevent trees from recruiting into the grasslands outside of the relatively well-buffered thermal environment of the forest, leading to large-scale mortality events, potentially killing entire cohorts of seedlings and providing a possible explanation for the abrupt nature of many tropical treelines (Fig. 2; Harsch & Bader, 2011).

Beyond temperature, the importance of precipitation in determining tropical treeline locations can not be overlooked because of the unique hydrology of cloud forest systems. Cloud forests occur in areas where cloud mist and immersion are frequent, and significant moisture inputs come from fog interception and horizontal precipitation (Grubb 1977, Bruijnzeel and Proctor 1995). The location of the “cloud immersion zone” may therefore be the ultimate determinant of cloud forest distributions. However, cloud patterns and associated precipitation vary widely among tropical mountains, with precipitation increasing with elevation at some sites (Kitayama 1992, Schawe et al. 2010) and decreasing with elevation at others (Veneklaas and Van Ek 1990, Rapp and Silman 2012). While cloud occurrence is generally more variable and less frequent at tropical

treelines compared to lower elevations, cloud immersion at and above treeline forests is still a regular event (Halladay et al. 2012a; pers. obs.).

A closed-canopy treeline cloud forest presumably creates a wetter microclimate than those found in the open grasslands, thereby potentially allowing for regeneration of drought-sensitive cloud forest species within the forest but not outside. Indeed, even with regular cloud immersion and significant moisture inputs above tropical treelines, the high mortality of seedlings recruiting into the grasslands has been attributed in part to water stress at some sites (Smith 1977, Wesche et al. 2008), but not at others (Rada et al. 1996). It has been posited that high-elevation tropical systems are generally more arid than temperate systems as a result of high evaporative demand and decreasing rainfall in tropical mountains (Leuschner 2000), which may partially explain the presence of water stress in grasslands above the treeline forest. In addition to the direct effects, water availability may indirectly influence treelines through natural or anthropogenically set fires (see more on fires below; Hemp 2005). Yet it is difficult to determine the exact role that precipitation and moisture play in influencing treeline elevations, as a result of the limited information available about water relations at tropical treelines (e.g., soil moisture data across tropical treelines ecotones) and the overall lack of long-term climatic records from tropical mountains.

Solar radiation

Relative to temperate systems, tropical treelines occur at higher elevations than in temperate sites. Exposure to solar radiation, especially UV, is thus elevated at tropical treelines since radiation increases with elevation and decreases with latitude. The regular

occurrence of freezing nights followed by intense morning light at tropical treelines may also lead to increased risk of low-temperature induced photoinhibition which can have a large effect on treeline dynamics (Bader et al. 2008). Bader et al. (2007) found that forest seedlings transplanted into the open grasslands above Ecuadorian treelines had higher survival and showed lower photooxidative tissue damage when provided shade than seedlings planted without shade. The abundance of adaptive mechanisms (e.g., increased antioxidants) that high elevation trees possess to deal with excess insolation (reviewed in Körner [2003]) suggests that light levels in tropical mountains play an important role in treeline dynamics. It is possible that adaptations to high light environments allow trees to persist within, or just outside of, the cloud forest canopy but are insufficient to protect trees in the open grasslands above tropical treelines where light intensities are high.

Forest species interactions with grasses

Given the harsh environmental conditions at and above treeline, ecological theory predicts that grasses should play a more facilitative than competitive role in seedling establishment within the grassland matrix above treeline (Bertness and Callaway 1994). It has even been argued that forests will advance upslope only in cases where ecological facilitation by alpine vegetation allows tree recruitment beyond the current treeline ecotone (Smith et al. 2003). However, grasses and alpine vegetation have been shown to play both facilitative and inhibitory roles in seedling establishment outside of some temperate treeline forests (Noble 1980, Ball et al. 2002, Callaway et al. 2002). It is difficult to apply observations from temperate to tropical treelines systems because of the

drastically different climatic and environmental conditions between the two regions (e.g. presence vs. absence of snow, strong vs. little seasonality).

Information specific to the role of plant interactions in determining the location and dynamics of tropical treelines is severely limited. At two separate Andean treeline sites, temperatures in the open grassland were colder near the ground than at the grassland canopy height or in the free atmosphere (Rada et al. 2009, Rehm and Feeley *In Press*), suggesting that grasses do little to buffer tree seedlings against the low temperature extremes that can occur outside of the closed forest canopy. In addition, Smith (1977) concluded that forest seedlings transplanted above a Venezuelan treeline suffered high mortality as a result of the combination of climatic stress and competition with grasses for water during the brief dry season. Grasses may however provide shading for seedlings, which can facilitate the establishment of tree seedlings above the established treeline forest (Bader et al. 2007). Conversely, grasses may also promote fire and increased grazing pressure, both of which have overall negative effects on the establishment of forest species in the grassland matrix (see below). Therefore grasses appear to have only minor facilitative effects on tree species recruitment and may actually play an inhibitory role in the recruitment of trees beyond the established tropical treeline - the opposite of what is believed to occur in most temperate treeline systems.

Seed dispersal, germination, and survival

Some research suggests that reduced dispersal and survival of tree seeds outside of the forest may potentially limit upslope shifts of cloud forest species and treeline with climate change (Dullinger et al. 2004). In Peru, Rehm and Feeley (2013) showed that

overall seed rain abundance and diversity decreased dramatically with distance above the treeline. Cierjacks et al. (2007) found that sowing additional seeds of two *Polylepis* species increased the number of recruiting seedlings for one species both inside and outside of the cloud forest while having no effect for a second species. These studies suggest that seed limitation and germination rates may restrict recruitment above the established treeline. Conversely, Körner (2012) argued that seed limitation and recruitment is unlikely to play an important role in determining treeline formation, but this argument was derived largely from studies of temperate sites. Our understanding of how seed limitations influencing the ranges of cloud forest species and tropical treelines is still very restricted and would greatly benefit from additional studies that investigate seed dispersal, survival, and germination across the treeline ecotone and in adjacent grasslands.

Soil

Potential limits to forest recruitment above tropical treelines associated with soil characteristics remain virtually unstudied. There are several descriptive accounts of soil characteristics either within the tropical treeline forests (Kitayama & Aiba, 2002; Körner & Paulsen, 2004; Cierjacks et al., 2008) or in the grasslands above treelines (Perez, 1987; Valencia et al., 2013), but only limited accounts describing changing soil conditions across the treeline ecotone (Hoch & Körner, 2005; Zimmermann et al., 2010). As far as we are aware, there has been no attempt to experimentally test for possible controls of tropical treeline position as a result of the response to varying edaphic conditions. In

addition, there has been very limited attempts to characterize the importance of mycorrhizal symbionts or other biotic components of soils above treeline

Soil temperatures are believed to be as important as air temperature for individual tropical treeline tree growth, as low temperatures directly limit growth of below- and above-ground biomass (Körner & Paulsen, 2004). Like air temperature, soil temperature fluctuations are larger in grasslands than adjacent treeline forests, largely because of decreased complexity of above ground vegetative structure and increased direct solar radiation at the grassland soil surface (Körner, 2012). This lack of forest canopy means that grassland soils, which are not shaded by forests, actually have warmer average temperatures than soils found in the adjacent treelines (Körner, 2012). This evidence suggests that at least mean soil temperatures do not limit treeline elevations, but soil temperature extremes have received little attention (Körner, 2012). Low soil temperature is also often linked to slowed soil nutrient cycling and mineralization rates at low temperature distribution limits such as tropical treelines. Below tropical treelines, it has been suggested that high soil moisture found in cloud forest systems further limits nutrient cycling and forest productivity, with nitrogen and phosphorous being the major limiting nutrients (Bruijnzeel & Veneklaas, 1998; Tanner et al., 1998; Benner et al., 2010). However, above tropical treelines, soil moisture measurements are usually limited, with studies lasting for one season or occurring at only one site (Perez, 1987; Valencia et al., 2013). It is plausible that soil moisture can play a role in determining tropical treeline elevations, but this is more likely because of periods of drought as opposed to soil moisture excess (Perez, 1987; Valencia et al., 2013; but see Rada et al., 1996). Given this limited information, it is difficult to determine how soil conditions such as temperature,

moisture, and nutrient availability across the treeline boundary affect tropical treeline positions and additional studies would greatly improve our understanding of these processes.

Human impacts

It has been argued that past and ongoing human disturbances have reduced the occurrence of tropical forest at high elevations and artificially lowered, or “depressed”, the elevation at which many tropical treelines occur (Ellenberg 1979, Young 2009). Human activities at high elevations are diverse and the temporal and spatial scale of such activities can have long-lasting and profound effects on cloud forests and their treelines (Young 2009). Probably the most prevalent activity around tropical treeline forests is the grazing of livestock within alpine grasslands (Ellenberg 1979, Young 2009). Livestock can directly limit tree recruitment into grasslands by the grazing and trampling of seedlings and saplings, and indirectly by increasing soil erosion. Fires associated with livestock grazing may have an even larger impact on tropical treeline location than the livestock themselves (Wesche et al. 2000, Hemp 2005). Fires are often used by pastoralists to stimulate new grass growth and reduce the presence of undesirable forage species such as woody species. If the return interval of fires is shorter than the the time necessary for the slow-growing trees to reach a sufficient height to escape the fire kill zone, then forest succession into the grasslands is essentially reset with each re-occurring fire (Wakeling et al. 2012). Even after fires are suppressed or removed, forest invasions into grasslands may be significantly delayed (Di Pasquale et al. 2008). In addition to livestock activities, clearing of tropical cloud forests to expand agricultural areas and

continuing cultivation of crops in the high elevation grasslands could further stabilize or even lower treeline elevations (Young 2009). Treelines that were once depressed by anthropogenic activities may be maintained at lower elevations even after human activities are halted as a result of inhibitory mechanisms (e.g., solar radiation, freezing events) that prevent tree establishment and forest encroachment into the grasslands, reinforcing the stability of the forest boundary (Harsch and Bader 2011).

All told, throughout the tropics the locations and dynamics of treelines are likely to be driven by a complex mix of past and present ecological and anthropogenic processes working at different spatial and temporal scales (Young and León 2007). Although the processes determining species ranges and treeline elevations are clearly complex, we may look at how natural factors and human activities have shaped tropical treeline distributions in the past as a means towards increasing our understanding of how tropical cloud forests and their treelines may be affected by ongoing and future climate change.

PAST AND PRESENT TROPICAL TREELINE SHIFTS AND CONDITIONS

Since the Last Glacial Maximum (LGM – 12,000 years before present), cloud forests and their associated treelines have shifted up and down slope as temperatures changed (Bush et al. 2004, Valencia et al. 2010). Even in regions where temperature fluctuations are highly correlated with past cloud forest shifts (e.g. the Andes), extended dry periods may represent a temporary switch to systems driven more by moisture than by temperature (Bush et al. 2004). Regardless, past cloud forest shifts are mostly attributed to fluctuations in temperature.

Beginning several thousand years ago, natural vegetation patterns in many tropical regions became masked and difficult to interpret from the palaeo-ecological record as a consequence of the increased influence of humans across the landscape (Hillyer et al. 2009, Valencia et al. 2010). Even though temperatures remained fairly stable or even increased during this period, increased human activities above treeline may have prevented cloud forests from shifting upslope, and possibly even driven them downslope (Di Pasquale et al. 2008, Bakker et al. 2008, Valencia et al. 2010). However, starting approximately 500 years ago, human influence over many tropical montane landscapes declined markedly with the influx of European colonizers (e.g., Spanish conquest throughout the Andes), leading to pronounced reductions of fire activity in some tropical grasslands over the last several centuries (Urrego et al. 2010). The trend may have been reversed over the last century as humans once again began to utilize high elevation areas for agriculture and livestock grazing. Even if human impacts had been reduced, at present, tropical treelines remain remarkably stationary even though climates have continued to become more favorable to upslope shifts of cloud forest species ranges (Harsch et al. 2009; but see Bakker et al. 2008). The lack of movement most likely indicates that inhibitory mechanisms other than temperature and/or the effects of past and continuing human activities may be stabilizing treelines by preventing cloud forest species from expanding their ranges to include areas currently under grassland.

Most recent analyses of cloud forest species migrations and treeline movements in response to contemporary climate change are restricted to the Andes. The mean annual temperature in the tropical Andes has increased by an average of 0.1- 0.39° C per decade since the mid-1900s, but warming has accelerated through time and was up to three times

faster during the end of the 20th and start of the 21st centuries than during previous decades (Vuille and Bradley 2000, Vuille et al. 2003). The means that temperature increases over the past several decades are occurring at rates faster than after the LGM, representing a major increase in the velocity at which cloud forest species must migrate upslope to track temperatures (Bush et al. 2004, Loarie et al. 2009).

In what we believe to be the most detailed assessment of tropical treelines in any region to date, Lutz et al. (2013) found that only 18% of the treeline segments that they examined in Peru had shifted upslope over a 42 year study period. The majority of treelines that shifted upslope were located within protected areas relatively free from human impacts. Even then, upslope treeline forest shifts were at a rate of < 2% of the pace required for forest species to remain at equilibrium with rising mean temperatures. In contrast to the protected areas, the majority of treelines in unprotected areas remained stationary or even retreated further downslope, possibly a result of continuing human disturbances (Lutz et al. 2013). These results indicate that even in protected areas with presumably decreased human disturbance, mean annual temperature may be a poor predictor of tropical treeline position and other factors are determining the location of the treeline ecotone. However, we are unaware of any attempt to model current or future tropical treeline positions using any climatic or environmental variable other than mean annual temperature.

The influence of precipitation has been severely understudied in tropical mountains with the exception of some in-depth analyses from the Andes. It appears that over the past half century, areas in northern Peru are becoming wetter overall while areas to the south are becoming drier (Vuille et al. 2003). There is also a trend of decreasing

cloud cover throughout the Andes with increasing north Atlantic sea surface temperatures and frequency of El Niño events (Halladay et al. 2012b). Of particular interest is that cloud cover, and hence precipitation, appears to be decreasing over the southwest Amazon during the dry season, which in turn reduces moisture in the cloud forest zone of Peru and Bolivia, intensifying the dry seasons experienced by tropical cloud forests (Halladay et al. 2012b). This research suggests that changes in precipitation and cloud inundation may be increasing water stress and fires near tropical treelines, perhaps preventing forest species from expanding into the drier, more seasonal grasslands, as has been documented in African mountains (Hemp 2005).

Deforestation and landuse change can also influence local and regional climates around treeline, especially temperature and water availability (Garcia-Carreras and Parker 2011). In the Peruvian Andes, cloud forests located near deforested areas had warmer and drier climates than areas far from deforested zones (Larsen 2012). In addition to deforestation within the cloud forest zones themselves, drying effects from deforested lowland landscapes may carryover and reduce precipitation in nearby cloud forests and associated treelines (Lawton et al. 2001). Therefore, aside from just global patterns of climate change, regional and local landuse change might have significant effects on local climate patterns, preventing forest species from establishing beyond the current treeline or even causing downslope shifts of the treeline. How these changes are affecting other biotic (e.g., competition with grass, seed dispersal) and abiotic (e.g., nutrient cycling) components of tropical treeline environments has yet to be addressed.

TROPICAL TREELINES IN THE FUTURE

The recent patterns of climate change are projected to intensify over the next century (IPCC 2013). In the tropical Andes, temperature is predicted to increase by an additional 4.5-5 °C by 2100, with faster warming at higher elevations (Vuille et al. 2008, Urrutia and Vuille 2009). If the distributions of cloud forest species and associated tropical treelines are determined primarily by temperature, then upslope cloud forest migrations of 900-1,000 vertical meters will be needed to track temperatures over the next 100 years (Vuille et al. 2003). However, this predicted migration does not account for changes in precipitation and cloud cover. In some tropical regions such as the Andes, precipitation is predicted to increase during the wet season and decrease during the dry season, increasing the seasonality experienced by cloud forest species (Vuille et al. 2008, Halladay et al. 2012b). Exacerbating this uncertainty varying precipitation patterns is the projection that cloud cover will continue to decrease throughout the tropical Andes into the future, especially during the dry season (Halladay et al. 2012b).

Precipitation and climatic patterns at local scales are also linked to regional changes in landuse. Human impacts in many tropical treeline systems have remained stable or even decreased because of shifting economic and social drivers of landuse (Aide et al. 2010), but effects of landuse change from adjacent lowland systems on the climates of tropical montane systems may intensify. For example, even with slowing deforestation rates (Hansen et al. 2013), any clearing of lowland rainforests could lead to further drying of adjacent tropical cloud forests and associated treelines. Reduced precipitation means that drought-sensitive cloud forest species will be exposed to longer and more intense dry periods in the future, especially near treeline. Furthermore, prolonged dry seasons will

increase the rate of natural and human-caused fires in the already fire-prone grasslands above treeline.

In addition to changes in temperature and precipitation, atmospheric concentrations of CO₂ are also increasing rapidly but it remains unclear how treeline vegetation will respond to increased CO₂ concentrations. Young trees of some species grown at and above temperate treelines experienced net carbon gains when exposed to elevated CO₂, but the enrichment effect diminished after just a few years (Dawes et al. 2011). This evidence of limited CO₂ enrichment effects, along with an analysis of non-structural carbohydrates in trees growing at treeline, suggests that the majority of individuals at treeline are not carbon limited and therefore are not predicted to benefit significantly from increased carbon availability (Hoch and Körner 2012). However, little to no effort has focused on testing the potential effects of CO₂ enrichment for tropical treeline species (but see Hoch and Körner 2005).

Unlike growth, freezing tolerances of many plant types, including trees at treeline, appears to be reduced by rising CO₂ concentrations (Woldendorp et al. 2008, Martin et al. 2010). This reduced freezing tolerance is largely due to changes in plant phenology (Repo et al. 1996) or alterations to physiological processes which in turn lead to reduced freezing tolerances (Loveys et al. 2006). Therefore, as CO₂ concentrations continue to rise, trees growing at treeline may become more susceptible to freezing events. However, freezing resistance is strongly linked to temperature (Sakai and Larcher 1987), so concurrent increases in temperatures at treeline elevations complicate the predictions of CO₂ effects.

Given our general lack of knowledge, it is also difficult to predict how other factors such as competition with grasses, seed dispersal, and soil nutrient cycling will change at and above tropical treeline forests during future climate change. Without additional studies addressing the basic ecological processes related to species distributions and ecotone formation, predictions of how these complex interactions will change with climate are tenuous at best.

STATIONARY TROPICAL TREELINES AND CLOUD FOREST BIODIVERSITY

Areas where cloud forests are currently distributed have served in the past, and could potentially serve in the future, as climate refugia for tropical lowland species requiring cooler climates in the face of increasing global temperatures (Bush et al. 2004, Feeley et al. 2012). Evidence of distributional shifts of cloud forest plant species is currently limited to two studies - one from Costa Rica and one from the Peruvian Andes (Feeley et al. 2011, 2013). Both of these studies found that cloud forest trees are currently shifting their distributions upslope 2 to 3 times slower than expected given concurrent warming (Feeley et al. 2011, 2013). It is not surprising that we find cloud forest species' upslope migrations lagging behind concurrent shifts in temperature because the majority of plant species globally are shifting at a slower pace than what is required to perfectly track mean temperature (Chen et al. 2011). The fact that the migration rates of many species are lagging behind warming creates the potential for a future extinction debt (Dullinger et al. 2004). Compounding the potential negative effects of slow species migrations, the leading edge of many high-elevation cloud forest species distributions are

not shifting upslope as indicated by stationary treelines. In the few cases where tropical treelines have been observed to shift upslope, they are moving at rates of 12 to over 100 times slower than the shifts in species mean elevations (Feeley et al. 2011, Lutz et al. 2013). This lack of change in species' upper elevational limit indicates that biome boundaries, such as the treeline ecotone, can pose formidable barriers to species migrations, further slowing or preventing species movements (Feeley et al. 2014).

One important aspect that remains unclear is how the inability of cloud forest species to expand their ranges to higher elevations will have cascading effects on other taxonomic and trophic groups. Cloud forest animals are generally shifting their ranges upslope faster than are plants (Freeman and Class Freeman 2014, Rehm 2014). These differential migration rates suggest that forest-dependent animal species are either more intolerant of rising temperatures and/or better capable of shifting their distributions to track climates. However, if trees do not shift their distributions upward, there will be no forest for these animal species to move into at higher elevations. What's more, there are a large number of plant species (e.g., arboreal epiphytes) that depend on cloud forest trees for structure and water capture, and presumably these species will only be able to shift to higher elevations after trees have expanded their ranges into the grasslands above current treeline.

Similar to other alternative stable state systems, it is possible that tropical cloud forests will respond to climate change in a punctuated fashion resulting in rapid upslope shifts of treeline, followed by relatively long periods of stasis (Wilson and Agnew 1992, Bader et al. 2008). It has also been posited that tropical cloud forests are still responding to temperature increases from the late-Holocene (Urrego et al. 2010). If this is true, then

tropical treeline shifts will be much too slow to keep pace with current and future warming. Given sufficient time, cloud forest communities and their associated treelines may reach elevations at which they are in equilibrium with climate. In the interim, discordant rates of species range shifts could have major implications for populations and range sizes.

An important, but often overlooked consideration is that in much of the neotropics, land area increases above current treeline because of the presence of large high elevation plateaus or hill-valley systems (Fig 1). If cloud forest species do indeed shift their ranges upslope past current treeline then the potential exists for some cloud forest species to gain habitat and potentially “benefit” from climate change in the future (Feeley and Silman 2010). The palaeo-ecological records indicate that such shifts have occurred in the past, but as already discussed here, treeline does not appear to be shifting upslope in response to modern climate change.

Stationary tropical treeline forests will result in one of two scenarios for cloud forest species’ ranges; 1) ranges can stay relatively stable and individuals can acclimate or adapt to the changing environmental conditions *in situ*, or 2) species can lose range area at their lower elevational limit while failing to gain area at their high elevational limit, resulting in decreasing range area and population sizes (Feeley et al. 2012). Current evidence suggests that acclimation or adaptation of tropical cloud forest trees is unlikely, especially considering the rate of current climate change and the long life span and generation times of most tree species (Clark et al. 2003, Feeley et al. 2012). In addition, many tropical cloud forest species are already exhibiting range shifts or contractions, especially at their low elevation limits, in response to rising temperature (Feeley et al.

2011, 2013). Therefore, the latter, more dire scenario appears to be the more likely of the two. For example, in the Andes, cloud forest species are predicted to experience average population losses of 45% if they are prevented from expanding into high-elevation grasslands above current treelines (vs. a potential gain of 20% if they do shift upslope into current grassland areas with warming; Feeley & Silman, 2010). Even if species are able to expand their ranges upwards in the future, many species will still be at increased risks of extinction or will experience severe population bottlenecks in the meantime.

Across the tropics, human impacts play a significant role in determining the location and dynamics of treeline. Yet even in protected areas that are relatively free from human disturbances, shifts in treeline elevation lag well-behind *a priori* expectations (Lutz et al. 2013). These patterns match those found in temperate montane systems, where treeline forests are observed to respond only slowly after the cessation of human disturbance (Camarero and Gutiérrez 2004). These observations suggest that tropical treeline forests may respond by shifting distributions upslope once free of human influences, but that even forests occurring well below their predicted climatic limit may, at best, respond slowly to rising temperatures.

Overall, we still have only a rudimentary understanding of the ecology of tropical cloud forests and their constituent tree species and how these diverse systems will respond to ongoing and future climate change. The Andes provides the most comprehensive understanding of cloud forest and their treelines to date. Yet even in this relatively well-studied region information remains extremely sparse. On the basis of the available information we can surmise that the grassland matrix found above treeline is a harsh environment for individual tree seedlings to establish and grow. Unlike at

temperate treelines, mean temperature does not appear to be the main driver of dynamics at tropical treelines. High temperature variation and extreme cold events above tropical treelines appear to reduce survival and recruitment of tree seedlings in the open grasslands. In addition to temperature, water stress during certain times of year may also negatively affect seedlings growing above treeline. High levels of solar radiation, competition with grasses, and low seed dispersal into the grasslands may also prevent forest seedlings from establishing beyond the forest canopy. Human activities above treeline, such as livestock grazing and fires, can further limit the ability of seedlings to recruit in the grasslands. All of these factors work to stabilize tropical treelines and may create a “grass ceiling” that will prevent cloud forest tree species from shifting their leading range edges upslope during climate warming.

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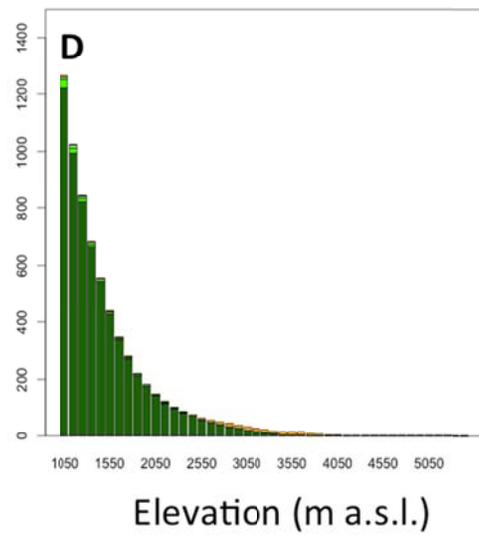
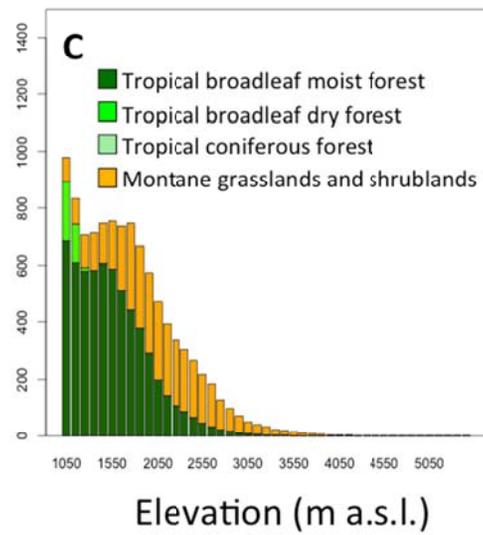
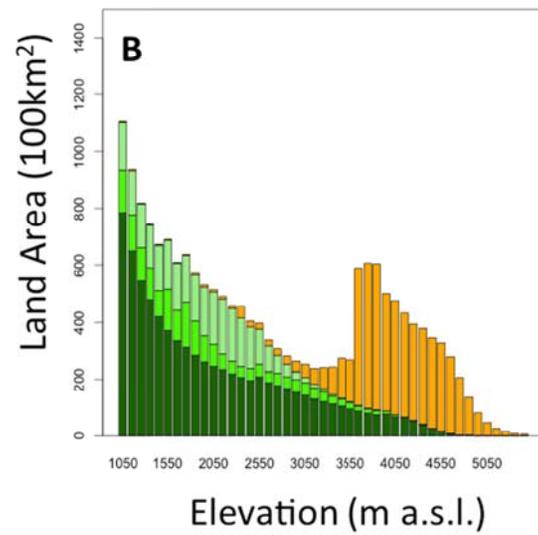
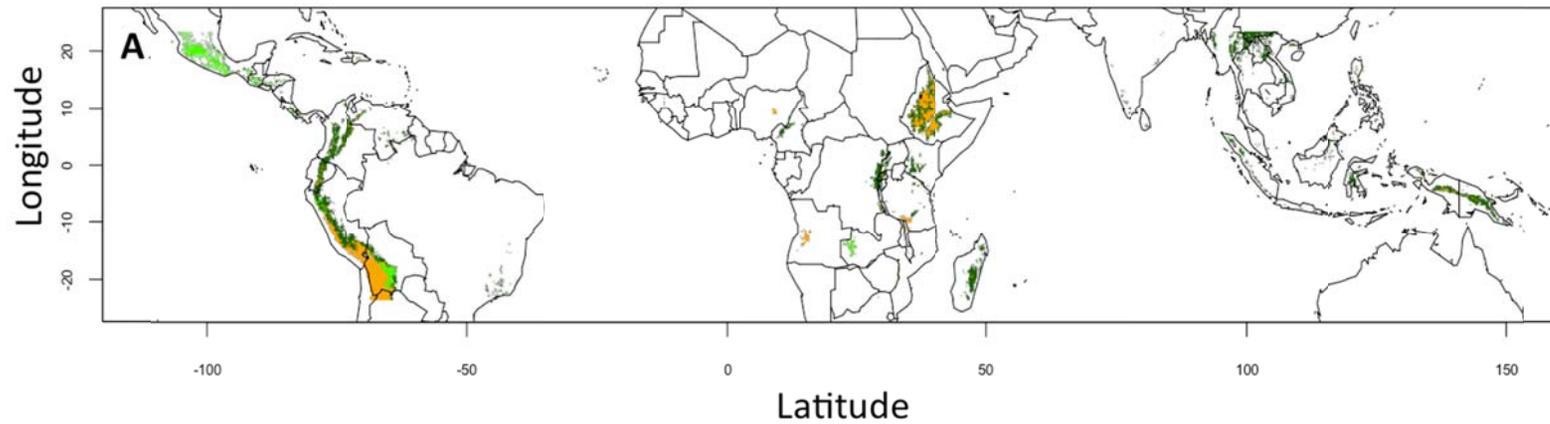


Fig 1.1 The area of land in the tropics and above 1000m a.s.l. (a) per 100 meter elevational bands under different habitat types in (b) the neotropics, (c) tropical Africa, Madagascar and southwest Asia, and (d) Tropical southeast Asia, Australia, and India. Elevations are based on the SRTM Digital Elevation Model (Rabus et al. 2003) with a resolution of 30 arc seconds and habitat types are based on WWF biome classifications (Olson et al. 2001). In the neotropics, the extent of land area increases at high elevations due to the shift from steep, forested slopes to a relatively-flat hill and valley topography dominated by montane grasslands (locally referred to as puna and páramo) above ~3600m a.s.l. Shading is consistent between all panels. For clarity we grouped the high elevation grasslands of South America (e.g. puna and páramo), Africa (e.g. Ethiopian Highlands and moorlands), and southeast Asia, Australia and India (e.g. subalpine heathlands) into one category of montane grasslands and shrublands.



Fig. 1.2 (a,b) Gradual and (c,d) abrupt treeline ecotones between tropical montane cloud forests and the alpine grassland habitats (puna) in the Peruvian Andes. Treeline (sometimes called timberline) is defined as the transition between the upper elevational limit of closed canopy forest with trees at least 3 m in height and the overlying grasslands (Körner 2012).

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CHAPTER II

FOREST PATCHES AND THE UPWARD MIGRATION OF TIMBERLINE IN THE SOUTHERN PERUVIAN ANDES.

Rehm, E.M. and K.J. Feeley. 2013. Forest patches and the upward migration of timberline in the southern Peruvian Andes. *Forest Ecology and Management*. 305: 204-211.

ABSTRACT

Montane plant species around the globe are predicted to shift their distributions upslope in response to increasing temperatures associated with climate change. In the tropical Andes, which are one of the most diverse and threatened of all biodiversity hotspots, many plant and animal species have already started to shift their distributions to higher elevations in response to warming. However, a variety of biotic and abiotic factors may stabilize Andean timberlines and halt further upslope migrations into the high elevation grasslands (referred to as “puna” in southern Peru) above the forest. One possibility is that small forest patches that occur above-timberline throughout the Andes may facilitate forest expansion into newly suitable areas in the puna, yet little is known about the ecology or function of these patches. In this study, I examined seedling recruitment patterns, seed dispersal, and microclimate at the timberline, in and around above-timberline forest patches, and in the puna. The above-timberline forest patches had similar patterns of seed dispersal as the timberline but overall < 1% of captured seeds were dispersed 10 m into the puna. At both the patch edges and the forest timberline, seedling abundances were lower in the puna relative to the adjacent forest and forest-puna ecotone. This reduction may be a result of reduced seed dispersal across the forest-puna ecotone and/or decreased germination of dispersed seeds due to the harsh microclimatic conditions occurring in the puna (daily temperature fluctuations are greatly elevated in the puna relative to the forest and frost events are more frequent and severe). While increasing temperatures associated with climate change may ameliorate some of the severe climatic conditions occurring in the puna, it will not directly affect other potential recruitment limitations such as reduced seed dispersal, high levels of UV

radiation, and anthropogenic activities in the puna (cattle grazing and fires). With the reduction of anthropogenic activities in the puna, above-timberline forest patches may serve as nucleating foci for future forest expansion. However, my results indicate that any upslope migration of the timberline into the puna will likely occur at a rate that is slower than what is required to keep pace with warming because recruitment is restricted to a narrow strip along the forest-puna borders. Slowed forest expansion into the puna could act as a barrier to the upslope migration of Andean cloud forest species leading to extreme losses of Andean biodiversity.

INTRODUCTION

Temperature is commonly believed to be one of the most important environmental factors determining the upper elevational limit of closed canopy forests, i.e., the timberline (Berdanier, 2010; Körner and Paulsen, 2004; Körner, 2012, 1998). Accordingly, with global warming, it is expected that montane forest species will shift their distributions upslope and colonize the currently unforested alpine zones (Grace et al., 2002; Harsch et al., 2009). However, in a recent meta-analysis conducted by Harsch et al. (2009), timberlines showed no consistent response to past climate warming; upward shifts were observed at 52% of sites, downslope shifts were observed at 1% of sites, and no shifts were observed at 47% of sites. While only a relatively small number of tropical sites were included in this meta-analysis (7 of 166), none shifted their timberline elevations through time (Harsch et al., 2009). More specifically for the tropical Andes, an analysis of remotely-sensed Landsat images taken from 1980-2010 has indicated no measurable change in the location of timberline despite the approximate 0.55 °C increase in temperatures that occurred over the same time period (assuming an approximate adiabatic lapse rate of $-5.5 \text{ }^{\circ}\text{C km}^{-1}$, this warming should have resulted in a ~100 m upslope shift in timberline; Zelazowski, 2010).

The reason that the timberline in the tropics, and specifically in the tropical Andes, is not migrating upslope despite rising temperatures remains unknown. However, it may be due to a combination of biotic and abiotic factors that prevent the recruitment and/or the growth of tree species above the timberline. For instance, increased frost severity and frequency (Körner and Paulsen, 2004; Wesche et al., 2008), elevated intensity of UV solar radiation (Bader et al., 2007a), reduced seed dispersal (Dullinger et

al., 2004; Primack and Miao, 1992), low germination rates of timberline species (Cierjacks et al., 2007a), competition with established grasses (Tilman, 1997), high levels of seed predation (Hillyer and Silman, 2010), altered edaphic conditions (Zimmermann et al., 2010), and anthropogenic disturbances such as livestock grazing combined with fire (Cierjacks et al., 2008) and agriculture clearing (Sarmiento and Frolich, 2002) can all potentially restrict forest tree recruitment above timberline. In addition, shifts in species distributions may lag behind temperature changes, and therefore it is possible that some timberlines are still responding to past climatic changes or have not yet begun to respond to more recent warming (Dullinger et al., 2012; Wardle and Coleman, 1992).

Regardless of the causes, if Andean timberlines continue to remain fixed despite future climate change, then this ecotone may act as a barrier halting the migration of high-elevation forest species (Feeley and Silman, 2010; Feeley and Rehm, 2012). This could have dire consequences to global biodiversity since the tropical Andean cloud forests are some of the most diverse ecosystems on Earth (Hobohm, 2003; Myers et al., 2000) and since many Andean cloud forest species have already been observed to be shifting their distributions upslope within the forests (Feeley et al., 2011; Forero-Medina et al., 2011; Feeley, 2012; Feeley et al., 2012b). Additionally, cloud forests are expected to act as future cool temperature refugia for species shifting their distributions upslope from the Amazonian lowlands (Feeley et al., 2012b), but are already highly threatened by other anthropogenic disturbances such as deforestation and conversion for other landuses (Sarmiento and Frolich, 2002; Purcell and Brelsford, 2004; Kintz et al., 2006; Cierjacks et al., 2008; Bruijnzeel et al., 2010; Martin et al., 2011; Román-Cuesta et al., 2011; Feeley and Rehm, 2012; Feeley et al., 2012a).

In the southeastern Peruvian Andes, the vegetation above the timberline is comprised mainly of a grassland matrix (hereafter referred to by the local name of “puna” a drier less-widespread version of “páramo”) that eventually gives way to alpine vegetation and bare rock, culminating in snow at extreme elevations. Within the puna matrix, there exist small mixed-species forest patches that are similar to *Polylepis spp.* forest patches growing up to several hundreds of meters above the principle timberline throughout the Andes (Kessler, 2002; Miehe and Miehe, 1994). The origin of these above-timberline forest patches is debatable, but they are often hypothesized to be the remnants of more extensive forests that were destroyed by past human activity (Kessler, 2002; Miehe and Miehe, 1994; Sarmiento and Frolich, 2002). Alternatively, they may be an advancing front of expanding forest vegetation halted through the recent increase of fire frequency associated with grazing (Di Pasquale et al., 2008). Regardless of their origin and history, these patches may play a crucial role in any future forest expansion above the current timberline, for example, by acting as nucleating foci for the establishment of forest seeds and seedlings in the puna (Schlawin and Zahawi, 2008; Yarranton and Morrison, 1974). As an analogy, patches of tropical lowland trees in abandoned fields act as seed sources, and areas in the immediate vicinity of “nurse” trees have higher rates of seedling establishment than occurs in nearby fields (Janzen, 1988; Nepstad et al., 1996; Turner and Corlett, 1996). These lowland forest patches also alter microclimate, promoting seed germination and forest expansion into surrounding pasture habitats. Similarly, the above-timberline forest patches in the Andes may potentially facilitate forest expansion into the puna under future climate change, especially if other human impacts (e.g., cattle grazing and fires) are decreased or completely removed from

the puna (Cierjacks et al., 2007). For example, in some central Andean forests, there are more seedling microsites available within forest patches than outside (Renison et al., 2004) and patches buffer against extreme climatic events, reducing seedling mortality (Cierjacks et al., 2007; Wesche et al., 2008). Furthermore, seedling abundance is often highest at the patch-puna ecotone (Byers, 2000; Cierjacks et al., 2007), indicating the potential for these patches to expand outwards.

To increase our understanding of the ecology of these above-timberline forest patches, as well as their potential role in facilitating the expansion of montane forests past the current timberline and into the puna of the southern tropical Andes, I investigated the structure and function of several above-timberline forest patches and adjacent timberline forests within Manu National Park, Peru. I specifically addressed the following questions: 1) Could changes in the patterns of seed dispersal and microclimate across the forest-puna ecotone limit forest expansion into the puna? 2) What are the current tree recruitment patterns at the forest to puna and patch to puna ecotones? and 3) Can forest patches potentially act as nucleating areas for future forest expansion above the current timberline into the puna? It is increasingly clear that high Andean landscapes have experienced long term and intensifying human alterations (Young, 2009; White, 2013). While human impacts were not an explicit aspect of this study, results are interpreted in the context of a human modified landscape.

MATERIALS AND METHODS

Study area

Data were collected along 14 transects located on three ridges on the eastern slope

of the Peruvian Andes in the Kosñipata Valley within Manu National Park, in the political department of Cusco in southeastern Peru ($\sim 13^{\circ}6'18''$ S, $71^{\circ}35'21''$ W). The three study ridges occur at the boundary between tropical montane cloud forests, which are characterized by heavy precipitation caused by orographic lifting of warm, moisture-laden air from the Amazon, and the relatively dry, unforested altiplano found to the west. Rainfall in the high elevation portions of the park averages $1900\text{--}2500$ mm yr^{-1} with a distinct wet season from October to April (Rapp and Silman, 2012). Forest soils below the timberline are characterized by a 20-70 cm thick organic layer over a 10 cm organic humic Ah layer, which are underlain by a 10-70 cm mineral layer (Zimmermann et al., 2010). Puna soils are much simpler with a 20-30 cm thick organic A layer underlain by a stony B layer (Zimmerman et al., 2010).

Manu National Park was designated as a national park in 1973, but even before this time the study areas were relatively conserved due to inaccessibility. Cattle grazing and associated fires do occur within the study area but cattle density and fire frequency is lower than in areas outside of Manu National Park and throughout other parts of the Andes (Gibbon et al., 2010). Although the study sites have had low levels of human impacts for at least the past 40 years relative to the surrounding landscape, current and past anthropogenic disturbances must be considered when investigating the timberline location. Therefore, results from the chosen study sites may be seen to offer a “best case scenario” for timberline shifts in the Peruvian Andes in response to climate change.

The 14 study transects were chosen in order to cover a wide range of timberline elevations (3146 – 3663 m a.s.l.) and by the requirement for nearby above-timberline forest patches. All transects extended from the closed-canopy montane cloud forest found

below timberline, across open puna, and into an above-timberline forest patch (Fig. S1). Forest patches ranged in size from 260 – 4100 m² and were located between 15 – 200 m from the nearest timberline. Full transect descriptions are provided in Table 1. At both the timberline and patch borders, the habitat switches from closed canopy tropical montane cloud forest (canopy height of ~5-12 m) to open puna grassland dominated by tussock grasses over a horizontal distance of approximately 5 m or less. This abrupt ecotone is unlike the more gradual transitions (> 50 m) from forest to grasslands that are commonly found in temperate zones and some other parts of the Andes (Körner, 2012).

Seed rain

During July-August 2011, 392 0.5 m² (70 x 70 cm) passive seed traps were deployed along the 14 study transects. Trap frames were constructed of PVC tubing with a square frame supported on four legs and placed level at a height of 0.5 m off the ground (Muller-Landau et al., 2002). Frames were covered with a 1 mm mesh netting with a weight placed in the center to prevent seeds from being blown out of the trap. Along each transect, 28 traps were installed in an unbalanced design with 12 traps at the timberline and 16 traps in and around the patch (Fig. S2). At the timberline, traps were placed in four transects that ran parallel to each other but perpendicular to the timberline. Trap transects were placed 3 m apart and seed traps were located at -10, -2, and 2 m relative to the timberline along each transect (negative values indicate distances extending into the forest, positive values indicate distances extending into the puna). Trap layout in the patches was more complicated, with traps placed along four transects laid out like a “+”, centered on the patch and extending from the patch interior into the puna (Fig S2). One

transect extended towards the nearest timberline, one transect extended away from the nearest timberline, and the two remaining transects ran perpendicular to these first two transects but in opposite directions. Along each trap transect, seed traps were placed at the patch center (distance from patch center to the patch border differed depending on patch size), -2, 2, and 10 m relative to the patch-puna ecotone. Traps were checked monthly over the course of one year and all seeds, reproductive material, leaf litter, and other debris were collected, dried, sorted, identified to lowest possible taxonomic rank, weighed, and measured.

Recruitment patterns

During August 2012, seedlings and saplings (individuals < 1.5 m tall or < 2.5 cm diameter at breast height) were censused in 0.5 m² (70 x 70 cm) seedling plots situated along transects running parallel and immediately adjacent to the seed trap transects described above. At the timberline, seedling transects extended from 20 m inside the forest to 10 m outside the forest, with seedling plots situated at -20, -10, -5, -2, -1, 1, 2, 5 and 10 m relative to the timberline. The plots at -10, -2 and 2 m were adjacent to seed traps and therefore all seed traps had paired seedling plots. At the above-timberline forest patches, seedling plots were located at the center of the patch, -10, -5, -2, -1, 1, 2, 5, and 10 m relative to the patch border. Plots at the center of the patch, -2, 2, and 10 m were adjacent to seed traps. Within these plots, all seedlings and saplings were identified to the lowest taxonomic level possible, and measured for height and diameter at ground level.

Microclimate

In May 2011, three of the 14 transects were arbitrarily chosen and HOBO ProV2 temperature and relative humidity data loggers (www.onsetcomp.com) were installed along each transects. Sensors were placed at 10 m within forest below timberline, at timberline, in the puna mid-way between the patch and timberline, at the patch-puna ecotone, and in the patch interior. Data loggers were shaded to protect from direct sun exposure and programmed to record the air temperature and relative humidity every 30 minutes over the course of one year.

Data analysis

Statistical analyses were carried out using R version 2.12.1 (The R Foundation for Statistical Computing, 2010). For clarity in the discussion I refer to locations along each of the 14 study transects as either timberline or patch but at both locations transects extended from the forest into the puna (Fig. 1). I also refer to the forest-puna ecotone, which encompasses both timberline and the borders between the patches and puna unless otherwise stated. I refer to seed traps found at -10 m within timberline forests and at the center of the patch to be interior traps even though they were sometimes at different distances from the forest-puna ecotone.

Within the transects, seed rain data for the entire year were averaged for each trap, and then traps were grouped based on location (timberline or patch) and distance from the forest-puna ecotone . Due to the skewed distribution of seed rain data, non-parametric label permutation tests with 1000 iterations were used to test for differences in: 1) seed rain abundance and 2) seed rain diversity (Shannon-Weaver index), for each combination

of location (timberline vs. patch) and distance from the forest-puna ecotone (interior vs. - 2 m vs. 2 m vs. 10 m) using pooled data from all 14 study transects.

Seedling abundances and distributions were compared within and between habitats to test for differences in: 1) overall seedling abundance between timberline and patches, and 2) seedling abundance with distance from the forest-puna ecotone. For each transect, seedling abundance was pooled for plots found within the same habitat and the same distance along a transect. A paired t-test was used to determine if overall seedling abundance differed between timberline and patches. Then seedling data was pooled for all transects for each habitat and label permutation tests with 1000 iterations were used to determine if seedling abundance varied with distance along transects.

The daily mean, daily mean high, and daily mean low temperatures were compared between habitats along each transect using label permutation tests with 1000 iterations.

RESULTS

Seed rain

Over the course of one year (2011/2012), a total of 100,439 seeds were captured in the 392 traps with 86% of seeds falling in timberline forest or forest patch traps, 14% in puna traps, and < 1% of seeds dispersing to traps 10 m into the puna. Seed rain was largely comprised of wind-dispersed seeds (94% wind dispersed vs. 6% vertebrate dispersed) with 66% of the total seed rain coming from species of the *Weinmannia* genera. The label permutation tests showed: 1) the abundance of seed rain in the forest patches was not different from the continuous forest ($p = 0.35$), 2) within the forests, seed

rain abundance did not vary with distance from the edge ($p = 0.32$, -10 m vs. -2 m within patch, $p = 0.11$, -10 m vs. -2 m within timberline forest), 3) the abundance of seed rain decreases immediately outside the forest, whether the forest is at timberline or a patch ($p = 0.002$), and 4) virtually no seeds are dispersed even 10 m into the puna ($p < 0.001$; Fig. 1). Seed rain diversity had similar patterns to seed abundance. Specifically I found that: 1) seed rain diversity does not vary between forest patches and timberline forests ($p = 0.45$), 2) within forests, seed rain diversity does not vary with distance from edge ($p = 0.08$, -10 m vs. -2 m within patch, $p = 0.47$, -10 m vs. -2 m within timberline forest), 3) seed rain diversity decreases just outside the forest but at varying degrees from timberline and patch borders ($p = 0.001$, -2 m within vs. 2 m outside patch, $p < 0.001$ -2 m within vs. 2 m outside timberline forest), and 4) seed rain diversity is extremely low even 10 m into the puna ($p < 0.001$; Fig. 1).

Seedling abundances

Total seedling abundance did not vary between timberline and patch transects ($t = -0.3626$, d.f. = 8, $p = 0.7263$). Differences in seedling abundance and transect position were present at both timberline and patches ($p = 0.001$), with fewer seedlings occurring further into the puna (Fig. 2). This pattern was more pronounced at patches than timberline. However, seedling abundance did not vary within patches or timberline forests (Fig. 2). The sharpest decrease in seedling abundance occurred between 1-2 m in the puna, but seedling abundance continued to decrease up to 10 m from the forest edge. At both timberline and patches, I found a slight increase of individuals at the forest-puna ecotone or just inside the forest (Fig. 2).

Microclimate

While results varied among transects, in general a very abrupt discontinuity in climate occurred at the forest-puna ecotone regardless of forest type (Table 2, Fig. 3). The diurnal temperature range was dramatically broader in the puna relative to the forest, and conditions at forest-puna ecotones were highly variable among sites, but were generally intermediate between forest and puna. Forest patches and timberline had similar temperature profiles (Table 2). Along the transects, the frequency of frost events was anywhere between 8-162 times higher in the puna than in the forest and the lowest recorded temperatures in the puna were on average 5.5 °C and 6.6 °C colder than in the timberline forests and patches, respectively (Table 2).

DISCUSSION

Timberlines worldwide do not show consistent directional shifts in response to recent climate warming, and in the tropics no studies have yet found evidence of upward shifts in timberline due to warming (Harsh et al., 2009; Zelazowski, 2010). In general, timberline shifts are prevented when the recruitment of forest species is restricted across the existing ecotone due to any of a large number of biotic and abiotic factors. As temperatures continue to rise, small forest patches found above the timberline may facilitate forest expansion into the puna by ameliorating some of these limitations. For example, forest patches may serve as additional seed sources in the puna and/or create microclimates more conducive to the recruitment and growth of forest tree seedlings. Furthermore, any improvement in conditions may extend beyond the patches themselves and into the surrounding puna. Despite their potentially important role in future forest

expansion, and thus the persistence of montane cloud forest diversity under warmer temperatures, the basic ecology and function of these patches remains very poorly understood.

One factor that likely limits forest recruitment above the Andean timberline is reduced dispersal of forest tree species into the puna. My results indicate that seed rain decreases sharply outside the forest, which is consistent with seed rain patterns at timberlines worldwide (Holtmeier, 2009 and references within). Reduced seed dispersal across the timberline could limit future forest expansion into the puna as has been shown in forest patches of *Polylepis spp.* occurring elsewhere in the Andes (Cierjacks et al., 2007; Torres et al., 2008). Likewise, Dullinger et al. (2004) demonstrated that low seed dispersal highly limited timberline shifts in the Northern Alps despite increased temperatures associated with climate change. Future expansion of cloud forests upslope into the puna could therefore be severely hindered unless seed dispersal limitation into the puna is somehow overcome.

In lowland tropical systems, nurse trees or small forest patches within grasslands can increase seed dispersal outside of the forest edge by serving as additional seed sources and attracting seed dispersers (Holl et al., 2000; Janzen, 1988; Nepstad et al., 1996; Turner and Corlett, 1996; Yarranton and Morrison, 1974; Zahawi and Augspurger, 2006). Seed rain patterns within and around the above-timberline forest patches that I studied were similar to those at the timberline yet seed dispersal was still extremely limited outside the forest patches. In the study area, birds serve as the primary animal seed disperser because other seed dispersers such as bats, insects, and many mammals are not present at timberline (Patterson et al., 2006). Forest patches had lower abundance of

seed dispersing birds than the continuous forest found below timberline (personal observations), which is consistent with observations of nearby *Polylepis spp.* forest patches where the overall abundance and movement of seed dispersers in patches was low (Lloyd and Marsden, 2011). In other words, even though forest patches act as additional seed sources, the lack of seed dispersal outside of patches and the lack of seed dispersers within patches may continue to restrict the ability of forest tree species to overcome seed dispersal limitation into the puna.

Beyond just seed dispersal, forest expansion into the puna will depend on seed germination and the survival and growth of seedlings to adult size. Seed viability is known to be extremely low for several timberline forming species throughout the Andes (Cierjack et al., 2007a; Domic, 2012) which may lead to reduced seedling establishment. Even if seedlings are able to establish, their growth and survival may be limited due to the harsh environmental conditions in the puna. For example, seedlings might grow well in the puna until a disturbance (i.e. fire or grazing) resets succession. Seedling growth may also be reduced above the height of the puna grasses because growing higher than the grasses would expose the seedlings to the high levels of UV radiation (Bader et al., 2007a) and the relatively harsh climatic conditions of the free atmosphere found above timberline (Callaway et al., 2002; Holtmeier, 2009). My seedling censuses showed that the abundance of woody stems is reduced in the puna relative to the forests (timberline or patch) and patch-puna ecotone but that seedlings are not entirely absent from the puna. This finding is consistent with the seedling abundance patterns that have been observed at most of the other puna-timberline studies conducted to date (Cuevas, 2000; Oosterhoorn

and Kappelle, 2000; Germino et al., 2002; Bader et al., 2007b; Cierjacks et al., 2007b; Munier et al., 2010; Walker et al., 2012).

At my study sites, there is a very marked change in diurnal temperature patterns between forests and puna, with forests having a narrower average daily temperature range and a greatly reduced frequency and severity of frost events. The forest-puna ecotone had intermediate temperature patterns between forest and open puna. At other tropical timberlines, the number and severity of frost occurrences raises seedling mortality and can severely restrict forest recruitment above timberline (Körner and Paulsen, 2004; Rada et al., 2009, 2001; Smith et al., 2009; Wardle, 1985; Wesche et al., 2008).

Although the minimum temperatures recorded along my transects were well above the freezing tolerances of many tropical timberline taxa (Bodner and Beck, 1987; Rada et al., 2001; Squeo et al., 1991), the increased number of freezing events and the large diurnal fluctuations in temperature in the puna could severely stress seedlings and saplings to a point where they do not establish or grow (Körner, 2012). Daily mean temperature at all locations was above the 5 °C tissue formation threshold, suggesting that individuals may not be growth limited, increasing the likelihood that severe climate events lead to higher mortality or tissue damage (Körner, 2008).

Mediation of the severe puna climate by forest is restricted to the forest interior and a narrow strip along the forest boundary, which coincides with the drop in seedling abundance with distance into the puna. At both temperate and tropical timberlines, individual trees or small forest patches buffer microclimate, promoting seedling establishment and survival (Bader et al., 2008; Cierjacks et al., 2007; Oosterhoorn and Kappelle, 2000; Stueve et al., 2011). For example, Cierjacks et al. (2007b) found that the

reduced frost frequency and narrower temperature fluctuations in Ecuadorian *Polylepis* spp. patches correlated with higher seedling establishment in forest patches than in adjacent grasslands. In this study, even the smallest forest patch (transect 3, 261 m², Fig 3) significantly buffered temperatures in the interior and patch-puna ecotone as compared to puna, suggesting that these forest patches alter microclimate in a way beneficial to seedling recruitment and survival. However, the scale of this buffering is limited and does not appear to extend far into the puna. Therefore, a lack of suitable microsites above timberline is another potential factor limiting forest expansion into the puna (Germino et al., 2002; Harsch et al., 2012; Renison et al., 2005; Schonenberger, 2001; Smith et al., 2003; Wesche et al., 2008). If harsh microclimatic conditions are in fact limiting tree recruitment and survival in the puna, rising temperatures should increase the availability of suitable microsites, especially near timberline and patch ecotones where the climate is already buffered relative to the puna, thereby facilitating future expansion of the forest.

An additional disturbance that cannot be overlooked, but that was not addressed explicitly in this study, is the presence of cattle grazing and human-lit fires throughout much of the Andean puna. While low intensity cattle grazing was found to have a positive impact on seedling recruitment in Ecuadorian *Polylepis* spp. stands (Cierjacks et al., 2008), fires associated with Andean grazing practices are believed to be a primary factor explaining current Andean timberline positions (Cierjacks et al., 2007b). For example, it has been argued that Andean timberlines are artificially depressed due to such activities (Sarmiento and Frolich, 2002; Sarmiento, 2002), and that the forest may expand into the puna, and analogous páramo ecosystems, if grazing and burning pressures are reduced or removed (Cierjacks et al., 2008). These anthropogenic disturbances likely play

a critical role in stabilizing timberlines and preventing upslope shifts in response to climate change. However, my study area in Manu National Park was specifically chosen due to the low intensity cattle grazing which rarely occurs within the puna-forest ecotone (Gibbon et al., 2010). In addition, the areas around the study transects have not burned for at least 20 years (and likely much longer) and cattle cannot access the majority of the study transects due to rough topography or natural barriers (personal observations). My results support the hypothesis that the recovery of cloud forests is generally slow and may take decades to centuries even in the relative absence of anthropogenic disturbance (Kappelle, 2001; Luna et al., 2001; Silver et al., 2001). I did find a substantial number of seedlings in the puna, which gives some indication that timberline and patches might be expanding outward, yet seedlings were rarely taller than the puna grasses suggesting that seedling growth may be limited, and thus preventing timberline shifts. Without detailed knowledge of past disturbance regimes in the study sites it is difficult to tease apart the natural and artificial explanations for the observed lack of timberline shifts. However, since the study area has been protected for >40 years, my results could be viewed as a “best case scenario” indicating how Andean timberline forests may respond to climate change with the reduction of other anthropogenic disturbances.

CONCLUSION

As a first step towards mediating the effects of global climate change on Andean forests, anthropogenic disturbances such as fires associated with cattle grazing should be reduced in the puna to increase the likelihood that forest species can shift their distributions upslope with increasing global temperatures. However, even at timberlines

that are relatively free of anthropogenic disturbances, such as those examined in this study, decreased seedling abundance, decreased seed dispersal, and increasing microclimate severity with distance into the puna, suggests that forest expansion will occur as a gradual diffusive process. While above-timberline forest patches may act as additional radiation points for forest expansion into the puna, active management will likely still be necessary to promote forest tree recruitment in the puna if forest expansion is to keep pace with the rapid warming predicted for this century.

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Table 2.1: Descriptions of 14 study transects, extending from forest below timberline to above-timberline forest patches in Manu National Park, Cusco, Peru.

Transect Number	Latitude (S)	Longitude (W)	Elevation (m.a.s.l.)	Patch Area (m ²)	Distance between patch and timberline (m)	Slope at patch (°)	Aspect (°)
1	13° 7'33.24"	71°36'56.66"	3617	1971	67	0	270
2	13° 8'32.24"	71°36'31.86"	3374	852	57	0	0
3	13° 6'43.63"	71°37'30.50"	3663	261	26	8	200
4	13°10'29.82"	71°37'6.53"	3387	795	43	0	0
5	13°10'11.50"	71°36'51.30"	3154	4139	100	0	0
6	13° 7'27.34"	71°37'4.94"	3614	1563	44	15	90
7	13°10'16.41"	71°36'50.96"	3223	271	200	7	25
8	13°10'10.96"	71°36'40.53"	3146	1021	74	20	330
9	13° 7'23.52"	71°37'5.72"	3628	1004	15	23	90
10	13° 7'5.20"	71°36'51.28"	3578	424	61	30	0
11	13° 6'57.36"	71°36'29.90"	3500	1941	180	30	310
12	13° 8'35.81"	71°36'36.97"	3333	935	28	0	0
13	13° 8'32.89"	71°36'48.42"	3330	604	20	0	0
14	13° 8'31.45"	71°36'47.30"	3333	952	60	0	0

Table 2.2: Daily mean, mean high and mean low temperature, overall minimum temperature and total number of days below freezing as measured at five habitats along three study transects over the course of one year (May 2011 – Apr 2012). Letters represent significant differences ($p < 0.05$) in temperature between habitats within a transect according to label permutation tests.

Transect	Location	Daily mean high temperature (°C ± 1 sd)	Daily mean temperature (°C ± 1 sd)	Daily mean low temperature (°C ± 1 sd)	Minimum temperature (°C)	Number of days below 0°C
1	Below timberline	12.8 ± 4.1 ^a	6.9 ± 1.0 ^{ad}	3.3 ± 2.0 ^a	-2.2	25
	Timberline	12.5 ± 2.7 ^a	6.8 ± 1.3 ^a	2.7 ± 2.3 ^{bd}	-4.0	46
	Open Puna	22.9 ± 7.4 ^c	8.1 ± 1.8 ^b	0.7 ± 3.2 ^c	-8.3	162
	Patch/puna ecotone	16.5 ± 5.6 ^d	7.4 ± 1.2 ^c	3.0 ± 2.1 ^{ad}	-2.8	29
	Interior Patch	11.7 ± 2.6 ^b	7.0 ± 1.0 ^d	4.3 ± 1.3 ^e	0.7	0
3	Below timberline	10.2 ± 1.8 ^a	6.9 ± 1.3 ^a	4.5 ± 1.6 ^a	-0.1	1
	Timberline	20.6 ± 6.6 ^b	8.5 ± 2.1 ^b	2.6 ± 2.6 ^b	-3.8	75
	Open Puna	19.2 ± 6.3 ^c	7.8 ± 2.0 ^c	2.3 ± 2.8 ^b	-7.3	88
	Patch/puna ecotone	16.2 ± 5.6 ^d	7.8 ± 2.0 ^c	3.5 ± 2.2 ^c	-2.2	21
	Interior Patch	11.1 ± 2.2 ^a	7.0 ± 1.4 ^a	3.9 ± 1.9 ^d	-1.2	10
5	Below timberline	14.5 ± 3.9 ^a	9.5 ± 1.2 ^a	6.5 ± 1.7 ^a	0.6	0
	Timberline	19.0 ± 6.3 ^b	9.7 ± 1.5 ^a	5.4 ± 2.1 ^b	-0.8	3
	Open Puna	24.4 ± 6.2 ^c	10.8 ± 1.6 ^b	3.9 ± 2.9 ^c	-3.7	34
	Patch/puna ecotone	21.7 ± 7.2 ^d	11.1 ± 1.5 ^c	6.8 ± 1.7 ^{ad}	1.0	0
	Interior Patch	13.9 ± 2.5 ^e	9.8 ± 2.3 ^a	7.1 ± 2.9 ^d	1.0	0

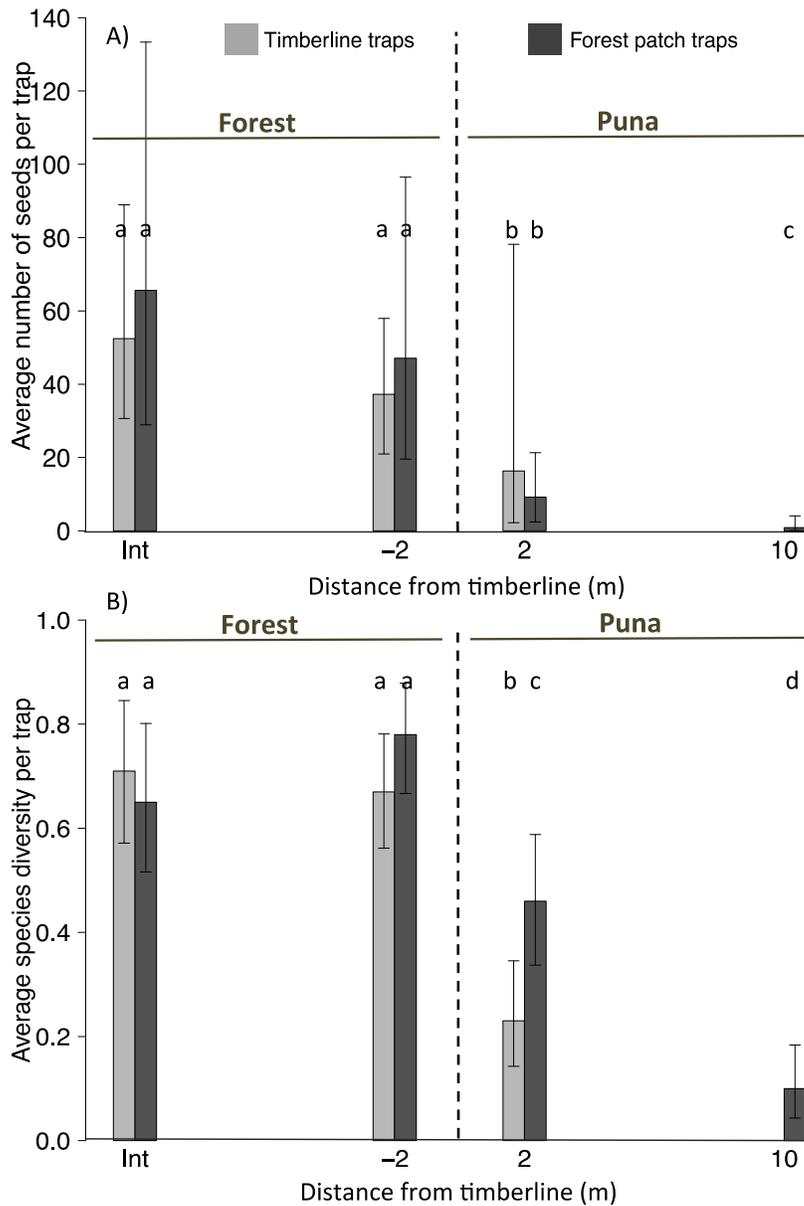


Fig. 2.1. The mean number of seeds collected and seed diversity (Shannon-Weiner index) per square meter of seed trap at different distances from the timberline (light gray) and patch (dark gray) as measured over a period of 12 months along 14 study transects. Letters indicate significant differences ($p < 0.05$) according to label permutation tests and vertical bars indicate 95% CIs.

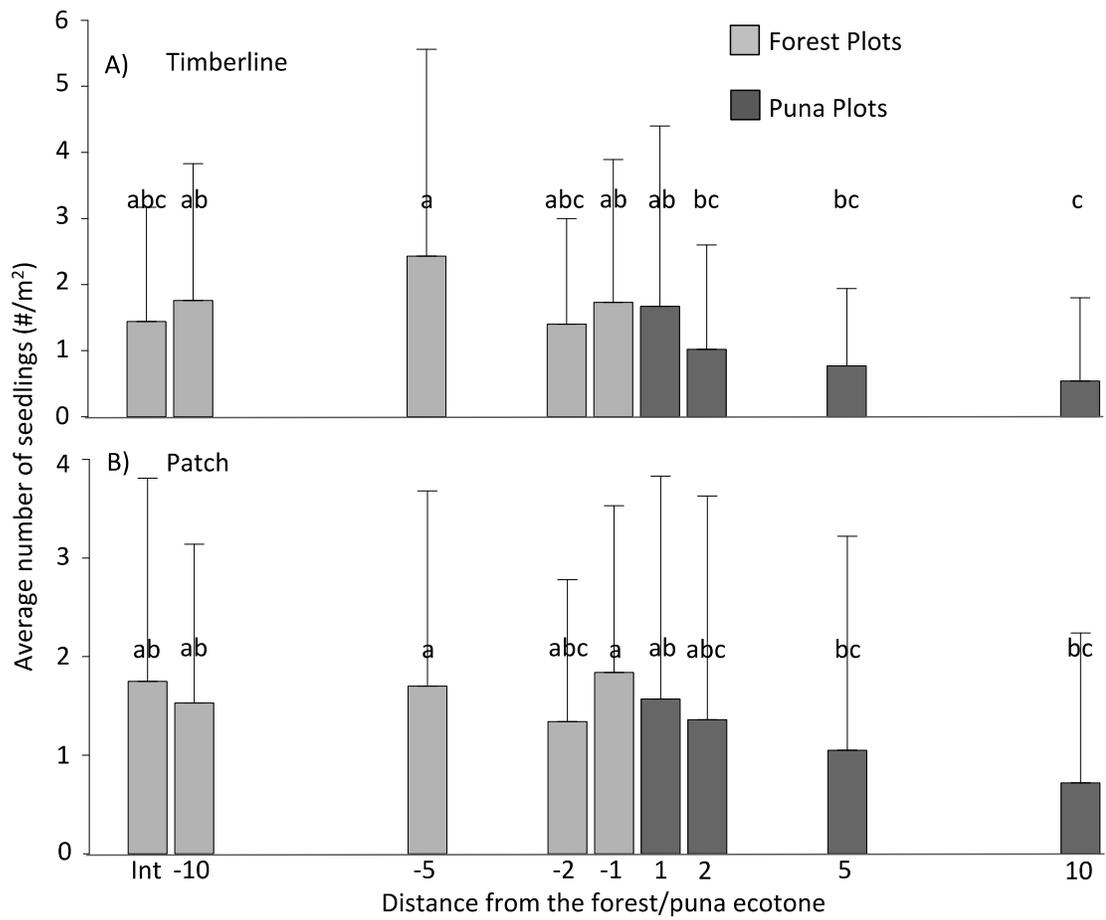


Fig. 2.2 Average number of seedlings per m² along timberline and patch transects (n = 14). Letters refer to significant differences ($p < 0.05$) according to label permutation tests and vertical bars indicate 1 s.d..

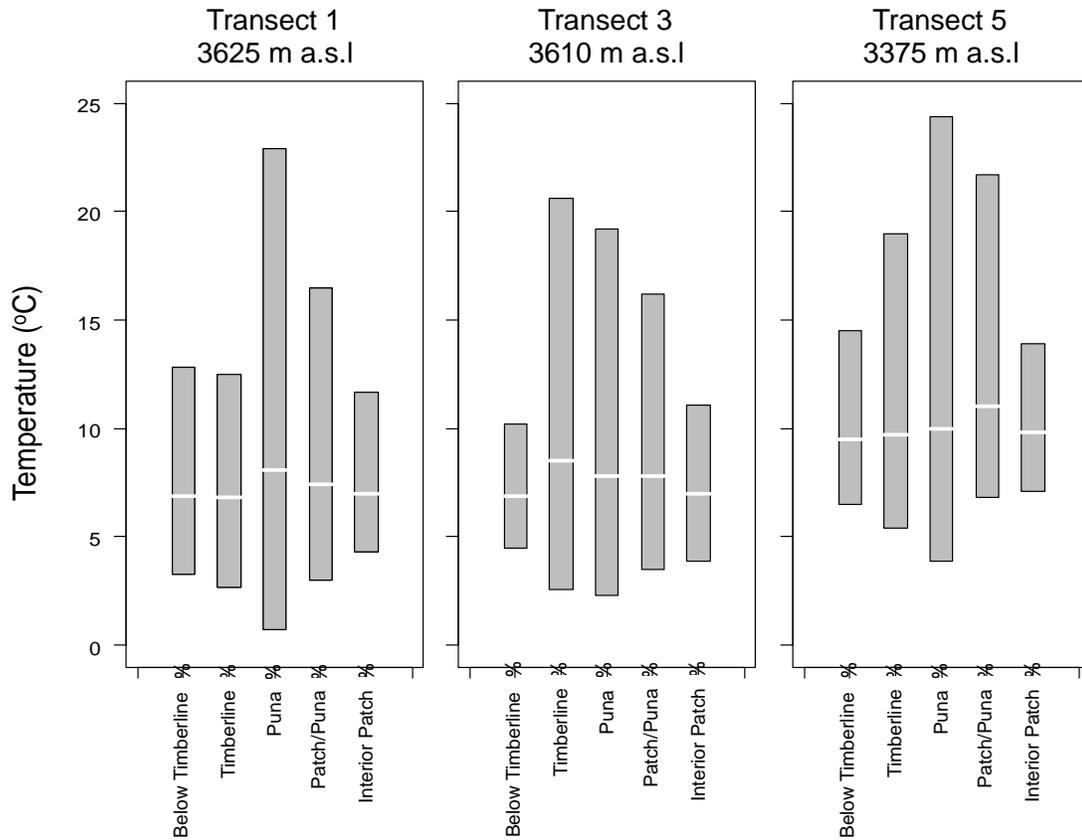


Fig. 2.3. Daily mean, daily mean high, and daily mean low temperatures within 5 different habitats (within forest, at forest-puna ecotone [timberline], in open puna, at puna-patch ecotone, within patch) along three arbitrarily chosen study transects. White lines represent mean temperatures.

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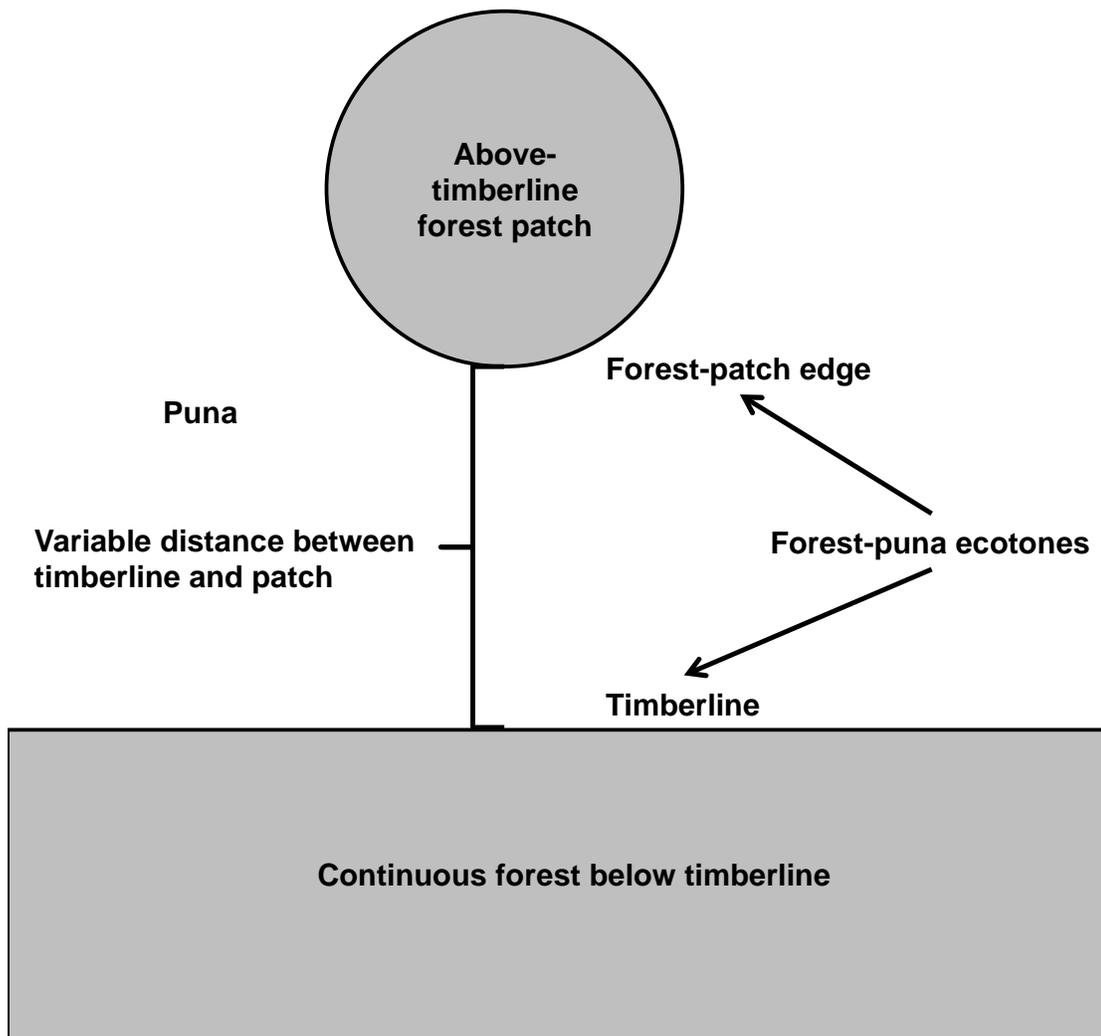


Fig. S2.1. Schematic diagram of sample transect showing locations of above-ground forest patches, timberline, the puna matrix, and associated habitat ecotones and edges. Distance between forest patch and timberline varied between transects. Forest-puna ecotone refers to the edge between forest and puna at both timberline and patches while forest-patch edge refers to only the forest patch and puna edge.

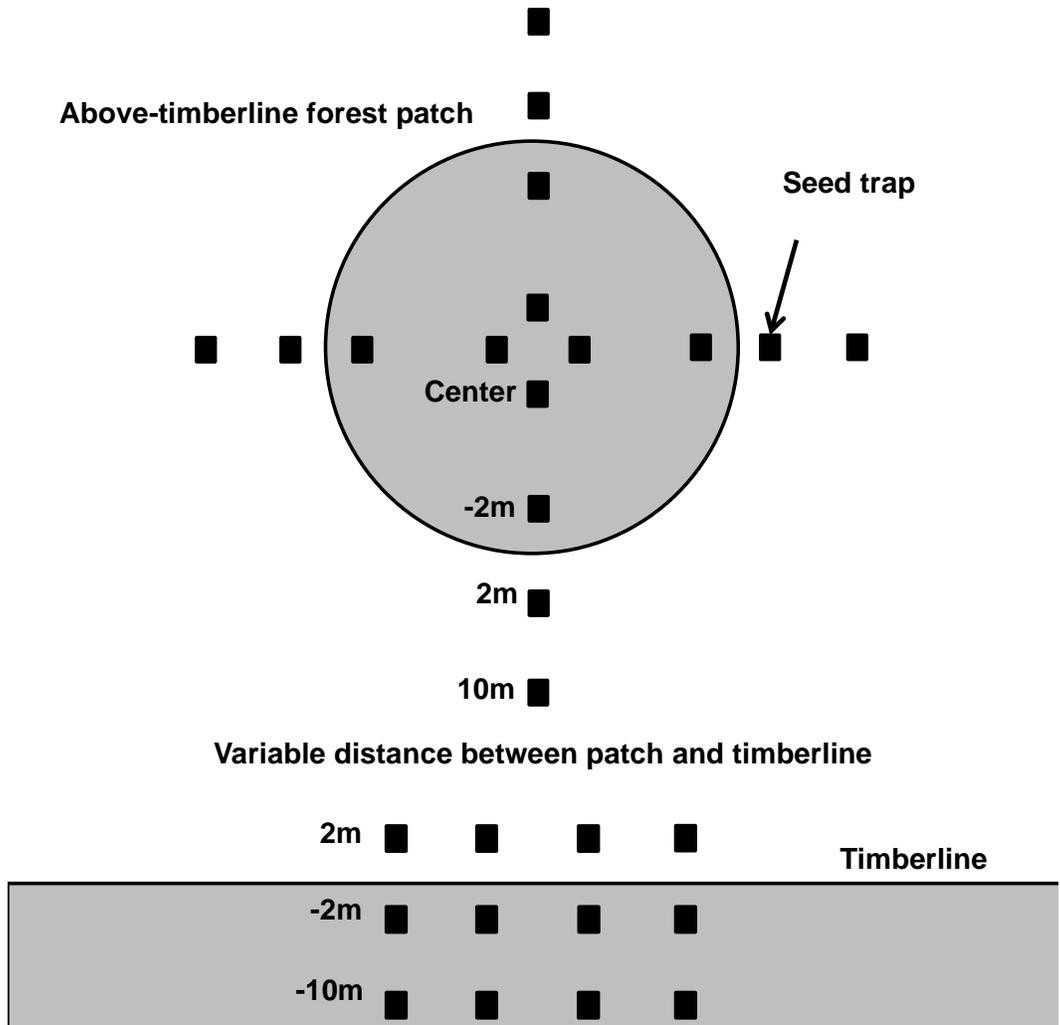


Fig. S2.2. Schematic diagram of seed trap transects at timberline and above-timberline forest patches. Traps were deployed in an unbalanced design with 16 traps at patches and 12 at treeline at each of the 14 study transects. Seed traps 10 m into the puna were only present at patches.

CHAPTER III
FREEZING TEMPERATURES AS A LIMIT TO FOREST RECRUITMENT
ABOVE TROPICAL ANDEAN TREELINES

Rehm, E.M. and K.J. Feeley. *In Press*. Freezing temperatures as a limit to forest recruitment above tropical Andean treelines. *Ecology*

ABSTRACT

The elevation of altitudinal treelines is generally believed to occur where low mean temperatures during the growing season limit growth and prevent trees from establishing at higher elevations. Accordingly, treelines should move upslope with increasing global temperatures. Contrary to this prediction, tropical treelines have remained stable over the past several decades despite increasing mean temperatures. The observed stability of tropical treelines, coupled with the drastically different temperature profiles between temperate and tropical treelines, suggests that using mean measures of temperature to predict tropical treeline movements during climate change may be overly simplistic. We hypothesize that frost events at tropical treelines may slow climate driven treeline movement by preventing tree recruitment beyond the established forest canopy. To assess this hypothesis, we measured freezing resistance of four canopy-forming treeline species (*Weinmannia fagaroides*, *Polylepis pauta*, *Clethra cuneata*, and *Gynoxys nitida*) at two life stages (juvenile and adult) and during two seasons (warm-wet and cold-dry). Freezing resistances were then compared to microclimatic data to determine if freezing events in the grassland matrix above treeline are too harsh for these forest species. Freezing resistance varied among species and life stages from -5.7 °C for juveniles of *P. pauta* to -11.1 °C for juveniles of *W. fagaroides*. Over a four year period, the lowest temperatures recorded at 10 cm above ground level in the grasslands above treeline and at treeline itself were -8.9 °C and -6.8 °C, respectively. Juveniles maintained freezing resistances similar to adults during the coldest parts of the year and ontogenetic differences in freezing resistance were only present during the warm season when temperatures did not represent a significant threat to active plant tissue. These findings

support the hypothesis that rare extreme freezing events at and above tropical treelines can prevent recruitment outside of closed canopy forest for some tree species and may significantly slow treeline advancement despite warming mean temperatures. Predictions of treeline shifts under climate change should be reevaluated to include species-specific climatic tolerances and measures of climatic variability.

INTRODUCTION

High-altitude treelines (hereafter referred to just as ‘treelines’) represent one of the most distinct and visible terrestrial ecotones, with closed canopy forests giving way to open alpine vegetation over a relatively short distance (Körner 2012). The abrupt ecotone transition at true climatic treelines is generally believed to occur at elevations where mean growing season temperatures directly limit the growth of upright woody trees (Körner 1998, 2008). This ‘growth limitation’ hypothesis suggests that treelines should shift upslope in response to rising global mean temperatures (Grace et al. 2002, Harsch et al. 2009). Contrary to this prediction, approximately half of the studied treelines worldwide have remained stable over the last century despite increasing mean temperatures (Harsch *et al.* 2009). Furthermore, rates of treeline shifts are spatially inconsistent, such that in some regions (e.g., in tropical mountains, New Zealand, and the temperate Andes) there have been relatively fewer documented upslope shifts in treeline (Wardle 2008, Harsch et al. 2009). While mean growing season temperature may be a good predictor of treelines at coarse geographic scales (Körner and Paulsen 2004, Paulsen and Körner 2014), the inconsistent patterns of treeline movements during global warming suggests that there may be additional climatic factors, such as temperature extremes, and non-climatic factors, such as biotic interactions, that modulate treeline movement at more local and regional scales (Rehm and Feeley *in Press*).

Compared to their temperate counterparts, tropical treelines experience a relatively mild year-round growing season with small seasonal temperature fluctuations but relatively large diurnal temperature swings, with warm days followed by freezing or near-freezing nights throughout the year (Körner and Paulsen 2004, Hoch and Körner

2005, Rehm and Feeley 2013, Kessler et al. 2014). Climatic variability is known to play a disproportionate role in shaping species' current ranges and future responses to climate change (Jentsch et al. 2007, Zimmermann et al. 2009, Thompson et al. 2013). Therefore, extreme temperatures may play a critical role in determining treeline movements during periods of climatic change especially if there are asynchronous rates of change in mean and extreme temperatures. For example, during climatically stable periods the ultimate position of treelines may be determined by mean growing season temperature but during periods of rapid climatic change, episodic extreme temperature events (e.g. freezing temperatures) could slow species' movements upslope or temporarily stabilize treelines.

One reason that low temperature extremes may play a major role in determining the upper distributional limit of tropical treeline-forming species is the fact that high-elevation tropical trees do not enter into a state of dormancy during cold seasons and therefore maintain their physiological activity throughout the entire year. As such, there is a greater potential for tissue damage due to freezing because active tissues are more susceptible to freezing damage than are dormant tissues (Rada et al. 1985, Sakai and Larcher 1987). Tissue damage may consequently occur even during the relatively mild freezing events that occur at tropical treelines. There is limited but growing evidence that the inability to avoid freezing damage may help to partially explain tropical treeline elevations and the pattern of the treeline ecotone, especially in regions where freezing temperatures regularly occur such as at treelines in the tropical Andes (Wesche et al. 2008, Harsch and Bader 2011).

Compared to species from other geographic regions, there is a relative dearth of information about the freezing resistances of treeline species from the tropics. The work

that has been done has largely focused on Andean species (for a review see Körner 2012). Adults of most tropical Andean treeline species exhibit freezing resistances between -6 to -13 °C (Goldstein et al. 1985, Rada et al. 1985, 2009, Cavieres et al. 2000), but one species, *Polylepis tarapacana*, the highest elevation treeline-forming species in the world, can tolerate temperatures down to -23 °C (Rada *et al.* 2001). Even at the same treeline, there can be large interspecific variation in freezing resistance. For example, Cavieres et al. (2000) found that the altitudinal limit of one Venezuelan treeline-forming species (*Podocarpus oleifolius*) was likely directly limited by freezing resistance while a second species (*Espeletia neriifolia*) could withstand more severe freezing temperatures. From this study, we can see that species forming current tropical treelines exhibit ecologically meaningful differences in freezing resistances, likely reflecting evolutionary and range specific climatic variation of individual species (Araújo *et al.* 2013). As such, low temperatures may directly limit some species' current elevation distributions while other species' ranges are limited by other factors (e.g. mean growing season temperature, precipitation or edaphic conditions). If increases in low temperature extremes lag behind rising mean temperature during climate change (Kodra et al. 2011), then low temperatures may continue to prevent some treeline-forming species from invading open habitats above treeline while other species that are relatively insensitive to low temperatures move upslope. Such a scenario could result in severe alterations to the structure and function of the treeline forest community and have cascading effects throughout the species rich cloud forest communities occurring below treeline (Feeley and Silman 2010).

Even within a species, low temperature resistance can vary with life stage and individual growth morphology. For example, it is generally believed that seedlings and saplings are less freezing resistant than conspecific adults (Larcher and Bauer 1981, Sakai and Larcher 1987). In addition, in an open grassland matrix such as that found above treeline, the height of individuals can play an important role in determining the exposure of plant tissue to extreme temperatures (Squeo et al. 1991, Sierra-Almeida and Cavieres 2012). The variation in freezing resistance due to ontogeny and differences in exposure due to plant height can be especially relevant for the upslope movement of treeline species because young plants growing close to the ground outside of the established forest will experience more severe and frequent frost events than taller individuals growing within closed canopy forests (Wesche et al. 2008, Rada et al. 2009, Rehm and Feeley 2013). Yet to our knowledge there has been no previous attempt to compare the freezing resistance of juvenile and adult trees at tropical treelines or to determine if low temperature extremes actually pose a significant threat to seedlings growing above treeline.

Before we can make accurate predictions about the dynamics and location of tropical treelines under climate change, we must first gain a comprehensive knowledge of how freezing events affect treeline species at different life stages and at different times of the year. As such, our goals in this study were to 1) determine if there are ecologically-meaningful differences in freezing resistance within a species at different times of year and in different life stages, 2) determine if freezing resistance varies among species after controlling for ontogeny and seasonal effects, and 3) relate species' freezing resistances to climate patterns to determine if low temperature extremes could plausibly limit tree

recruitment above treeline for four tropical Andean treeline-forming species (*Weinmannia fagaroides*, *Polylepis pauta*, *Clethra cuneata*, and *Gynoxys nitida*).

METHODS

Study sites

This study was conducted at two high-elevation tropical treelines in the southeastern Peruvian Andes within Manu National Park, Cusco department, Peru (~13°6'18" S, 71°35'21" W). Treeline elevation within the Park varies, but the two treeline sites used in this study occur at 3620 meters above sea level (m.a.s.l.) with a mean annual air temperature of 6.8° C (Rehm and Feeley 2013), which is consistent with the global treeline isotherm (Körner and Paulsen 2004, Paulsen and Körner 2014). As the treelines in this study represent a life form change from closed canopy tropical montane cloud forest to non-woody grassland vegetation (locally known as puna), we consider the treeline center as the point closest to the forest where tussock grasses begin to grow. For both treeline sites, this point was clearly visible as tussock grasses do not invade the shaded understory of the forest and the transition from forest to grasslands occurred over a distance of < 5 m. At the treeline center, the forest canopy overhangs the grassland vegetation by 1-2 m but then quickly gives way to open sky. Treeline sites were situated on flat ground on the opposite sides of a 100 m wide section of cloud forest; with treeline site 1 facing towards the North/Northeast and treeline site 2 towards South/Southeast. Temperature and moisture seasonality at treeline in the southeastern Peruvian Andes can be characterized by two distinct seasons, a relatively cold-dry season occurring from

May-September and a relatively warm-wet season from November-March (Rapp and Silman 2012). Diurnal temperature fluctuations are much greater than seasonal temperature variation and frosts can occur almost any time of year.

Throughout the tropics, anthropogenic disturbances can have strong effects on local and regional treeline positions and dynamics (Young and León 2007). Human activities such as cattle grazing and fire have certainly played a role in the vegetative dynamics around our study sites in the past. However, our study sites have been protected for at least 40 years and continuing human disturbance in the area is restricted to a few locations where light cattle grazing occurs only during certain times of the year (Gibbon et al. 2010), with no evidence of new fires (e.g. fire scars or charred tree stumps) occurring for at least 25+ years (pers. comm. Manu National Park officials). Furthermore, mean annual temperatures at our study sites fall very close to the global treeline isotherm suggesting that the selected treelines are indeed true climatic treelines and are not depressed by anthropogenic disturbances (Körner and Paulsen 2004, Paulsen and Körner 2014). The species included in this study are believed to represent true treeline-forming species and, as such, we treat the relationship between species-specific freezing resistance and temperature profiles at our treelines as representative of climatic treelines in the tropical Andes.

Freezing Resistance

Freezing resistance was determined for adults (≥ 10 cm dbh & ≥ 3 m tall) and small juveniles (≤ 1.0 cm diameter at ground level & ≤ 50 cm tall) of the four most dominant canopy-forming treeline species in the study region; *Weinmannia fagaroides*,

Polylepis pauta, *Clethra cuneata*, and *Gynoxys nitida*. Adult trees were sampled from the treeline center (as defined above) and leaves were taken from a height of 2-5 m above the ground. Juveniles were sampled within the grassland matrix within 3-10 meters of the treeline center. Juveniles did not grow to heights above the grass canopy. To test for seasonal variation in freezing resistance, sampling occurred at the beginning of the warm season in November 2013 and during the peak of the cold season in July 2014. To determine if freezing resistance varied between life stages, six adults and six juveniles from each species were sampled during each sampling period. From each individual, at least six intact mature leaves were harvested (for juveniles leaves were collected 5-20 cm height above the ground) and, in order to avoid major alterations to leaf water content, harvested leaves were immediately placed in sealed polyethylene bags and transported to Cusco, Peru, in an insulated container at 3-6 °C. Leaves were harvested shortly after sunrise between 06:00 and 08:00 a.m. and placed into freezing treatments by mid- to late-afternoon within eight hours of initial harvest. For each sampling period, we sampled 4 species x 2 life stages x 6 individuals x 6 leaves for a total of 288 leaves.

One leaf from each individual was cut at the petiole and randomly assigned to one of six target treatment temperatures: 4, -3, -6, -9, -12, -30 °C. Leaves assigned to the same treatment temperature were then packaged together. Leaves designated to the -3, -6, -9, -12 °C treatments were placed inside polystyrene foam boxes inside a single manually controlled commercially available freezer (Electrolux model EFCW152NSKG) which had been pre-cooled to 4 °C. Temperature was then lowered at a rate of 2-3 °C h⁻¹ until reaching the first target temperature of -3 °C. Temperature was held at -3 °C for two hours. After two hours those leaves designated to the -3 °C temperature treatment were

removed. Temperature in the freezer was then lowered to the next target temperature of -6 °C and the process was repeated for each target temperature. As leaves were removed from the freezer they were placed inside a separate refrigerator and thawed at 3-5 °C h⁻¹ until reaching 4 °C.

The 4 and -30 °C treatments served as references for live and dead leaf material, respectively. Leaves assigned to the 4 °C reference were kept in a separate refrigerator (Electrolux model ERD092UBGS) maintained at 4 °C throughout the entire freezing process. In order to create a dead leaf reference, assigned leaves were rapidly cooled (> 4 °C h⁻¹) to -30 °C and held at that temperature for 24 hours.

Within each package of leaves, temperature was recorded every 10 seconds and checked in real-time every 15 minutes (12-bit Temperature Smart Sensor; S-THB-M006, accuracy ±0.2 °C, Onset computer corporation, Massachusetts, USA) throughout the freeze/thaw process. At each 15-minute interval the freezer thermostat was manually adjusted to ensure consistent cooling rates and to maintain stable temperatures once a target temperature was reached. A circulating fan was placed inside the freezer to ensure homogeneity of air temperature inside the freezer. During freezing treatments, temperature fluctuated around target temperatures by an average of ±0.3 °C.

After treatment, all leaves were held at 4 °C for 72 h to allow necrosis development in leaf material. Individual leaves were then visually assessed for tissue damage/discoloration at 5% increments (Sakai and Larcher 1987). Following visual observations, samples were placed in distilled water in individual plastic test tubes and kept at 4 °C for 48 h. Electric conductivity was then measured using a handheld conductivity meter (Greisinger GMH 3430 conductivity meter, Greisinger electronic,

Germany) as a measure of electrolyte leakage (Flint et al. 1967, Burr et al. 1990). To kill living tissue, samples were then boiled in a hot water bath at 90 °C for 15 min and allowed to equilibrate for 72 h before taking a second measurement of maximum electrolyte leakage.

Temperature Data

In order to gain a general understanding of the low temperatures experienced at and around treeline, an array of seven HOBO ProV2 temperature/relative humidity data loggers (U23-001; accuracy: $\pm 0.2^{\circ}\text{C}$, Onset computer corporation, Massachusetts, USA) were deployed May 2011-Aug 2013 and May-July 2014 at the two treeline study sites (Rehm and Feeley 2013). Data was unavailable from September 2013-April 2014 due to logger failure. Data loggers were located approximately 10 cm above the soil surface, which was the same height as the majority of leaves collected from juveniles used in this study, and shielded from direct sunlight with a well-ventilated polystyrene cover. At treeline site 1, two loggers were placed at the treeline center point and two loggers were placed in the open ground between tussock grasses approximately 10 m from the treeline edge. At treeline site 2, two loggers were placed at the treeline center point but only one logger was placed in the open grassland. Temperature for all loggers was recorded at 30 min intervals. Data from these loggers were screened to find the lowest recorded temperatures at the field sites over several years. The extreme lows were used as estimates of the lowest temperatures experienced by individual plants growing near the treeline ecotone. These estimates are conservative since temperatures may have reached lower temperatures over the lifespan of the sampled plants.

In order to quantify patterns of microclimate across treeline in more detail, additional temperature sensors were established along one transect at each of the two treeline sites from 24 July to 4 September 2014. Each of these transects consisted of four HOBO Microstation dataloggers (H21-002, Onset Computer Corporation, Massachusetts, USA) each equipped with three Air Temperature/Relative Humidity Smart Sensors (S-THB-M006; accuracy: $\pm 0.2^{\circ}\text{C}$, Onset Computer Corporation, Massachusetts, USA). Relative to the treeline center and along each transect, a single datalogger was placed at 10 m within cloud forest, directly at the treeline center, 7 m into the open puna and 15 m into the open puna. Temperature sensors attached to each datalogger were placed at 10 cm above the ground, at the top of the grass canopy (~ 50 to 70 cm above the ground), and at 2 m above the ground. All temperature sensors were covered with a well-ventilated polystyrene cover to shield sensors from direct sunlight. This design allows for comparison of microclimate variation within each transect horizontally across the treeline ecotone and vertically at each sample point. Temperatures were recorded every 15 min.

Statistical Analysis

In this study, freezing resistance is expressed as the threshold temperature at which 50% of sample tissues die (LT_{50} ; Sakai and Larcher 1987). LT_{50} was calculated for each individual and then averaged across conspecifics of the same life stage during the same season ($n = 6$ for each life stage, season, species combination). For visual assessments, LT_{50} was calculated using logistic regression and for electrolyte leakage LT_{50} was calculated using nonlinear Gompertz models (Lim et al. 1998). LT_{50} values for the two methods were significantly correlated ($R^2 = 0.8$, $p < 0.001$, Pearson's correlation)

but values were generally colder for the electrolyte leakage method. For clarity, only results from the electrolyte leakage method are presented as they represent the more conservative estimate (i.e., colder LT_{50} values = greater freezing resistance) in the context of this discussion.

Since our goals were to determine freezing resistance variation due to the individual effects of life stage, season and species, differences in LT_{50} between species, life stages and seasons were analyzed using a series of generalized linear models built in R 3.0.3 (R Development Core Team 2013). To test for ontogenetic differences in freezing resistance with life stage within a species, individual models were built for each species and season combination with life stage (juvenile or adult) as the sole predictor variable. A Posthoc Tukey honestly significant difference (HSD) test was then run to look for differences in LT_{50} between life stages within season and species using the Multcomp package in R (Hothorn et al. 2008). Similarly, to test for seasonal differences in freezing resistance between individuals of the same life stage within a species, individual models were built for each species and life stage combination with season (cold or warm) as the sole predictor variable followed by HSD tests to determine differences in LT_{50} between seasons within life stage and species. To test for variation in LT_{50} among species, individual models were built for each season and life stage combination with species as the predictor. When a significant effect ($p < 0.05$) of species was present, HSD tests were run to determine which species-life stage groups exhibited differences in LT_{50} .

RESULTS

Lower or more negative LT_{50} values represent greater freezing resistance than the higher or less negative LT_{50} values. Freezing resistance in leaves varied from a low of -5.7 °C in juveniles of *P. pauta* during the warm season to a high of -11.1 °C in juveniles of *W. fagaroides* during the cold season. There was no consistent trend in the relationships between leaf freezing resistance, life stage, and season as these relationships varied among and within species.

Freezing resistance of leaves for *C. cuneata* did not vary with season within a life stage (Fig. 1) but there were differences between life stages, with juveniles maintaining higher freezing resistance than adults during the warm season (Table 1). For *G. nitida* and *P. pauta* freezing resistance of leaves varied with both season and life stage. For both of these species, adults had higher freezing resistances than juveniles during the warm season (Table 1). Within life stage, juveniles of *G. nitida* maintained higher freezing resistance during the cold season than the warm season while adults of *P. pauta*, maintained higher freezing resistance during the warm season than the cold season (Fig. 1). For *W. fagaroides*, no difference was found in freezing resistance between leaves of juveniles and adults during either season (Table 1). However, there was a difference across seasons within life stage, with juveniles having higher freezing resistance in the cold season than juveniles during the warm season (Fig. 1).

Freezing resistance varied among species with season but only at the juvenile life stage (Fig. 2). During the warm season, *C. cuneata* maintained higher freezing resistance than all the other species. During the cold season, *P. pauta* maintained lower freezing

resistance than all other species. There were no differences in freezing resistance among adults during either season.

Based on the array of seven data loggers deployed since May 2011, we found that absolute minimum temperature at 10 cm above ground level at our study sites was -8.9°C in the grassland while the minimum temperature at the treeline center reached -6.8°C , more than 2°C warmer despite being separated by only 10 meters. For both the grassland and treeline center this absolute lowest temperature reading occurred during the same morning on August 10, 2012 at treeline transect 1. Absolute minimum temperatures recorded across the more detailed treeline microclimate transects from July 24 to September 4, 2014 were milder than those recorded from the individual data loggers deployed since May 2011 (likely due to the shorter measurement period). However, there was a clear pattern of more severe nighttime low temperatures at 10 cm above ground level versus the grass canopy height (50-70 cm) or 2 m above ground at almost all distances from the treeline along both transects (Table 2). Mean nighttime minimum temperatures followed similar patterns, with lowest temperatures occurring at 10 cm above ground level in the open grassland (15 and 7 m from treeline) and with highest temperatures occurring within the cloud forest and at heights above the grass canopy (Table 2). Similarly, mean nighttime minimum temperatures at 10 cm above ground level were significantly lower than temperatures either at the top of the grass canopy or when coupled to the free atmosphere at 2 m above the ground (Table 2). Mean nighttime minimum temperatures were generally lowest in the open grasslands and highest in the forest interior when measured at the same height.

DISCUSSION

We found that for several dominant tropical treeline-forming species, the freezing resistances of leaves were insufficient to withstand the low temperature extremes recorded near the ground in the grasslands above treeline. Even when we consider that the LT_{50} of attached leaves *in situ* may be 1-2 °C colder (Taschler and Neuner 2004), all species had, at best, very small safety margins between their maximum freezing resistance and minimum recorded temperatures. What's more, given a longer climatic dataset, it is highly likely that absolute minimum temperatures would be well below the -8.9 °C minimum that we recorded over our study period. These findings indicate that juvenile plants growing at or near ground level in the grasslands are exposed to periodic extreme freezing events that can kill their leaves, or potentially entire plants. If this is the case, then our findings strongly suggest that the expansion of some tree species' upper range edges past the current treeline will be inhibited by rare extreme freezing events, even as mean temperatures increase.

Low temperature extremes were not uniform within the grassland habitat, with the most extreme low temperatures occurring at ground level where the juvenile trees maintained the majority of their leaves. These data are consistent with similar microclimatic measurements at other tropical Andean treelines (Hertel and Wesche 2008, Rada et al. 2009). The lower temperatures at ground level can be attributed to nighttime radiative cooling. Once above the grassland canopy, free air-mixing results in milder minimum temperatures. Therefore, as juveniles grow taller they may escape the severe freezing temperatures that occur near the ground. The probability of a plant establishing and reaching heights where exposure to extreme low temperatures is minimized will

depend on the species-specific growth rate and the return interval of extreme freezing events (Wakeling et al. 2012). Trees grow relatively slow at tropical treelines suggesting that it may take several decades for individuals to grow to heights above the dangerous cold air layer close to the ground (Hoch and Körner 2005). Our study supports this “escape hypothesis” and demonstrates the high likelihood that, in addition to mean temperature, low temperature extremes play a significant role in determining seedling survival near the ground in the grasslands above treeline.

This study represents the first attempt to quantify freezing resistance of different life stages within tropical treeline-forming tree species. The ontogenetic patterns in leaf freezing resistance that we found indicate that juveniles of the studied species maintain freezing resistances comparable to adults. Similarly, Vitasse *et al.* (2014) found that juveniles and adults in temperate European forests had the same freezing resistances during similar periods of leaf developmental stage. The lack of ontogenetic differences in leaf freezing resistance during the coldest portion of the year in our study likely represents a critical need for all individuals to maintain high freezing resistance during periods of low temperature extremes regardless of life stage or position relative to the closed-canopy treeline forest (Körner 2003).

Converse to the cold season, we did find ontogenetic differences in leaf freezing resistance for three of the four species during the warm season. However, these statistically different freezing resistances may be ecologically irrelevant because freezing events likely do not reach critical thresholds during the warm season. For two species, *G. nitida* and *P. pauta*, the reduction in leaf freezing resistance of juveniles relative to adults during the warm season may represent a physiological adjustment in juveniles to

maximize growth during periods when freezing temperatures pose a minimal threat to active plant tissues. For *C. cuneata* the high leaf freezing resistance of juveniles relative to adults during the warm season is difficult to explain but may represent disparities in the phenological cycles within species. Actively growing tissue tends to be less freezing resistant than dormant tissue (Öquist and Huner 2003) so the patterns of leaf freezing resistance found in our study may reflect differences in the timing of growth between life stages (Vitasse 2013, Vitasse et al. 2014). A more detailed knowledge of phenological patterns (e.g. timing of growth or flowering) at tropical treelines would allow us to disentangle the intricacies of freezing resistance patterns within species and age class.

Species exhibited ecologically meaningful differences in the ability of their leaves to withstand the microclimatic conditions above treeline, suggesting that not all of the current treeline-forming species will be able to establish in the grasslands above current treeline unless extreme low temperatures increase or become less frequent. For example, we found that low temperature extremes have the potential to directly limit the ability of *P. pautia* to grow beyond treeline. Conversely, freezing temperatures may be insufficient in explaining the upper elevational distribution limit for *W. fagaroides* as this species' freezing resistance was slightly colder than the observed minimum temperatures. These species-specific freezing resistances may be reflected in the variable patterns of recruitment occurring at the treeline ecotone, with *W. fagaroides* being relatively more abundant and *P. pautia* being relatively scarce (unpublished data).

Understanding which species are most likely to establish beyond the current treeline during climate change is important when making predictions about future treeline shifts. One of the main pathways for rapid upslope treeline shifts during contemporary

climate change will be through the establishment of individuals or groups of seedlings at relatively large distances (> 20 m) beyond the current treeline. These seedlings can then alter microclimate, creating areas conducive to additional seedling recruitment and act as nucleating foci for further forest expansion throughout the grassland. Of our four study species, only *W. fagaroides* seems capable of withstanding the relatively harsh freezing regimes of the open grasslands. However, in our study area the number of juveniles of all species, including *W. fagaroides*, decreases sharply with distance into the grassland indicating that seedling recruitment in the grassland is most likely limited to just a short distance (< 10 m) from the treeline itself (Rehm and Feeley 2013). Therefore, for some species, freezing temperatures may directly inhibit seedling recruitment above treeline while other species that are better able to withstand freezing temperatures may ultimately be limited by additional factors (e.g. low temperature limitations on growth).

The freezing resistance and microclimate data presented here coupled with current recruitment patterns at our study sites suggest that treeline shifts in response to current climate change are occurring in a relatively slow and diffuse manner, possibly hindered by periodic freezing events. Indeed, documented cases of treeline shifts in and around our study sites are rare, and even when treelines are moving upslope they do so at an extremely slow pace, lagging well behind current shifts in mean temperature (Lutz et al. 2013). Similarly, slow rates of treeline shifts were documented in some non-tropical areas where recruitment above treelines may be limited by freezing events (Wardle and Coleman 1992, Wardle 2008). Slow rates of tropical treeline shifts could have major impacts on biodiversity in the species-rich cloud forest community, as many species occurring below tropical treelines have already begun to shift their distributions upslope

(Freeman and Class Freeman 2014, Rehm 2014). If current treeline forming species do not also shift upslope into areas currently under grasslands, then cloud forest species will continue to lose habitat at their lower, warm range edge while failing to gain habitat at their upper, cold range edge, leading to severe range contractions and elevated extinction risks in this biodiversity hotspot (Feeley and Silman 2010). Even when treeline shifts do occur, it is likely that treeline species will shift at discordant rates depending on species-specific factors which limit recruitment above treeline, such as freezing resistance or growth sensitivity to low mean temperatures. In this scenario, uneven shift rates in treeline species will likely result in altered species assemblages and forest composition, which may have cascading effects on the function and dynamics of cloud forest systems.

The freezing resistance of tropical treeline species in the Andes could become even more relevant in the future because the severity and duration of low temperature extremes may stay relatively constant during current climate change despite rising mean temperatures (Vavrus et al. 2006, Kodra et al. 2011). In addition, future increases in mean temperature and CO₂ concentrations can alter phenology and reduce overall freezing resistance of trees (Woldendorp et al. 2008, Martin et al. 2010, Augspurger 2013). Paradoxically, this means that tropical treeline-forming trees may become even more susceptible to extreme freezing events in the future despite mean temperature increases. We show that the upper distributional limits of several tropical treeline species already occur at elevations where freezing temperatures may directly limit survival and recruitment of juveniles in the open terrain above the current treeline. Any reductions in freezing resistance capacities caused by climate change could result in further recruitment limitations at tropical Andean treelines unless there is a concurrent increase in absolute

minimum temperatures. As such, based on our findings and the relative stability of treelines in the tropics despite significant climate warming, we may need to reevaluate predictions of upward tropical treeline shifts during climate change, at least in regions where freezing temperatures regularly occur. Furthermore, additional factors beyond just temperature (e.g. water stress, edaphic conditions, and solar radiation) may also play important roles in determining cloud forest expansion into the grasslands above treelines (Rehm and Feeley *in Press*). Yet to date, most of these other factors have received relatively little attention in tropical treeline systems, making predictions about tropical treeline shifts and the fate of tropical cloud forests even more uncertain.

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Table 3.1: Estimated freezing resistances (LT_{50} ; $^{\circ}C \pm (1 \text{ SE})$) of four dominant treeline species measured during two different seasons (cold and warm) at two life stages (adult and juvenile). $n = 6$ for each species, life stage, and season combination. Significant differences (posthoc Tukey Honestly Significant Difference tests, $p < 0.05$) are shown in bold and represent difference in freezing resistance within the same season and species but between life stage.

Species Season	Life Stage		p-value
	Adult	Juvenile	
<i>C. cuneata</i>			
Cold	-8.5 (0.6)	-8.8 (0.7)	0.778
Warm	-7.8 (0.8)	-10.7 (0.6)	0.015
<i>G. nitida</i>			
Cold	-8.0 (0.8)	-9.1 (0.8)	0.362
Warm	-10.3 (0.9)	-6.0 (0.6)	0.004
<i>P. pauta</i>			
Cold	-6.5 (0.7)	-6.3 (0.6)	0.775
Warm	-9.3 (0.6)	-5.7 (0.4)	<0.001
<i>W. fagaroides</i>			
Cold	-9.2 (0.9)	-11.1 (0.4)	0.082
Warm	-7.5 (0.9)	-7.8 (0.9)	0.818

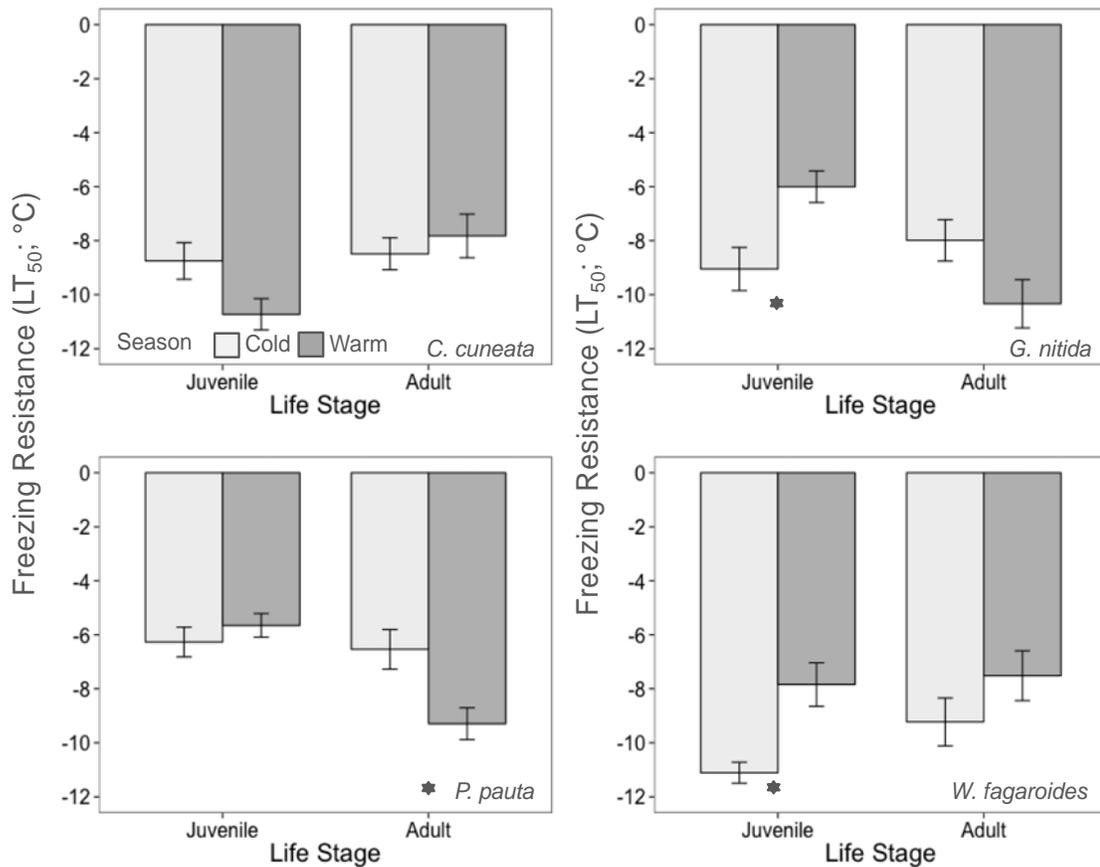


Fig. 3.1. Variation of freezing resistance (LT₅₀; °C ± (1 SE)) for each species at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Asterisks represent significant differences (posthoc Tukey Honestly Significant Difference tests, $p < 0.05$) in freezing resistance within the same species and life stage but during different seasons.

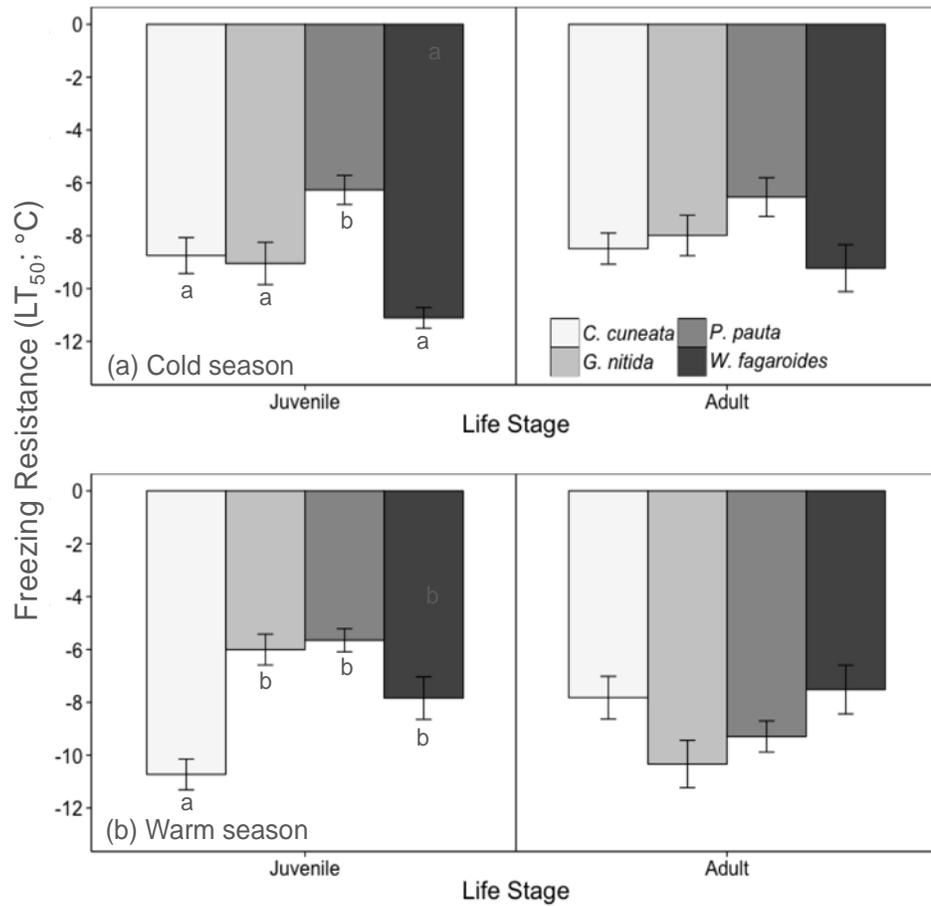


Fig. 3.2. Variation of freezing resistance (LT_{50} ; °C \pm (1 SE)) among species at two different life stages during two seasons ($n = 6$ for each species, life stage, and season combination). Letters represent significant differences (posthoc Tukey Honestly Significant Difference tests, $p < 0.05$) in freezing resistance within a season and life stage but among different species. For adults, there were no significant differences among species for either season.

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CHAPTER IV

EXPERIMENTAL SEEDLING TRANSPLANTS SHOW THAT FACTORS OTHER THAN MEAN TEMPERATURE MAY DETERMINE TROPICAL TREELINE DYNAMICS

ABSTRACT

Tropical treelines often represent the boundary between the upper distributional limit of the species rich montane cloud forests and the high-elevation grassland community. Using global treeline patterns, the elevations at which tropical treelines occur are believed to represent the point where low mean temperatures limit growth in upright woody trees. Therefore the upper distributional edge of cloud forest species (i.e., treeline) should shift to higher elevations with increasing global mean temperatures. However, treelines throughout the tropics have remained stationary over the last several decades despite increasing global temperatures suggesting that mean temperature alone may be insufficient in explaining tropical treeline locations at more local and regional scales. The limited evidence from the tropics suggests that other factors such as extreme freezing events and shading may also play an important role in tropical treeline dynamics. Our goal was to conduct a large-scale seedling transplant study using four dominant canopy-forming treeline species (*Weinmannia fagaroides*, *Symplocos baehinii*, *Clethra cuneata*, and *Gynoxys nitida*) to build a more comprehensive understanding of the effects of mean temperature, low temperature extremes, shading, and their interactions on seedling survival at and above a tropical treeline in the Peruvian Andes. We found species-specific and often contradictory responses of seedling survival to changes in mean temperature. Two of the studied species (*W. fagaroides* and *S. baehinii*) showed a negative relationship between transplanted seedling survival and mean temperature, the opposite of what is predicted to occur under warmer climates. Conversely, *C. cuneata* showed increased survival at higher mean temperatures but higher seedling survival also depended on concomitant increases in low temperatures and the presence of shade. Finally, shade was

the only treatment that increased survival of *G. nitida* seedlings, supporting previous claims that shading may be more important than temperature in determining tropical treeline elevations. These findings show that several variables besides just mean temperature can determine the upper distributional limit of tropical treeline forming species. If cloud forests are unable to expand into the high elevation grasslands found above current treelines, there may be severe species loss in this biodiversity hotspot. Therefore, we may need to re-evaluate predictions of future local and regional tropical treeline shifts as current predictions are largely based only on global correlations between treeline location and mean temperature.

INTRODUCTION

Many species are shifting their distributions to higher latitudes and altitudes in response to increasing global mean temperatures (Parmesan and Yohe 2003, Chen et al. 2011). In order to remain at equilibrium with climate and avoid severe range contractions species must shift their ranges at a pace similar to that of temperature shifts (Thomas et al. 2004, Colwell et al. 2008). However, the majority of shifting species, and especially plants, are lagging behind concurrent changes in temperature (Chen et al. 2011, Freeman and Class Freeman 2014, Rehm 2014). The relative rates of species range shifts and temperature change are especially important in the tropics given there is often high levels of biodiversity and endemism in relatively small geographic areas (Myers et al. 2000).

One such biodiverse area is the tropical Andean montane cloud forest (Myers et al. 2000). Tree species in the Andes are shifting their mean elevations upslope in response to climate warming, but shifts are at a rate of 30-40% of the predicted rate based on changes in temperature alone (Feeley et al. 2011). In contrast to species' mean elevations, the upper elevational distribution limits of many cloud forest species appear to be stable through time as indicated by relatively stationary tropical treeline (Harsch et al. 2009, Rehm and Feeley *in Press* b). Stable treelines suggest that cloud forest species are unable to expand or shift their ranges to include the higher elevation areas that are currently under grassland (Rehm and Feeley *in Press* b). Understanding why cloud forest species are shifting their mean elevations upslope while their upper range edges (i.e., treeline) remain stationary will have important implications for the conservation of this biodiversity hotspot.

Despite their importance in understanding future cloud forest biodiversity, tropical treelines are relatively poorly studied. It is believed that at a global scale treelines occur at elevations where mean annual growing season temperature is low enough to inhibit tree growth (Körner 1998, 2012). Therefore treelines are predicted to respond relatively rapidly to increasing global temperatures by shifting upslope (Grace et al. 2002, Harsch et al. 2009). However, the limited experimental evidence that exists from the tropics suggests that other factors such as shading and extreme temperature events are more important than mean annual temperature in determining tree growth and recruitment above treelines at the regional and local scale (Smith 1977, Smith et al. 2003, Bader et al. 2007a, Rehm and Feeley *in Press a*).

To date, only two studies have explicitly tested the limitations of seedling establishment above tropical treelines. Smith (1977) found that almost all tree seedlings transplanted into the open grasslands above a Venezuelan treeline died within a year. The high mortality was attributed to a combination of competition with grassland species, moisture stress, and the temperature extremes experienced in the open grassland. Bader et al. (2007) also transplanted tree seedlings into open grasslands but concluded that shading, not temperature or competition, was the most significant factor in determining seedling establishment above their treeline site in Ecuador. Potentially confounding Bader et al.'s (2007) results is the fact that shade can affect seedling establishment through different mechanisms. Shade can reduce the likelihood of photoinhibition in cold-temperature and high-light environments such as tropical treelines (Ball et al. 1991). In addition, the natural or artificial canopy needed to create shade can buffer plants against extreme temperatures fluctuations relative to more open, un-shaded

microhabitats. As such, based on the limited current evidence, it can be difficult to untangle the importance of shade *per se* versus the buffering effects against temperature extremes that canopy coverage provides in determining seedling establishment success above treelines.

Extreme temperatures are known to play a disproportionate role in shaping species' current range limits and responses to climate change (Jentsch et al. 2007, Zimmermann et al. 2009, Thompson et al. 2013). Extreme low temperatures and the physiological threat they pose to plant tissues are potentially more important at and above tropical treelines than temperate treelines since tropical treeline trees do not enter into a true state of dormancy. Therefore these trees maintain actively growing tissue throughout the year that is at relatively high risk of damage during even mild freezing events experienced at tropical treelines (Sakai and Larcher 1987). In the Peruvian Andes, Rehm and Feeley (*in Press a*) demonstrated that several tropical treeline-forming species may be prevented from establishing above treelines due to extreme low temperatures experienced in the grasslands outside of the closed-canopy cloud forest. This finding is congruent with the results of Cavieres et al. (2000) who found that freezing temperatures may pose a species-specific threat to seedlings growing above a Venezuelan treeline. Therefore, the occurrence of even rare freezing events may help to explain the lack of upslope shifts in tropical treelines despite increasing mean temperatures.

Due to the relative dearth of studies at tropical treelines, it is unclear how microclimatic stressors and non-climatic factors work to promote or inhibit seedling establishment above the current treeline. As such, our goals in this study were to build a more comprehensive understanding of the specific and possibly unique factors

controlling seedling establishment at tropical treelines by conducting a large-scale seedling transplant at a treeline in the Peruvian Andes. Specifically we wanted to test the effects of 1) mean temperature, 2) absolute low temperature, 3) shading, and 4) the interactions between temperature and shading on the survival of tree seedlings transplanted into the grasslands above treeline. In addition, due to the predominant focus on mean growing season temperatures as an explanation of treeline form and location, we also wanted to simulate climatic warming by planting seedlings in locations with mean temperatures several degrees higher than they currently experience to test the effects that changes in mean temperatures during climate change will have on seedling recruitment at treeline.

METHODS

Study site

This study was conducted in the southeastern Peruvian Andes at treeline sites located within Manu National Park in the Department of Cusco (in the areas of 13°6'18" S, 71°35'21" W). Treeline elevations in the study area range from approximately 3100 to over 3650 meters above sea level (m.a.s.l.). The treeline ecotone can be characterized by a sharp transition from closed-canopy tropical montane cloud forest to open grassland (locally known as *puna*). This ecotone transition occurs over distances of one to 10 m. Seasonality at the treeline sites is characterized by two distinct seasons, a relatively dry-cold season occurring from May to July and a relatively wet-warm season from October to March (Rapp and Silman 2012). For a full description of the study sites see Rehm and

Feeley (2013) and for a description of climate in the study area see Rapp and Silman (2012).

Transplant experiment

Seedlings (≤ 50 cm tall and ≤ 1.5 cm diameter at ground level) of four common canopy-forming treeline species (*Weinmannia fagaroides*, *Gynoxys nitida*, *Clethra cuneata*, *Symplocos baehni*) were collected at three different treeline sites located at approximately 3650 m.a.s.l.. The majority of seedlings were encountered at treeline or directly outside of treeline (< 5 m into the grassland) except for *S. baehni*, which were only found inside closed canopy cloud forest. Seedlings were carefully removed from the ground with sufficient soil left within the root matrix to allow any associated mycorrhizae to be transplanted with the seedling. Seedlings were then transplanted to one of four treeline transplant sites: two high- (3650 m.a.s.l.), one mid- (3325 m.a.s.l.) and one low-elevation site (3150 m.a.s.l.). Mean temperatures at the low-elevation and mid-elevation sites were roughly 2.0 to 3.5 °C and 1 to 1.5 °C warmer, respectively, than the high elevation sites (Table S1). The 2.0 to 3.5 °C higher mean temperatures at the low-elevation site relative to the high-elevation sites are the same as the predicted increase in temperature to occur on the eastern slope of the Peruvian Andes over the next 40-70 years (Urrutia and Vuille 2009).

At each transplant site, seedlings were planted under one of four treatments: treeline (control), open grassland, 60% artificial shade, and 60% artificial shade with enhanced nighttime warming. To quantify the microclimate at all treatment plots and transplant sites a HOBO ProV2 temperature/relative humidity data logger (U23-001;

accuracy: $\pm 0.2^{\circ}\text{C}$, Onset Computer Corporation, Massachusetts, USA) was placed in the center of each plot 10 cm above the soil surface, shielded from direct sunlight by a well ventilated polystyrene cover and programmed to record temperature every 30 min.

Seedlings were naturally occurring along the treeline; seedlings assigned to the treeline treatment were therefore moved between adjacent areas to create a baseline control treatment accounting for any transplant effect. Transplant areas within the treeline occurred along the forest edge and were shaded by the forest canopy during the majority of the day, receiving direct sunlight only during the afternoon. Treeline seedlings were therefore classified as “shaded” for statistical analyses (see below) because shade is likely most important during the morning when low temperatures are coupled with high light intensities leading to photoinhibition (Ball et al. 1991, Bader et al. 2007).

In the open grassland treatment, seedlings were transplanted 20 m into the open grassland in the open spaces between grass tussocks. While seedlings in the open grassland treatment received some shading from the tall tussock grasses (average grass canopy height = 60-80 cm), this shading would be the typical amount of shade provided for individuals naturally growing within the grassland matrix away from treeline. As such, seedlings transplanted into the open grassland were classified as “not shaded” during statistical analyses.

Both artificial shade treatments consisted of a shade cloth stretched across a frame one meter above the soil surface and were adjacent to the open grassland plots. In the shading with enhanced nighttime warming treatment, an aluminum landscape cloth was used as this material reflects long-wave radiation, raising mean and absolute low nighttime temperatures of the underlying ground and vegetation. Microclimate conditions

under this treatment were roughly similar to those found at the treeline treatment at the same transplant site (for a full description of treatment microclimates see Results and Table S1). The 60% shade treatment with minimal nighttime warming used a standard non-metallic shade cloth. This shade cloth did result in slightly elevated nighttime temperatures but temperature profiles remained intermediate between the open grassland and treeline treatments (Table S1).

The number of available seedlings for transplant varied between species from a high of $n = 689$ for *W. fagaroides* to a low of $n = 179$ for *S. baehinii*. In order to use a minimum of 25 individuals per species under each treatment and transplant site combination, complete replication was only possible for *W. fagaroides*. For the remaining species (*S. baehinii*, *C. cuneata*, and *G. nitida*), seedlings were only transplanted to high-elevation site A where they were subjected to all treatments and to the low-elevation site where seedlings were only subjected to the treeline and open grassland treatments (Table S2).

For *W. fagaroides*, two groups of seedlings were transplanted, the first during May and June 2012 at the beginning of the dry-cold season and the second from September and October 2012 at the beginning of the wet-warm season. For all other species transplants were only conducted between September and October 2012 at the beginning of the wet-warm season. Within a season, all individuals were transplanted within a 21-day period. Microclimatic measurements (i.e., mean and absolute low temperatures) for each species, treatment and transplant site combination were taken starting from the day individuals were transplanted which resulted in variation of microclimate at some treatment and transplant site combinations within a species. In

other words, even though individuals of the same species were transplanted to the same treatment within the same transplant site, groups of individuals may have been transplanted on different days within the same season resulting in slightly different values for mean and absolute low temperatures (e.g. *G. nitida* seedlings transplanted to the treeline treatment at high-elevation site A).

Survival of all individuals was monitored until 24 July 2014. Individuals were considered alive if they maintained at least one green leaf at the end of the study period. Individuals that dropped all their leaves shortly after the initial transplant would often sprout new leaves over the next 2-6 months and were therefore also considered alive if they had at least one green leaf at the end of the experiment.

Statistical Analysis

Due to the variation in experimental design between species, two different statistical analyses were utilized to test the effects of temperature, shade and their interaction on seedling survival. Survival for *W. fagaroides* was analyzed using generalized linear mixed-effects models (GLMMs) from the lme4 package (Bates et al. 2014) in R 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria). In the GLMMs, survival was modeled as a binomial response variable with a logit link function. To test how temperature and shade affected survival of transplanted seedlings, models included fixed effects of mean temperature since transplant, absolute low temperature since transplant, transplant season (dry-cold or wet-warm season), shade (shaded or not shaded) and the interactions between shade and absolute low temperature and shade and

mean temperature. The identity of the transplant site was included in the models as a random effect.

We first fit a full model including all main and interaction terms. We then used the Likelihood ratio test (LRT) to eliminate non-significant variables following a backward selection procedure. Main effects could only be excluded if the interaction terms including the main effect were first eliminated. The significance of random effects was not evaluated as they were a required part of the experimental design and therefore were included in all models.

For the remaining three species (*S. baehni*, *C. cuneata*, and *G. nitida*), survival was modeled using generalized linear models (GLMs) within the lme4 package. Model formation and model selection procedures were similar to those for *W. fagaroides*, but for these species we did not include a random effect of transplant site as seedlings were only transplanted into two of the four sites (high-elevation site A and the low-elevation site). In addition, the transplant season was not included as these three species were only transplanted during the wet-warm season. A second analysis was run including the number of days since different groups of seedlings were transplanted, but results were almost identical to the GLMs presented here. As such, the simpler models were preferred and the results of these models are discussed here. Therefore, GLMs for the remaining three species modeled survival against the main effects of mean temperature since transplant, absolute low temperature since transplant, shade, and the interactions between shade and mean temperature and shade and absolute low temperature. Model selection then proceeded as for *W. fagaroides*, first fitting a full model and then using the LRT to determine the significance of fixed effects.

RESULTS

Mean temperatures within a transplant site (high-elevation A, high-elevation B, mid-elevation, and low-elevation) did not vary markedly among treatments. Absolute low temperature did vary among treatments with absolute low temperatures under the open grassland and 60% shade with minimal nighttime warming (standard shade cloth) being on average 3.5 and 3.6 °C colder than at the corresponding treeline sites, respectively (Table S1). Absolute minimum temperatures under the metallic 60% shade (i.e., with enhanced nighttime warming) were generally intermediate between the open grassland and treeline sites, with temperatures being 1.7 °C colder than the treeline. Therefore, between the various elevations and treatments we created a range of microclimatic conditions, in terms of both means and extreme low temperatures, that could be used to assess the effects of temperature on seedling survival.

We assume that background mortality due to any transplant effect would be comparable across controls, treatments and locations. Therefore we discuss seedling survival in relative terms as opposed to absolutes. Seedling survival varied greatly among species and treatments. Overall fewer than half of all transplanted seedlings survived to the end of the experiment (565 of 1323 seedlings) with *C. cuneata* having the highest survivorship (140 of 225 [62%] of individuals alive) and *S. baehni* with the lowest survivorship (13 of 179 [7%] of individuals alive).

For *W. fagaroides*, the species for which we had the greatest sample size and most extensive set of treatments, survival was negatively affected by increasing mean temperature (Table 1, Figure 1). Only 41 of 230 *W. fagaroides* individuals (18%) survived to the end of the experiment at the low elevation site where mean temperatures

were highest for all treatments. Survival was highest at the mid-elevation site (84 of 131, 64%) where mean temperature for each treatment was generally intermediate between the high- and low-elevation sites. Survival at the two colder high-elevation sites was 68 of 198 (34%) and 76 of 130 (58%) for high-elevation sites A and B, respectively. In addition to the effects of mean temperature on survival, seedlings transplanted during the dry-cold season had significantly higher survival (68 of 145, 47%) than those transplanted during the wet-warm season (208 of 544, 38%; Table 1). Neither shade nor absolute low temperature had significant effects on seedling survival for *W. fagaroides*.

S. baehnii had poor survival when transplanted from their natural habitat in the cloud forest interior to any of the experimental treatments. Yet similar to *W. fagaroides*, *S. baehnii* seedling survival decreased with mean temperature (Figure 2a). In addition, seedling survival increased as low temperature increased (Figure 2b). The majority of surviving *S. baehnii* seedlings across all treatments and transplant sites were at the treeline treatment at high-elevation site A, with 11 of 25 (44%) seedlings being alive at the end of the experiment. Only one of 25 individuals survived under the 60% shading and nighttime warming treatments at high-elevation site A and open grassland treatment at the low-elevation site. No individuals survived under the 60% shading treatment with minimal nighttime warming or open grassland treatment at high-elevation site A or the treeline treatment at low elevation.

Survival of *C. cuneata* seedlings also depended on mean temperature but the relationship was opposite of that of *W. fagaroides* and *S. baehnii*, with higher mean temperatures having a positive effect on survival (Figure 3a). Furthermore, survival of *C. cuneata* had a positive relationship with increasing low temperature (Figure 3b). In

addition to just temperature, seedling survival was higher when seedlings were provided with shade (Figure 3c), but there were no interactions between temperature and shade on seedling survival (Table 1).

In contrast to the other species, shade was the only factor found to affect seedling survival of *G. nitida* (Table 1). For *G. nitida* seedlings, 114 of 150 (76%) shaded seedlings survived compared to 34 of 80 (44%) individuals transplanted into the open grassland without shade (Figure 4).

DISCUSSION

By conducting a seedling transplant experiment using multiple high elevation tree species that are common at treeline, we were able to demonstrate that upslope shifts in the leading range edges of many forest species, and hence the treeline, in response to global climate change will be dependent on both climatic and non-climatic factors. Current predictions of upslope shifts in tropical treelines may be incorrect as these predictions are largely based largely on projected changes in mean temperature but local scale treeline processes may be dependent on species-specific responses to changes in mean temperature as well as additional climatic and non-climatic factors. For example, our current understanding of temperature controls on treeline leads to the prediction that all treeline species will experience increased survival at higher mean temperatures. In contrast to this prediction we found that 2 of 4 species included in this study, *W. fagaroides* and *S. baehni*, showed decreased seedling survivorship with increased temperatures.

On the other hand, *C. cuneata* did show an increase in seedling survival with increasing mean temperature, supporting the contention that low mean temperature negatively affects recruitment at treeline but mean temperature only partially explained survival of this species. These contradictory findings illustrate that in order to comprehensively explain the location and dynamics of tropical treelines we need to look beyond just mean temperature.

The fact that survival of *W. fagaroides* decreased with mean temperature was contrary to expectation since this is the most common and abundant species at our study sites at both the adult and juvenile life stage. If low mean temperatures were indeed having a negative effect on individuals at treeline, then we would expect to find higher survival and hence higher fitness with increasing mean temperature for this dominant canopy-forming treeline species. Yet our findings show the opposite. The reduced survival at higher mean temperatures may be a reflection of the narrow climatic niche breadth exhibited by *W. fagaroides* and many other tropical cloud forest species (Myers et al. 2000, Rapp et al. 2012). Indeed, the current range of *W. fagaroides* in the region extends from 3700 to 3250 m.a.s.l., meaning that when moving individuals downslope to 3150 m.a.s.l. we transplanted them outside of the species' current realized climatic niche (Rapp et al. 2012). The low-elevation site was on average 2.0 to 3.5 °C warmer than the site where individuals were collected from, suggesting that even a mild increase in mean temperatures within the current range of *W. fagaroides* may be outside of the climatic tolerance of this species. Our findings demonstrate that the conservative prediction of a 3.5 °C mean temperature increase in the Andes by 2100 (Urrutia and Vuille 2009) may

severely reduce survival and recruitment of the current dominant treeline-forming species, further slowing upslope shifts in treeline.

Seedling survival of *S. baehinii* also showed a negative relationship with mean temperature but this relationship might be best explained by the fact that this species appears to be predominantly a forest-interior species. Mean temperature within the closed canopy cloud forest tends to be lower than the open grasslands, with mean temperature at treeline being intermediate between the two (Rehm and Feeley 2013). Moving individuals from the forest interior to any treatment outside of the forest or to sites downslope may have resulted in high mortality due to the higher mean temperature relative to the forest interior. The positive relationship between *S. baehinii* seedling survival and low temperature also supports the idea that this species does best in the forest interior and experiences high mortality even at the forest edge. As a forest-interior species we would expect that individuals of *S. baehinii* would maintain relatively poor freezing resistance because they would rarely be exposed to sub-zero temperatures within the forest interior (Sakai and Weiser 1973). Accordingly, transplanting individuals away from the forest interior to the relatively harsh freezing regimes of the treeline or open grassland likely exposed them to potentially lethal frost events. Therefore *S. baehinii*, which is a canopy-forming species at treeline, is an unlikely candidate to expand on its own into the grasslands except at rare microsites where temperature patterns mimic those of the forest interior.

C. cuneata was the only species to show increased seedling survival when exposed to higher mean temperatures. This relationship could support the proposed role of mean temperature in determining treeline elevation and form. However, the positive

relationship of *C. cuneata* seedling survival with low temperature also highlights the need to incorporate temperature extremes when explaining local and regional treeline dynamics. Indeed, frost events and other climate extremes have already been elicited to explain the abrupt form of some treelines in the tropics (Harsch and Bader 2011). *C. cuneata* has previously been shown to be sensitive to extreme frost events occurring in the open grasslands above treeline (Rehm and Feeley, *in Press a*). Therefore, while growth processes may be inhibited by low mean temperatures at treeline (Körner 2012), rare frost events may periodically kill individuals growing above treeline, further preventing this species' expansion into the open grasslands (Rehm and Feeley, *in Press a*). It appears that *C. cuneata* will need to experience both increased mean and low temperatures during future climate change before it may expand its range beyond the treeline boundary. Mean temperature will continue to increase throughout the Andes (Urrutia and Vuille 2009) but concurrent increases in low temperature extremes are less certain and will largely depend on shifts in cloud base, which themselves are difficult to predict (Quintana-Gomez 1999, Kodra et al. 2011, Halladay et al. 2012). Complicating matters even further is the fact that *C. cuneata* seedlings had higher survival when provided with shade, which indicates that factors other than temperature can also play a significant role in explaining tropical treeline dynamics.

In addition to *C. cuneata*, shade also significantly increased survival of transplanted seedlings of *G. nitida*. This finding supports previous work showing that forest seedlings transplanted above treeline had higher survival when provided with shade (Bader et al. 2007). At our treeline sites, chronic photoinhibition may occur during mornings when sub-zero temperatures are combined with high light intensities, likely

limiting the ability of seedlings to establish in the open grasslands (Ball et al. 1991, Bader et al. 2008). Although many treeline species maintain adaptations that specifically protect them from excess solar radiation and photoinhibition, the exposed environment of the open grassland treatment may have been unsuitable due to light intensities beyond what treeline species can tolerate. The decreasing trend in cloud cover throughout much of Andes suggests that solar radiation loads and the likelihood of frequent photoinhibition will increase in the future making the already harsh grassland environment even more difficult for trees to invade (Halladay et al. 2012).

An upslope shift in treeline can occur when just a single species establishes at elevations higher than the current treeline. It is possible that additional treeline-forming species not included in this study may see large increases in seedling survival as a result of increasing mean temperatures and therefore act as pioneers in invading the high elevation grasslands. In such a case, we may expect treeline to advance in a slow, diffuse manner where forest invasion into the grasslands follows a successional pattern where many species can establish above treeline only after significant microclimatic buffering and possibly shade is provided by a pioneer species. If such a pioneer species does exist, it should dominate current recruitment patterns because mean temperatures in our study sites have increased by ~ 0.15 °C per decade since the 1950s resulting in more favorable climatic conditions (Vuille and Bradley 2003). Yet the species included in this study were specifically chosen because they represented the most dominant and abundant species at the juvenile stage at the treeline ecotone. Only one species, *C. cuneata*, showed a positive affect on seedling recruitment due to higher mean temperatures, but even then, low temperature extremes and shade also significantly altered seedling survival rates.

Collectively these findings suggest that the most likely candidates for species expansion into the grasslands are those included in this study. However, the included species exhibit species-specific responses to their local environment, making predictions of treeline shifts based on mean temperature alone tenuous at best. Complicating matters further is the fact that there is a large suite of additional factors not considered here that may further limit forest expansion above tropical treelines, including other climatic variables such as moisture seasonality, as well as non-climatic variables such as edaphic conditions (Rehm and Feeley *in Press* b). Clearly it would be highly informative for future studies to test the complementary or contradictory ways in which mean temperature and additional factors affect recruitment of tropical treeline-forming species.

These results have important implications for global biodiversity as the cloud forests of the Andes are one of the most diverse ecosystems on earth and species below treeline have already begun to respond to increasing mean temperatures by shifting the centers of their distributions upslope (Feeley et al. 2011, Forero-Medina et al. 2011). As species move upslope they will generally abandon habitats at lower elevations and hence will need to gain area at higher elevations in order to avoid severe range contractions and biotic attrition (Colwell et al. 2008). If Andean cloud forest species are able to expand their ranges upslope then they may actually increase their range area and population sizes because land area increases at high elevations in the Andes (Feeley and Silman 2010). However, our results support the idea that various factors, some of which may not necessarily change during future climate change (e.g. shade), will impede species from expanding their ranges beyond the current forest boundary to higher elevations, resulting in a stationary treeline. This expectation is supported by the fact that no studies have

recorded upslope shifts in tropical treeline over the past several decades despite significant warming (Harsch and Bader 2011, Rehm and Feeley *in Press* b). Therefore our current understanding of the factors that determine species range limits and the dynamics of tropical treeline may be overly simplistic, leading to unrealistic projections of how these diverse systems will respond to climate change.

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Table 4.1. Results of generalized linear mixed-effects models (GLMMs) and generalized linear models (GLMs) testing which predictor variables best explain survival of transplanted seedlings. GLMMs were used for *W. fagaroides* as there were sufficient seedlings available to create full replication across all treatments and study sites. GLMMs included fixed effects of mean temperature since transplant, absolute low temperature since transplant, transplant season, shade, and the interactions between shade and mean temperature and shade and low temperature and transplant site entered as a random effect. GLMs were used for *G. nitida*, *C. Cuneata*, and *S. baehni* because seedlings were available for transplant at only two of four transplant sites. GLMs included the same fixed effects used in the GLMMs except for transplant season because all seedlings were transplanted during the same season. For both GLMMs and GLMs variable selection followed a backward selection procedure using the Likelihood Ratio Test and $p = 0.05$.

	Estimate	SE	z-value	P
<i>(a) W. fagaroides</i>				
Intercept	4.57	1.55	2.95	0.003
Mean Temperature	-0.46	0.17	-2.82	0.005
Transplant Season	-0.84	0.26	-3.23	0.001
Transplant Site [R]*	0.06	0.34		
<i>(b) S. baehni</i>				
Intercept	17.04	3.99	4.27	<0.001
Mean Temperature	-2.04	0.45	-4.50	<0.001
Low Temperature	0.77	0.30	2.50	0.012
<i>(c) C. cuneata</i>				
Intercept	-9.29	3.77	-2.47	0.014
Mean Temperature	0.94	0.32	2.92	0.003
Low Temperature	0.59	0.21	2.78	0.006
Shade	3.57	0.80	4.47	<0.001
<i>(d) G. nitida</i>				
Intercept	-0.25	0.23	-1.12	0.265
Shade	1.40	0.30	4.75	<0.001

- Transplant Site was included as a random effect only for this species' analysis

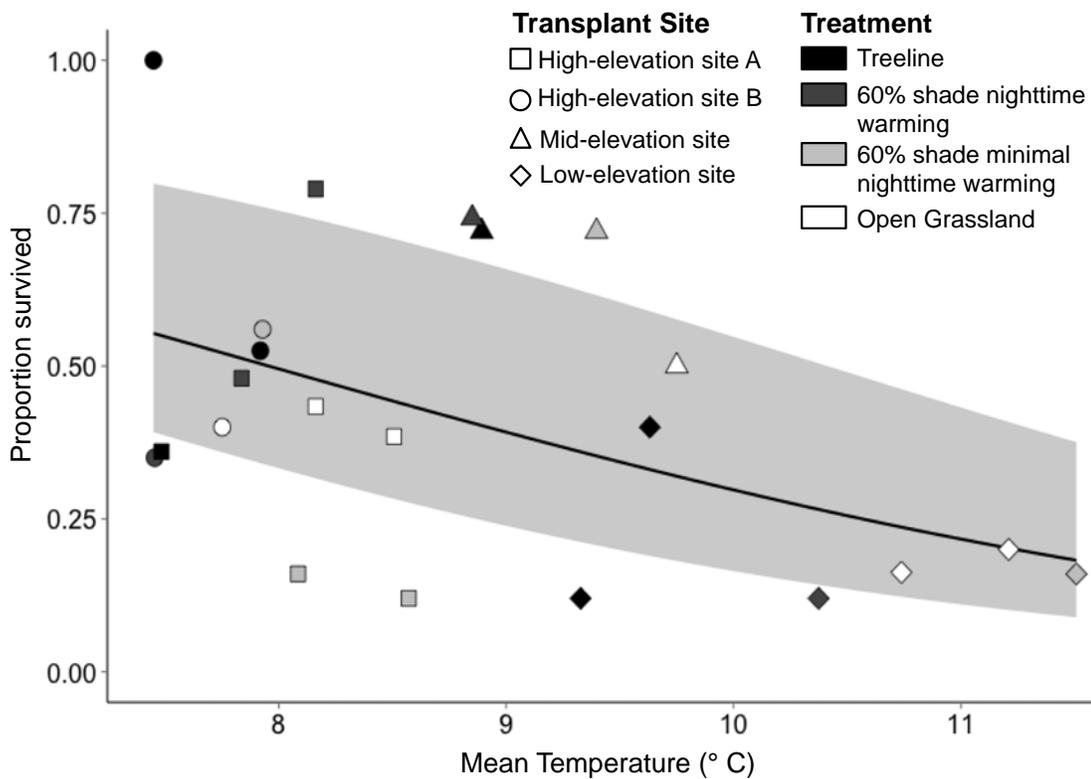


Fig 4.1. Mean (solid line) and 95% confidence intervals (shaded area) for the modeled relationship between *W. fagaroides* seedling survival and mean temperature across all transplant sites and treatments according to generalized linear mixed-effects models. Points indicate observed results of experiments. Different symbols distinguish results from different transplant sites while shading distinguishes different experimental treatments. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.

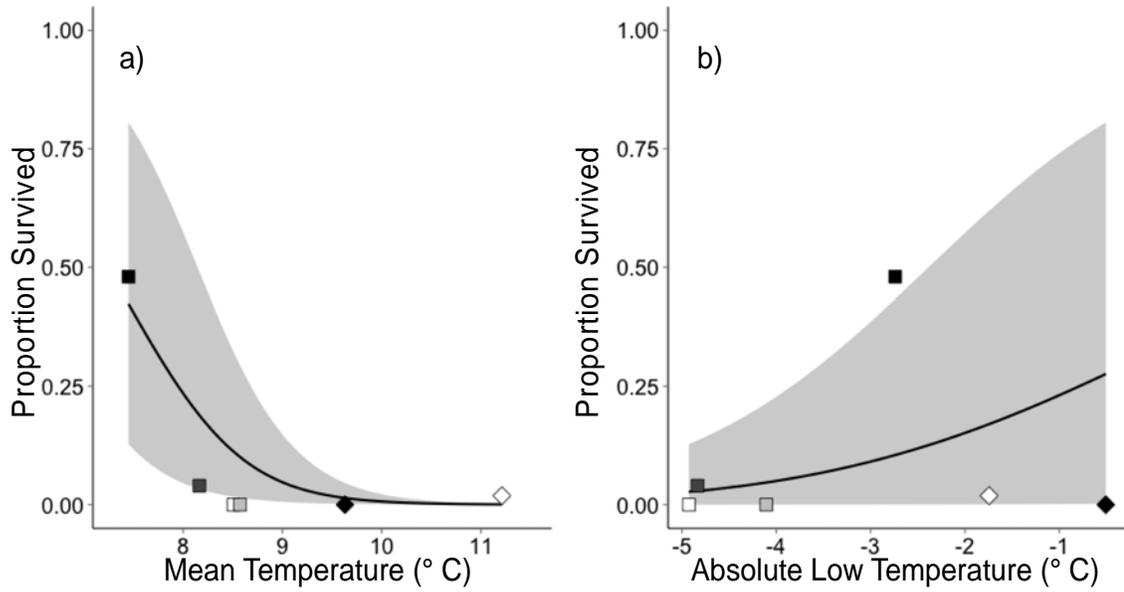


Fig 4.2. Modeled relationships between *S. baehni* seedling survival and a) mean temperature and b) absolute low temperature. Solid lines represents the modeled mean and the shaded area is the 95% confidence intervals as determined by generalized linear models. Squares are results from the high-elevation site A while diamonds are results from the low-elevation site. Different shades of symbols represent different treatments; black = treeline, dark gray = 60% shade with nighttime warming, light gray = 60% shade with minimal nighttime warming, and white = open grassland.

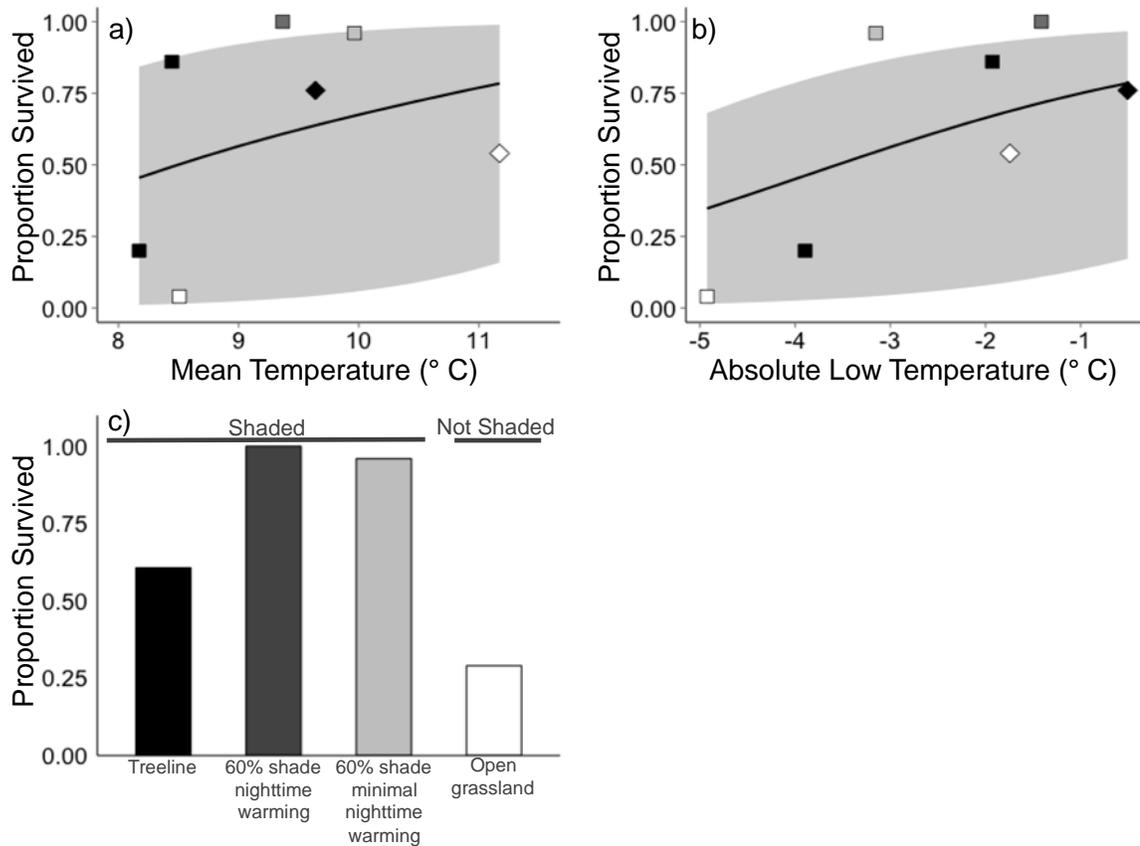


Fig 4.3. *C. cuneata* seedling survival and a) mean temperature, b) absolute low temperature and c) shade. Solid lines in a) and b) represent the modeled mean and the shaded area is the 95% confidence intervals as determined by generalized linear models. Squares are results from the high-elevation site A while diamonds are results from the low-elevation site. Different shades of symbols represent different treatments; black = treeline, dark gray = 60% shade with nighttime warming, light gray = 60% shade with minimal nighttime warming, and white = open grassland. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.

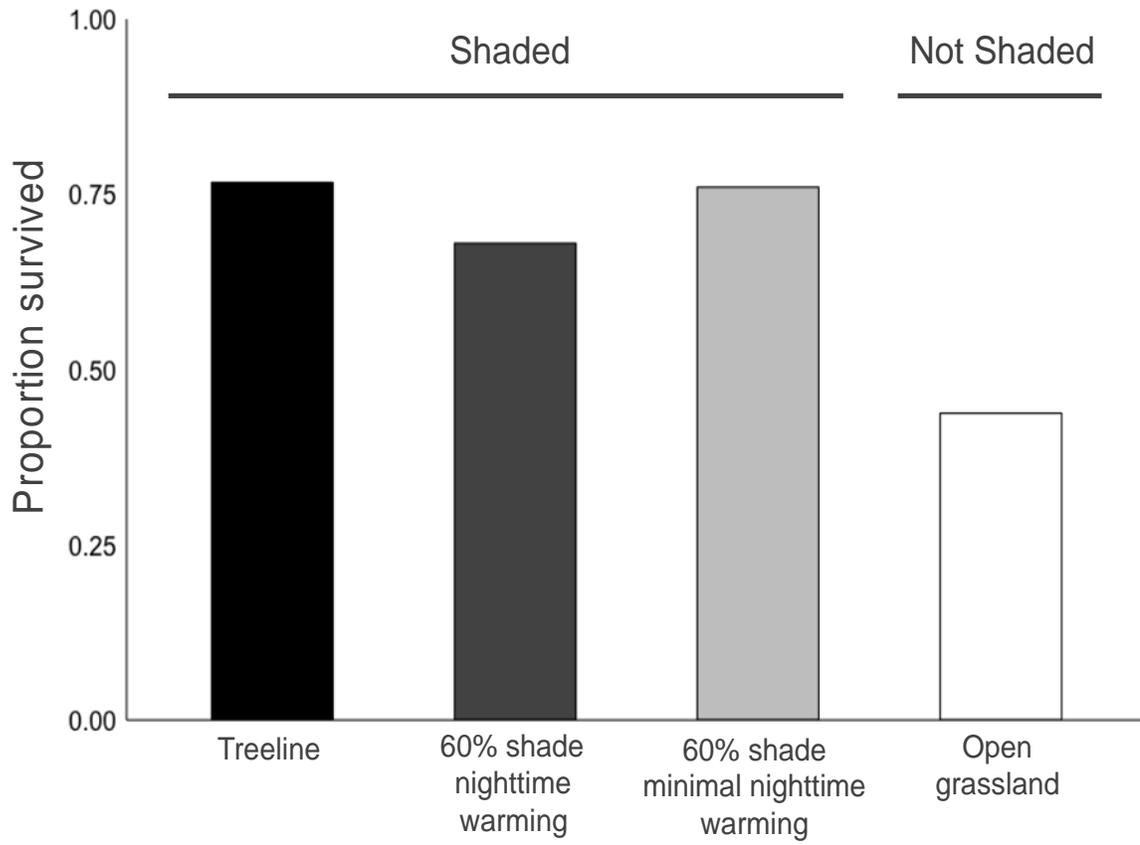


Fig 4.4. *G. nitida* seedling survival under various treatments. Seedlings planted into the treeline, and two shade treatments were classified as being Shaded and seedlings transplanted into the open grassland were classified as Not Shaded.

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Table S4.1. Mean and absolute low temperatures of each treatment for each transplant site from 1 May 2012 – 24 July 2014. Temperatures are given to provide a reference for the efficacy of the experimental treatments. Note that temperature measurements given here are from the entire experimental period but actual temperature measurements for each species, treatment, and transplant site combination may vary because microclimatic measurements were taken from the day individuals were transplanted which was often shorter than the full measurement period.

	Mean Temperature (°C)	Low Temperature (°C)
<i>High-elevation site A</i>		
Treeline	7.5	-6.8
Open puna	8.2	-8.9
60% shade nighttime warming	8.1	-6.3
60% shade minimal nighttime warming	7.8	-7.7
<i>High-elevation site B</i>		
Treeline	7.9	-3.3
Open puna	7.8	-7.9
60% shade nighttime warming	7.5	-5.7
60% shade minimal nighttime warming	7.9	-6.5
<i>Mid-elevation site</i>		
Treeline	8.9	-2.8
Open puna	9.8	-5.4
60% shade nighttime warming	8.9	-3.5
60% shade minimal nighttime warming	9.4	-7.3
<i>Low-elevation site</i>		
Treeline	9.3	-1.7
Open puna	10.7	-6.3
60% shade nighttime warming	10.4	-5.7
60% shade minimal nighttime warming	11.5	-7.4

Table S4.2: Summary data for seedlings transplants of all species under all treatments and transplant sites. Note that variation in planting date within a season led to groups of individuals planted under the same treatment at the same transplant site to have different mean temperatures resulting in multiple points for some treatment and transplant site combinations.

Species	Treatment	Transplant site	Transplant season	Shade (Y = shaded, N = not shaded)	Low Temperature (°C)	Mean Temperature (°C)	Number alive	Total number transplanted
<i>Weinmannia fagaroides</i>	Treeline	High-elevation B	Dry/cold	Y	-2.7	7.4	25	25
<i>Weinmannia fagaroides</i>	Treeline	High-elevation B	Wet/warm	Y	-3.3	7.9	21	40
<i>Weinmannia fagaroides</i>	Treeline	High-elevation A	Wet/warm	Y	-6.8	7.5	9	25
<i>Weinmannia fagaroides</i>	Treeline	Mid-elevation	Wet/warm	Y	-2.8	8.9	18	25
<i>Weinmannia fagaroides</i>	Treeline	Low-elevation	Dry/cold	Y	-0.5	9.6	10	25
<i>Weinmannia fagaroides</i>	Treeline	Low-elevation	Wet/warm	Y	-1.7	9.3	6	50
<i>Weinmannia fagaroides</i>	60% shade and nighttime warming	High-elevation B	Wet/warm	Y	-5.7	7.5	7	20
<i>Weinmannia fagaroides</i>	60% shade and nighttime warming	High-elevation A	Dry/cold	Y	-4.8	8.2	15	19
<i>Weinmannia fagaroides</i>	60% shade and nighttime warming	High-elevation A	Wet/warm	Y	-6.3	7.8	12	25
<i>Weinmannia fagaroides</i>	60% shade and nighttime warming	Mid-elevation	Wet/warm	Y	-3.5	8.9	23	31
<i>Weinmannia fagaroides</i>	60% shade and nighttime warming	Low-elevation	Wet/warm	Y	-5.7	10.4	3	25
<i>Weinmannia fagaroides</i>	Open Grassland	High-elevation B	Wet/warm	N	-7.9	7.8	8	20
<i>Weinmannia fagaroides</i>	Open Grassland	High-elevation A	Dry/cold	N	-4.9	8.5	10	26

Table S4.2 Continued

Species	Treatment	Transplant site	Transplant season	Shade (Y = shaded, N = not shaded)	Low Temperature (°C)	Mean Temperature (°C)	Number alive	Total number transplanted
<i>Weinmannia fagaroides</i>	Open Grassland	High-elevation A	Wet/warm	N	-8.9	8.2	23	53
<i>Weinmannia fagaroides</i>	Open Grassland	Mid-elevation	Wet/warm	N	-5.4	9.8	25	50
<i>Weinmannia fagaroides</i>	Open Grassland	Low-elevation	Dry/cold	N	-1.7	11.2	5	25
<i>Weinmannia fagaroides</i>	Open Grassland	Low-elevation	Wet/warm	N	-6.3	10.7	13	80
<i>Weinmannia fagaroides</i>	60% shade, minimal nighttime warming	High-elevation B	Wet/warm	Y	-6.5	7.9	14	25
<i>Weinmannia fagaroides</i>	60% shade, minimal nighttime warming	High-elevation A	Dry/cold	Y	-4.1	8.6	3	25
<i>Weinmannia fagaroides</i>	60% shade, minimal nighttime warming	High-elevation A	Wet/warm	Y	-7.7	8.1	4	25
<i>Weinmannia fagaroides</i>	60% shade, minimal nighttime warming	Mid-elevation	Wet/warm	Y	-7.3	9.4	18	25
<i>Weinmannia fagaroides</i>	60% shade, minimal nighttime warming	Low-elevation	Wet/warm	Y	-7.4	11.5	4	25
<i>Symplocos baehnii</i>	Treeline	High-elevation A	Wet/warm	Y	-2.7	7.4	12	25

Table S4.2 Continued

Species	Treatment	Transplant site	Transplant season	Shade (Y = shaded, N = not shaded)	Low Temperature (°C)	Mean Temperature (°C)	Number alive	Total number transplanted
<i>Symplocos baehnii</i>	Treeline	Low-elevation	Wet/warm	Y	-0.5	9.6	0	25
<i>Symplocos baehnii</i>	60% shade and nighttime warming	High-elevation A	Wet/warm	Y	-4.8	8.2	1	25
<i>Symplocos baehnii</i>	Open Grassland	High-elevation A	Wet/warm	N	-4.9	8.5	0	26
<i>Symplocos baehnii</i>	Open Grassland	Low-elevation	Wet/warm	N	-1.7	11.2	1	53
<i>Symplocos baehnii</i>	60% shade, minimal nighttime warming	High-elevation A	Wet/warm	Y	-4.1	8.6	0	25
<i>Clethra cuneata</i>	Treeline	High-elevation A	Wet/warm	Y	-3.9	8.2	5	25
<i>Clethra cuneata</i>	Treeline	High-elevation A	Wet/warm	Y	-1.9	8.4	43	50
<i>Clethra cuneata</i>	Treeline	Low-elevation	Wet/warm	Y	-0.5	9.6	19	25
<i>Clethra cuneata</i>	60% shade and nighttime warming	High-elevation A	Wet/warm	Y	-1.4	9.4	25	25
<i>Clethra cuneata</i>	Open Grassland	High-elevation A	Wet/warm	N	-4.9	8.5	1	25
<i>Clethra cuneata</i>	Open Grassland	Low-elevation	Wet/warm	N	-1.7	11.2	27	50
<i>Clethra cuneata</i>	60% shade, minimal nighttime warming	High-elevation A	Wet/warm	Y	-3.2	10.0	24	25

Table S4.2 Continued

Species	Treatment	Transplant site	Transplant season	Shade (Y = shaded, N = not shaded)	Low Temperature (°C)	Mean Temperature (°C)	Number alive	Total number transplanted
<i>Gynoxys nitida</i>	Treeline	High-elevation A	Wet/warm	Y	-3.9	8.2	16	25
<i>Gynoxys nitida</i>	Treeline	High-elevation A	Wet/warm	Y	-3.9	8.1	41	50
<i>Gynoxys nitida</i>	Treeline	Low-elevation	Wet/warm	Y	-0.5	9.6	21	25
<i>Gynoxys nitida</i>	60% shade and nighttime warming	High-elevation A	Wet/warm	Y	-2.8	8.1	17	25
<i>Gynoxys nitida</i>	Open Grassland	High-elevation A	Wet/warm	N	-4.9	8.5	24	55
<i>Gynoxys nitida</i>	Open Grassland	Low-elevation	Wet/warm	N	-1.7	11.2	11	25
<i>Gynoxys nitida</i>	60% shade, minimal nighttime warming	High-elevation A	Wet/warm	Y	-3.6	8.3	19	25

CHAPTER V
CONCLUSIONS AND FUTURE DIRECTIONS

Treelines have long been of interest to researchers because they are one of the most abrupt and visible terrestrial ecosystems (Humboldt, 1854). Throughout this dissertation I have shown that treelines will continue to act as important study systems, especially in terms of ecosystem responses to climatic change. However, in certain areas like the tropics, our understanding of treeline dynamics is still severely limited. As tropical treelines often represent the upper distributional limit of the hyper-diverse cloud forest ecosystem, understanding tropical treeline dynamics will improve our ability to predict how this cloud forest system will respond to ongoing climate change.

Chapter I represents the first attempt to summarize all the existing literature related to tropical treelines and discuss tropical treelines in a climate change context. Other comprehensive treeline works largely focus on temperate treelines and search for a global explanation of treeline form and position with little attention given to differences between tropical and temperate treelines (e.g. Holtmeier, 2009; Körner, 2012). While the number of studies taking place at tropical treelines is still extremely limited, some general patterns do begin to emerge. One of the most striking differences between tropical and temperate treelines are varying climate regimes. Tropical treelines experience relatively mild annual temperature fluctuations but extreme daily fluctuations whereas temperature patterns at temperate treelines follow extreme seasonal variation with most temperate treelines experiencing a severe winter where trees enter into a state of dormancy. Given the hypothesized importance of temperature on treeline position (Körner, 1998; Paulsen & Körner, 2014), these differences in climatic patterns may play an important role in modulating treeline positions at local and regional scales. In addition, In Chapter I I show that it may be necessary to look beyond just mean temperature when explaining tropical

treeline position and migrations under climate change. Indeed, all evidence from the tropics suggests that tropical treelines have remained stable despite significantly warming mean temperatures over the past decades and century (Harsch et al., 2009; Lutz et al., 2013). There seems to be a suite of climatic (e.g. extreme low temperature events, moisture seasonality) and non-climatic (e.g. anthropogenic disturbances) factors that may limit upslope shifts of tropical treelines due to increases in global mean temperatures. Geographic research biases mean that relatively few studies have taken place at tropical treelines and therefore we still have a very limited understanding of the ecology of tropical treelines. Future studies should focus on microclimate, edaphic conditions, cloud immersion and potential moisture stress (both drought and water logging), biotic interactions between tree seedlings and grassland vegetation (and possibly mycorrhizal associations), photoinhibition of seedlings, and mycorrhizal associations at and above tropical treelines.

To begin to address some of these potential factors controlling tropical treelines, I first looked towards small patches of forest that occur above the current continuous closed-canopy forest limit. The origin of these forest patches is debatable. Patches may be remnant sections of more extensive forests that have been destroyed by past human activities (Kessler, 2002; White, 2013) or they may represent an advancing forest front that has only recently been slowed by increased fire activities above treeline (Di Pasquale et al., 2008). In either case, these forest patches appear to occur in areas where microclimate is suitable for tree establishment and growth. During periods of climate change, the patches may act as nucleating foci for forest expansion into the puna by altering microclimate and act as an additional seed source in the puna. Yet to date, these

forest patches do not seem to be expanding outwards and seedling recruitment is limited to a narrow strip (< 5 m wide) around treeline, suggesting that treeline forests may provide a buffering effect for forest seedling recruitment in the grassland matrix.

The potential buffering effect of the treeline forest on microclimate was particularly evident in Chapter III. Seedlings growing above treeline are subject to a relatively harsh microclimatic regime typified by extreme cold events near the ground in the grassland matrix away from forest. Seedlings of at least three of the four study species likely experience tissue loss and possibly whole plant death due to extreme low temperatures that occur at irregular intervals in the grassland just tens of meters away from the forest. In order for seedlings growing at distances greater than a few meters from the treeline forest to survive and reach adulthood, they would need to grow to a sufficient height (greater than grass height; 50-70 cm) in order to escape the damaging freezing zone close to the ground. As far as I am aware there has been no attempt to follow individual tree seedlings near tropical treelines over time so we have a very limited knowledge of growth rates and patterns in young recruits. Adults of at least some tropical treeline-forming species appear to exhibit a conservative and extremely slow growth rate (Hoch & Körner, 2005) suggesting that seedlings may exhibit this same slow growth pattern. Slow growth in seedlings means that seedlings will stay in the dangerous freezing layer near the ground, with periodic freezing episodes repeatedly killing tissues. In such a case, seedlings may never reach sufficient heights unless low temperature extremes experience significant increases. Clearly more detailed studies investigating microclimatic conditions across tropical treelines are needed.

Long-term climatic records from climate stations in tropical mountains are rare and do not quantify microclimatic conditions at treelines themselves. Before we can make accurate predictions as to the role of freezing events in future treeline migrations, we need to understand how low temperature extremes occur at varying temporal and spatial scales. In addition, there is a need to quantify growth patterns in seedlings of a suite of treeline forming species. Furthermore, efforts should be made to determine freezing resistances of the majority of treeline forming species to help understand how the treeline forest community may be altered by low temperature events during climate driven treeline migrations.

In Chapter IV I attempted to create a more comprehensive understanding of tropical treeline dynamics under climate change by incorporating the findings of Chapters I-III into a large-scale seedling transplant experiment. Specifically I wanted to test the variable controls of mean temperature, low temperature extremes, and solar radiation (using shading treatments) on seedling recruitment outside of treeline in four dominant treeline forming species. These findings further suggest that mean temperature alone is insufficient to explain seedling recruitment at and above treeline. Seedling survival exhibited species-specific patterns with regards to the experimental treatments. Mean temperature played a significant role in only three of four study species, and in two of these species, increasing mean temperature actually had a negative effect on seedling survival (the opposite of what is predicted based on the growth limitation hypothesis). These findings show that our predictions on treeline movements under changing climates are far too simplistic (Grace et al., 2002; Harsch et al., 2009). It is possible that the ultimate positions of treelines are indeed determined by low mean temperature limitations

on growth but treeline movements due to climate change will likely be determined by various controlling factors.

This body of work presents a clear message that there is a critical need for further investigations concerning tropical treeline responses to climate change. Future studies can focus on virtually any aspect of treeline ecology ranging from leaf level processes such as photoinhibition up to mechanisms determining community dynamics. We know that cloud forest species occurring below treeline are responding to climate warming through upslope migrations (Freeman & Class Freeman, 2014; Rehm, 2014). However, treeline itself, which represents the upper distributional limit of dozens of cloud forest species' ranges, does not appear to be moving upslope. Species that are migrating upslope within the cloud forest belt may experience large range contractions and elevated extinctions risks unless the cloud forest community expands into the grasslands located above current treeline (Feeley & Silman, 2010). With this dissertation I hope to improve our understanding of current and future tropical treeline form and function. We should shift our focus away from mean temperature in order to gain a more comprehensive understanding of tropical treeline dynamics. Yet to date, other factors that may control treelines have received relatively little attention in tropical treeline systems, making predictions about tropical treeline shifts and the fate of tropical cloud forests even more uncertain.

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