

Seedling transplants reveal species-specific responses of high-elevation tropical treeline trees to climate change

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Abstract The elevations at which tropical treelines occur are believed to represent the point where low mean temperatures limit the growth of upright woody trees. Consequently, tropical treelines are predicted to shift to higher elevations with global warming. However, tree-lines throughout the tropics have remained stationary despite increasing global mean temperatures. The goal of the study reported here was to build a more comprehensive understanding of the effects of mean temperature, low-temperature extremes, shading, and their interactions on seedling survival at tropical treelines. We conducted a seedling transplant study using three dominant canopy-forming treeline species in the southern tropical Andes. We found species-specific differences and contrasting responses in seedling survival to changes in mean temperature. The most abundant naturally occurring species at the seedling stage outside the treeline, *Weinmannia fagaroides*, showed a negative relationship between the survival of transplanted seedlings and mean temperature, the

opposite of a priori expectations. Conversely, *Clethra cuneata* showed increased survival at higher mean temperatures, but survival also increased with higher absolute low temperatures and the presence of shade. Finally, the survival of *Gynoxys nitida* seedlings was insensitive to temperature but increased under shade. These findings show that multiple factors can determine the upper distributional limit of species forming the current tropical treeline. As such, predictions of future local and regional tropical treeline shifts may need to consider several factors beyond changes in mean temperature. If the treeline remains stationary and cloud forests are unable to expand into higher elevations, there may be severe species loss in this biodiversity hotspot.

Keywords Timberline · Freezing tolerance · Ecotone · Climate change · Species migration

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 climate change velocities (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 that there are often high levels of biodiversity and endemism in relatively small geographic areas (Myers et al. 2000).

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One system of particular interest in terms of biodiversity is the tropical Andean montane cloud forest, which is one of the most biodiverse geographic regions on earth (Myers et al. 2000). Tree species in the Andes are shifting their mean elevations upslope in response to climate warming, but shifts are significantly slower than the predicted rate based on changes in mean temperature alone (Feeley et al. 2011; Duque et al. 2015). 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 treelines (Harsch et al. 2009; Rehm and Feeley 2015a). Stable treelines suggest that cloud forest species are prevented from expanding or shifting their ranges to include the higher elevation areas that are currently occupied by alpine grasslands (Rehm and Feeley 2015a). Understanding why cloud forest species are shifting their mean elevations upslope while their upper range edges (i.e., the treeline) remain stationary will have important consequences for the conservation of this biodiversity hotspot under future climates.

Despite their importance in determining future cloud forest biodiversity, tropical treelines are relatively poorly studied in comparison to temperate treelines. It is believed that at a global scale, treelines occur at elevations where low mean annual growing-season temperatures 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 body of experimental evidence that is available from the tropics suggests that other factors, such as shading and extreme low-temperature events, may be more important than mean annual temperature in determining tree growth and recruitment above treelines at the regional and local scales (Smith 1977; Smith et al. 2003; Bader et al. 2007).

To date, only two studies have explicitly tested the factors limiting tree seedling establishment above tropical treelines. Smith (1977) found that almost all tree seedlings transplanted into the open grasslands above a Venezuelan treeline died within 1 year. This author attributed the high mortality to a combination of competition with grassland species, moisture stress, and the low-temperature extremes occurring 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 important factor determining seedling establishment above the treeline at their study site in Ecuador. Potentially complicating the interpretation of 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 temperature fluctuations relative to more open, unshaded microhabitats. As such, it is difficult to untangle the importance of shade per se versus its buffering effects against temperature extremes and, in particular, low temperatures.

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; Rehm and Feeley 2015b). 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 trees do not enter into a true state of dormancy during colder periods. Tropical treeline trees maintain actively growing tissue throughout the year that is at relatively high risk of damage during even mild freezing events (Sakai and Larcher 1987). In the Peruvian Andes, Rehm and Feeley (2015b) demonstrated that several tropical treeline-forming species may be prevented from establishing above current treelines due to the extreme low temperatures occurring 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 threat to seedlings of some tree species 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.

It is still unclear how microclimatic stressors and other environmental factors work to promote or inhibit seedling establishment above tropical treelines as treeline studies are biased towards temperate systems (Rehm and Feeley 2015a). As such, our goals in this study were to build a more comprehensive understanding of the factors controlling seedling establishment at tropical treelines under current and future climates by conducting a large-scale seedling transplant study at a treeline in the southern Peruvian Andes. Our specific objectives were 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 the treeline.

Materials and 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 (13°6'18"S, 71°35'21"W). Treeline elevations in the study area range from

approximately 3100 to over 3650 m a.s.l.. The study sites have been protected for at least the last 40 years, and therefore recent anthropogenic disturbances at and around the treeline study sites are minimal. In addition, mean annual temperatures at our treeline sites (6.8–9.7 °C) fall within the natural variation of the global treeline isotherm, suggesting that the selected treeline sites are not significantly depressed by human activities (Körner and Paulsen 2004; Paulsen and Körner 2014).

The treeline ecotone can be characterized by a sharp transition from closed-canopy tropical montane cloud forest to open grasslands (locally known as *puna*). This ecotone transition occurs over horizontal distances of 1–10 m, with treeline ecotones present in the study area from approximately 3100 to 3650 m a.s.l. but not forming one continuous treeline across elevations. The grassland above the treeline is dominated by grasses ranging from 0.5 to 1.0 m in height (compared to grasses only 10–20 cm tall in nearby non-protected areas) that form distinct tussocks surrounded by open ground. Some woody shrubs also occur near the treeline, but these were not considered in the study because they were uncommon in the study area and seedlings did not naturally occur under the shelter of shrub canopies. 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). Soil moisture at 10 cm below the soil surface was almost identical between open grassland and the understory of adjacent treeline cloud forest (unpublished data), suggesting that soil moisture may not be limiting treeline position in our study area. Soil properties can be variable across the treeline ecotone (Zimmermann et al. 2010) but aside from soil temperature, edaphic conditions are not generally believed to be an ultimate determinant of treeline dynamics (Körner 2012) and were therefore not taken into account in our study. 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 (defined here as plants with stems ≤ 50 cm tall and ≤ 1.5 cm diameter at ground level) of three common canopy-forming treeline species (*Weinmannia fagaroides*, *Gynoxys nitida*, *Clethra cuneata*) were collected at three different treeline sites located at approximately 3650 m.a.s.l. All three species exhibit traits common to alpine tree species. They were chosen as study species due to their high relative abundance as seedlings along the treeline ecotone, suggesting that these three species may be future pioneer invaders of the grasslands above the treeline. Further studies are needed to completely characterize the

traits of these species, but all three species are wind dispersed, have small leaves relative to congenics at lower elevations, exhibit leaf traits indicative of high-light and/or low-temperature environments (e.g. tough sclerophyllous leaves, tomentose hairs on leaves and bark), and can grow as short, stunted, shrub-like trees in extremely harsh climatic conditions. Species' elevational ranges are poorly documented throughout the tropics, but each of the study species occurred naturally as seedlings and adults at all transplant and collection sites used in the study, except for *G. nitida*, which did not occur as seedlings at the lowest transplant site but was present in the adult stage. However, *G. nitida* occurs as seedlings at other treeline sites at similar elevations not used in the current study, suggesting that all of the study sites included here fall within the natural range of the three study species.

Seedlings were collected in an approximately 100-m-long strip immediately adjacent to the treeline (at the forest edge or up to 5 m into the grassland). Mycorrhizal associations for high-elevation tropical species are unknown, but seedlings were carefully removed from the ground with sufficient soil left within the root matrix so we assume that any associated mycorrhizae were transplanted with the seedling. Seedlings with extensive or disproportionately long root systems were not used as these seedlings may be vegetative ramets and respond to transplanting differently than individuals germinated from seed. Seedlings were 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.). It should be noted that one of the seedling collection sites also served as one of the high-elevation transplant sites [high-elevation site A; Electronic Supplementary Material (ESM) Fig. S1]. The inclusion of additional transplant sites was not possible due to the insufficient number of naturally occurring seedlings in the study area. Mean temperatures at the low-elevation and mid-elevation sites were 2.0–3.5 °C and 1.0–1.5 °C warmer, respectively, than that at the high-elevation sites (ESM Table S1). These higher mean temperatures of 2.0–3.5 °C at the low-elevation site relative to the high-elevation sites are the same as the increases in temperature predicted 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 with minimal nighttime warming, and 60 % artificial shade with enhanced nighttime warming. Within a treatment, seedlings were planted at least 30 cm apart, and therefore the size of each treatment plot varied according to the number of seedlings being planted. To quantify the microclimate at all treatment plots and transplant sites, we placed a HOBO ProV2 temperature data logger (U23-001; Onset Computer Corp., Bourne, MA) with an accuracy of

± 0.2 °C in the center of each plot at 10 cm above the soil surface. The temperature data loggers were shielded from direct sunlight by a well-ventilated polystyrene cover and programmed to record temperature at 30-min intervals.

Seedlings assigned to the treeline treatment can be viewed as a baseline control treatment to account 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 late afternoons. 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 away from the treeline into the open grassland in the open spaces between grass tussocks. While seedlings in the open grassland treatment received some shading from the taller tussock grasses (average grass canopy height at transplant sites 60–80 cm), especially in the morning and evening, this shading would be the typical amount of shade provided to any individuals growing naturally within the grassland matrix away from treeline. As such, seedlings transplanted into the open grassland were classified as “not shaded” for statistical analyses.

Both artificial shade treatments were adjacent to the open grassland plots and consisted of a permeable shade cloth stretched across a frame at 1 m above the soil surface. Adjacent tussock grasses were often taller than the artificial shade canopy and formed a vegetative barrier to light penetration from the sides of the shade cloth during the morning and evening hours. In the 60 % artificial shade with enhanced nighttime warming treatment, an aluminum-coated landscape cloth was used as this material is designed to reflect long-wave radiation, raising mean and absolute low nighttime temperatures of the underlying ground and vegetation at night. Microclimate conditions under this treatment were roughly similar to those found at the treeline treatment at the same transplant site (for a full summary of mean and low temperatures for various treatments see the “Results” and ESM Table S1). A standard non-metallic shade cloth was used for the 60 % artificial shade treatment with minimal nighttime warming. This shade cloth did result in slightly elevated nighttime temperatures, but temperature profiles remained intermediate between the open grassland and treeline treatments (ESM Table S1). In both of the artificial shade treatments, individuals were planted towards the center of the shade cloths to avoid potential edge effects.

The number of seedlings available for the transplant experiments varied between species from a high of $n = 689$ for *W. fagaroides* to a low of $n = 225$ for *C. cuneata*. In

order to use a minimum of 25 individuals per species under each treatment by transplant site combination, complete replication across all transplant sites was only possible for *W. fagaroides*. For *C. cuneata* and *G. nitida*, seedlings were only transplanted to one high-elevation site (high-elevation site B) where they were subjected to all treatments and to the low-elevation site they were only subjected to the treeline and open grassland treatments (ESM 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 *C. cuneata* and *G. nitida*, seedlings were transplanted only between September and October 2012 at the beginning of the wet–warm season. Therefore, only statistical models for *W. fagaroides* included transplant season as a predictor of seedling survival. Due to the remote location and rugged terrain of the study sites, it was often necessary to transplant individuals of the same species to the same transplant site over multiple days, but all individuals were transplanted within a 21-day period within a season. Microclimatic measurements (i.e., mean and absolute low temperatures) of individuals for each species, treatment, and transplant site combination were taken starting from the day individuals were transplanted. This 21-day planting schedule resulted in variation of recorded microclimate at some treatment and transplant site combinations within a species. Specifically, even though individuals of the same species were transplanted to the same treatment within the same transplant site within the same season, groups of individuals may have been transplanted on different days within the 21-day planting period, resulting in different values for mean and absolute low temperatures (e.g. *G. nitida* seedlings transplanted to the treeline treatment at high-elevation site A; ESM Table S2). Low-temperature measurements could be quite variable during the 21-day planting window so we only included data for the temperatures that an individual actually experienced post-transplantation rather than characterizing each treatment by an average low temperature measurement.

Survival of all individuals was monitored until 24 July 2014. Individuals were classified as alive if they maintained at least one green leaf at the end of the study period. Individuals that dropped all of their leaves shortly after the initial transplant would often sprout new leaves over the next 2–6 months and were therefore also classified as alive based on their condition at the end of the experiment period. We assessed survival rather than changes in height since growth is extremely slow in the cold temperature treeline environment, and any growth would likely have been smaller than any measurement error and therefore inconclusive.

Statistical analysis

Due to the variation in experimental design between species, we utilized two different statistical analyses 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@ 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 the survival of transplanted seedlings, models included fixed effects of mean temperature since transplant, absolute low temperature since transplant, transplant season (as a binomial variable; dry–cold = 0 or wet–warm = 1), shade (as a binomial variable; not shaded = 0 or shaded = 1), and the interactions between shade and absolute low temperature and shade and mean temperature. The identity of the site to which seedlings were transplanted was included in the models as a random effect.

We first fit a full model including all main effects and interaction terms. We then used the likelihood ratio test (LRT) to eliminate non-significant variables following a backward selection procedure. Main effects were only 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 two species (*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 because it was not possible to separate site effects from treatment effects as seedlings were only transplanted into two sites (high-elevation site B and the low-elevation site). In addition, the transplant season was not included as these two species were only transplanted during the wet–warm season. A second analysis was run which included 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 *C. cuneata* and *G. nitida* 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*, with first fitting a full model and then using the LRT to determine the significance of fixed effects and interactions.

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 within a transplant site, with absolute low temperatures under the open grassland and 60 % shade with minimal nighttime warming treatments being on average across all sites 3.5 and 3.6 °C colder, respectively, than at the corresponding treeline site (ESM Table S1). Absolute minimum temperatures under the 60 % shade with enhanced nighttime warming treatment were generally intermediate between those at the open grassland and treeline sites, with temperatures being on average 1.7 °C colder than at the treeline site. Therefore, between the various elevations and treatments we created a range of microclimatic conditions in terms of both mean and extreme low temperatures that could be used to assess the effects of temperature on seedling survival.

As we assumed that background mortality due to any transplant effect would be comparable across controls, treatments, and locations, seedling survival is discussed 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 (551 of 1144 seedlings), with *C. cuneata* having the highest survivorship [140 of 225 (62 %) of individuals surviving] and *W. fagaroides* having the lowest survivorship [276 of 689 (40 %) of individuals surviving].

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; Fig. 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 %) individuals] where mean temperature for each treatment was generally intermediate between those of the high- and low-elevation sites. At the two colder high-elevation sites, i.e., high-elevation sites A and B, 68 of 198 (34 %) and 76 of 130 (58 %) seedlings survived, respectively. In addition to the effects of mean temperature on survival, seedlings transplanted during the dry–cold season had a 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 the survival of *W. fagaroides* seedlings. Survival of *C. cuneata* seedlings also depended significantly on mean temperature, but the relationship was opposite of that for *W. fagaroides*, with higher mean temperatures having a

Table 1 Results of generalized linear mixed-effects models and generalized linear models testing which predictor variables best explain the survival of transplanted seedlings of three Andean treeline species from May 2012 to July 2014

Predictor variables	Estimate	Standard error	z value	p
<i>Weinmannia 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]	Intercept			
High elevation A	0.03			
High elevation B	-0.71			
Mid-elevation	1.11			
Low-elevation	-0.43			
<i>Clethra 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>Gynoxys nitida</i>				
Intercept	-0.25	0.23	-1.12	0.265
Shade	1.40	0.30	4.75	<0.001

GLMMs were used for *W. fagaroides* as there were sufficient seedlings available to create full replication across all treatments and study sites. Generalized linear mixed-effects models (GLMMs) included fixed effects of mean temperature since transplant, absolute low temperature since transplant, transplant season (binomial, dry-cold = 0 or wet-warm = 1), shade (as a binomial variable; not shaded = 0 or shaded = 1), and the interactions between shade and mean temperature and shade and low temperature and transplant site entered as a random effect. Generalized linear models (GLMs) were used for *C. cuneata* and *G. nitida* because seedlings were transplanted to 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 $p = 0.05$

positive effect on survival (Fig. 2a). Furthermore, there was a positive relationship between survival of *C. cuneata* and increasing low temperatures (Fig. 2b). In addition to just temperature, seedling survival was higher when seedlings were provided with shade (Fig. 2c), 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 significantly 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 (Fig. 3).

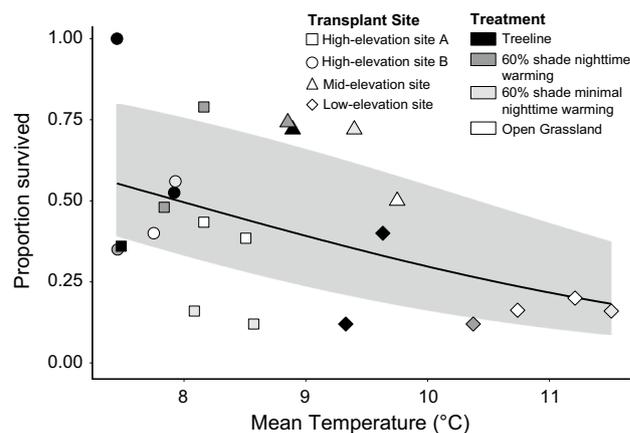


Fig. 1 Mean (solid line) and 95 % confidence intervals (shaded area) for the modeled relationship between *Weinmannia fagaroides* seedling survival and mean temperature (May 2012–July 2014) across all transplant sites and treatments in the southern Peruvian Andes according to generalized linear mixed-effect models (GLMMs). Shading of symbols distinguishes different experimental treatments, and shapes of symbols distinguish different 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

Discussion

The result of our seedling transplant experiment using multiple high-elevation tree species that are commonly found at the treeline demonstrate that upslope shifts in the leading range edges of some dominant canopy-forming treeline species, in response to global climate change, will possibly be dependent on both climatic (e.g., temperature) and other factors (e.g., shade from other vegetation). Since local-scale treeline processes may be dependent on species-specific responses to changes in mean temperature as well as additional factors, the current predictions of a general upslope shift in tropical treelines may be incorrect as these predictions are based largely, or solely, on projected changes in mean temperature.

Our observation that survival of *W. fagaroides* decreased at the highest mean temperature was contrary to a priori expectations 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 of this dominant canopy-forming treeline species under conditions of increased mean temperature. Yet our findings show the opposite effect of mean temperature. The reduced survival at higher mean temperatures may be a reflection

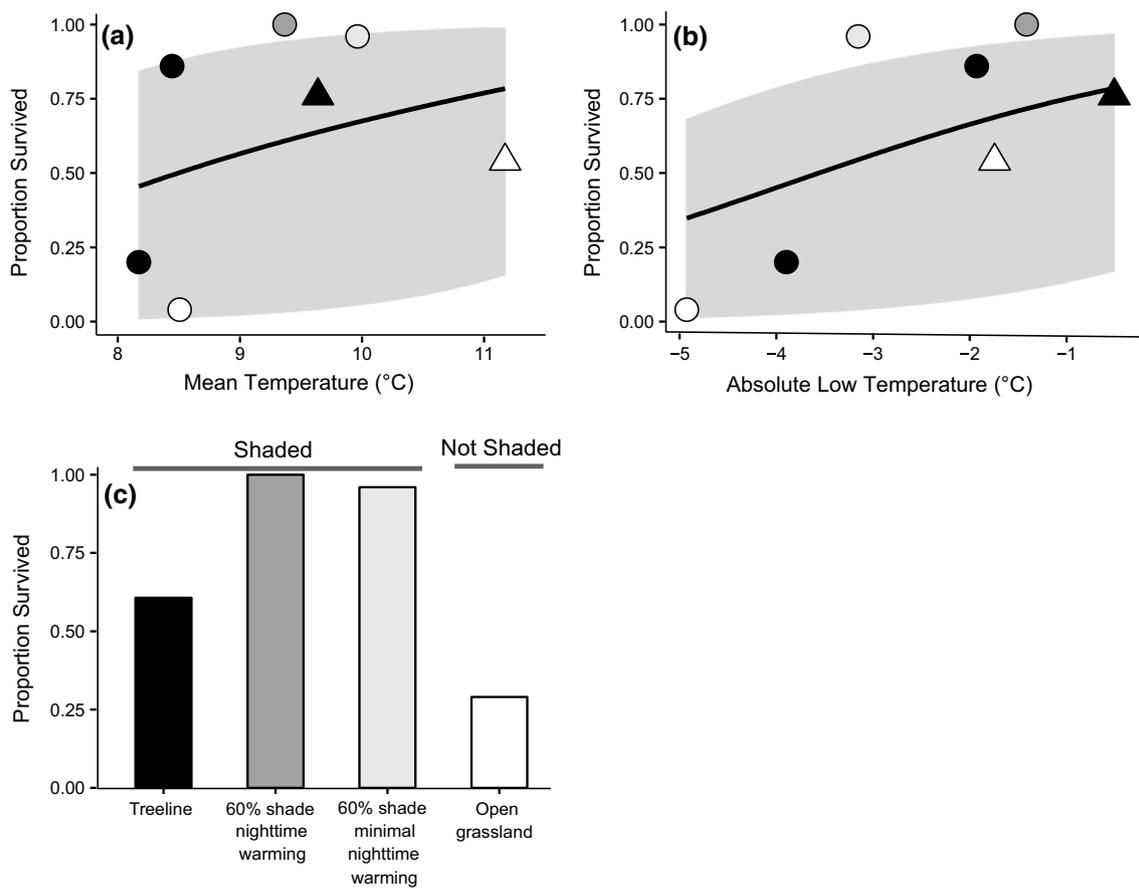


Fig. 2 Transplanted *Clethra cuneata* seedling survival in the southern Peruvian Andes (September 2012–July 2014) with respect to mean temperature (a), absolute low temperature (b), and shade (c). Solid lines in a and b represent the modeled mean and the shaded area is the 95 % confidence intervals as determined by generalized linear models. Different symbols represent different transplant sites: circles high-elevation site B, triangles low-elevation site. Shading of symbols represents different treatments: black treeline, dark gray 60 % shade with enhanced nighttime warming, light gray 60 % shade with minimal nighttime warming, 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. In c only the open grasslands and treeline treatments were replicated at both high- and low-elevation sites; bars for these two treatments therefore represent the average proportion which survived at the two sites, whereas in the shading treatments the bars only represent the proportion which survived at high-elevation site B

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).

The current known range of *W. fagaroides* in the region extends from approximately 3150 to 3650 m.a.s.l. (Rapp et al. 2012, unpublished data). As such, when we transplanted seedlings of this species downslope to 3150 m.a.s.l., we may have transplanted individuals which were acclimated to the upper cold-range margin to the very warm-edge of the species' current realized climatic niche. Without further experimentation it is difficult to determine if our findings represent truly poor survival under warmer mean temperatures or whether they are simply a result of artificially elevated mortality from transplanting cold-acclimated phenotypes to warmer, downslope sites.

The conservative prediction of a 2.0–3.5 °C increase in mean temperatures in the Andes over the next several decades indicates that temperatures will significantly rise over the lifetime of individual trees. It is possible that a more gradual warming rate may allow individuals to adjust their metabolic rates, resulting in higher survival under future warmer temperatures than the rates reported here. However, current warming rates are faster than historical rates and will likely accelerate in the future, allowing limited time for individuals to adjust. In addition, Rapp et al. (2012) found that growth of individuals across the range of several *Weinmannia* species occurring below our study area did not increase with increasing temperature, suggesting that higher temperatures do not necessarily result in higher growth and survival of individuals. Throughout the tropical

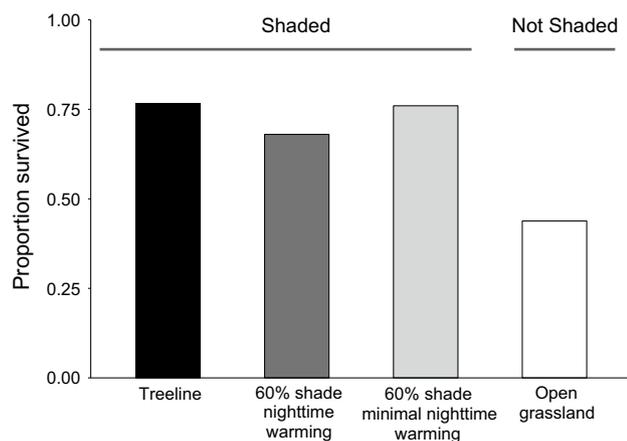


Fig. 3 Proportion of transplanted *Gynoxys nitida* seedling survival in the southern Peruvian Andes (September 2012–July 2014) with respect to *shade*. Seedlings planted in the treeline, and two shade treatments were classified as being shaded and seedlings transplanted into the open grassland were classified as not shaded. Only the open grasslands and treeline treatments were replicated at both high- and low-elevation sites; bars for these two treatments therefore represent the average proportion which survived at the two sites, whereas in the shading treatments the bars only represent the proportion which survived at high-elevation site B

Andes, species are often restricted to a narrow elevational bands, suggesting that individuals have a limited ability to acclimate to climatic conditions outside of a species' current range. Therefore, we assume that transplanting individuals downslope represents realistic increases in mean temperature that current treeline individuals will experience over the course of their lifetime. We suggest that even a mild increase in mean temperature may be outside of the acclimation range and climatic tolerances of seedlings of this important species. In such a case, increasing mean temperatures under future climates may severely reduce the survival and recruitment of the current dominant treeline-forming species, further slowing upslope shifts in the treeline.

Converse to *W. fagaroides*, *C. cuneata* did show an increase in seedling survival with increasing mean temperature, supporting the contention that low mean temperatures negatively affect recruitment of this species at or beyond the treeline. However, the positive relationship of *C. cuneata* seedling survival with absolute low temperatures 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 proposed to explain the abrupt form of some treelines in the tropics (Harsch and Bader 2011), and *C. cuneata* has previously been shown to be sensitive to the extreme frost events that can occur in the open grasslands above the treeline (Rehm and Feeley 2015b). Taken together, these results suggest that

growth processes may be inhibited by low mean temperatures at the treeline (Körner 2012) and that establishment may be limited by rare frost events, further preventing this species' expansion into the open grasslands (Rehm and Feeley 2015b). It therefore appears that *C. cuneata* will need to experience increases in both mean and low temperatures during future climate change before it can expand its range beyond the established treeline boundary. Mean temperatures 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 the cloud immersion zone, which is also difficult to predict (Quintana-Gomez 1999; Kodra et al. 2011; Halladay et al. 2012). Complicating matters even further is the fact that in our experiment *C. cuneata* seedlings showed higher survival when provided with shade, indicating that factors other than temperature can also play a significant role in explaining the dynamics of this important treeline species.

As with *C. cuneata*, we observed that shade also significantly increased the survival of transplanted seedlings of *G. nitida*. This finding supports previous work showing that forest seedlings transplanted above a tropical treeline had higher survival when provided with shade (Bader et al. 2007). Unlike the forest canopy which is buffered against extreme cold temperatures, chronic photoinhibition may occur close to the ground during mornings when sub-zero temperatures (due to cold air pooling) combine 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 high light intensities combined with extreme low temperatures beyond what seedlings at treeline can tolerate. The trend of decreasing cloud cover throughout much of Andes (Halladay et al. 2012) 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.

An upslope shift in treeline can occur when individuals of just a single species establish at elevations higher than the current treeline. It is possible that additional treeline-forming species not included in this study may experience 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 scenario, we may expect the treeline to advance in a slow, diffuse manner, with forest invasion into the grasslands following a successional pattern where many species would establish above the treeline only after significant microclimatic buffering and possibly shade is provided by pioneer species. If

such pioneer species do exist, they should dominate current recruitment patterns at and beyond the treeline since mean temperatures in our study sites have already increased by approximately 0.15 °C per decade since the 1950s (Vuille et al. 2003). Yet the species included in this study were specifically chosen because they represent the most dominant and abundant treeline species in our study area at the both the juvenile and adult stages. Only one species, *C. cuneata*, showed a positive effect on seedling recruitment due to higher mean temperatures, but even then, low temperature extremes and shade also significantly altered seedling survival rates. If the species included in this study are indeed the most likely to act as pioneer species invading the grasslands above the treeline, then the species-specific responses to the local environment exhibited in this study makes predictions of treeline shifts based on mean temperature alone tenuous at best.

We are limited in our ability to determine how individuals across a species range will respond to future conditions due to the study design which involved only transplanting individuals from higher to lower elevation sites. The lack of seedlings at low-elevation sites suggests that conditions towards the lower range margins of the studied treeline species may already restrict seedling growth and survival. Our understanding of seedling dynamics would potentially be improved if a more typical reciprocal transplant study could be conducted that would allow us to determine if response of individual survival to various environmental conditions is uniform across a species range or varies with local phenotypes.

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. For example, there is evidence from temperate treelines that higher mean temperatures can lead to increased water stress in treeline individuals (Moyes et al. 2013, 2015), but the relationship between increased mean temperatures and moisture availability has yet to be tested at tropical treelines. In addition, the roles of biotic interactions, edaphic conditions, and increased CO₂ concentrations on local and regional tropical treeline dynamics have remained virtually unaddressed in tropical montane systems (Rehm and Feeley 2015a). Clearly it would be highly informative for future studies to test the complementary or contrasting ways in which mean temperature and other factors can affect recruitment of the study species as well as other tropical treeline-forming species.

It has been argued that many tropical treelines have been artificially depressed due to human activities (Young 2009). Our study sites have experienced anthropogenic influences in the past but have been protected and relatively free from these influences for at least the past 40 years due to their

location in Manu National Park. However, even choosing sites within the national park boundary does not guarantee that our study area was completely free from human disturbances. We specifically selected transplant sites based on their inaccessibility and the lack of evidence of recent human disturbance, such as fires or cattle grazing. Therefore, the findings from this study may provide some explanation as to why tropical treelines have not shifted upslope even after human activities have been greatly reduced and despite concurrent increases in mean temperatures. Furthermore, our study sites could be viewed as a “best case scenario” because in other parts of the Andes and tropics, species may have to contend with both changes in climate and ongoing anthropogenic activities.

These results have important implications for global biodiversity as the cloud forests of the Andes are one of the most diverse ecosystems on earth. Many cloud forest tree species below the treeline have already begun to respond to increasing mean temperatures by shifting the centers of their distributions upslope (Feeley et al. 2011; Duque et al. 2015). 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 range contractions (Lenoir and Svenning 2015). However, our results support the notion that various climatic and environmental factors, some of which may not necessarily change as a direct result of future climate change (e.g. shading), will impede some of the current treeline-forming species from expanding their ranges beyond the current forest boundary and into higher elevations, likely resulting in a stationary treeline. This expectation is supported by the fact that no studies have recorded significant upslope shifts in tropical treelines over the past several decades despite significant warming (Harsch et al. 2009; Lutz et al. 2013; Rehm and Feeley 2015a). Therefore our findings support the contention that mean temperature is a poor predictor of tropical treeline shifts during periods of climatic instability, at least at local and regional scales. We clearly must improve our understanding of the varied factors that determine species' range limits and the dynamics of tropical treelines in order to create more realistic projections of how these diverse systems will respond to future climate change.

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Author contribution statement ER and KF conceived and designed the project. ER analyzed the data. ER and KF wrote the manuscript.

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