

**IDENTIFICATION OF FUNCTIONAL GROUPS IN PÁRAMO PLANTS AND EVALUATION  
OF THEIR SUSCEPTIBILITY TO GLOBAL CLIMATE CHANGE**

By

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## **Abstract**

Above treeline in the mountains from the Northern Andes, there is an ecosystem of restricted distribution locally called as páramo. Páramos are tropical ecosystems of high altitude where there are special conditions that make them and their flora unique, such as greatly dynamic cloud conditions, excessive amounts of UV radiation, rapid changes of incident sunlight and pronounced temperature fluctuations during each day, among others. They are critical as a source of water in the region, are important carbon sinks, are home to a vast endemic flora, and possess some of the fastest rates of diversification in the world. Although the ecosystemic services of páramos depend to their high spatial abiotic and biotic heterogeneity that generates complex mosaics of plants associations; unfortunately, little work has been done on the susceptibility of páramo plants to climate change. Human-induced climate change is affecting all the Earth's biomes, including páramos, where models predict that temperatures will increase, while the volume and frequency of precipitation will decrease to the end of this century. All research indicates that warmer and drier environments will drive vegetation upward, as plants did in the past, during interglacial periods, rather than adapting to the new conditions. Plants can respond to climate change by adaptation, migration, or local extinction; adaptation implies that plants can acclimate its physiology to the new conditions, migration requires reproductive traits that favored dispersal and colonization of new areas, and it is well established, that species with limited ranges of distribution, such as those restricted to the páramo, have a higher risk of extinction. However, adaptation and migration capacities are not enough studied facing global warming in plants of the humid Colombian páramo. For that reason, this thesis aims: 1) To functional classify an important group of páramo plant species to select some of them 2) to explore the thermal acclimation of photosynthesis and plant respiration (capacity of adaptation) and 3) to assess the effect of warming on germination traits (migration capacity). This information could be used to understand species behavior in future global change scenarios. In the introduction section páramo ecosystems are defined along with their climatic and biological singularity, as well as the main current threats to the ecosystem, especially the effect of climate change. In the first chapter, new findings in plant functional classification are presented that allow us to conclude that although the harsh conditions of páramos promote a huge variety of morphological adaptations, it is also the cause of a smaller number of functional responses, and since functional responses are closely linked to growth forms, in páramos it could be useful to use growth forms as a proxy of plant functional types (PFT) in all cases and with caution in the case of shrubs. In the second chapter, thermal acclimation of photosynthesis and respiration was evaluated and, in addition to the limited functional response, it was found that páramo plants have a limited capacity for thermal acclimation, but although páramo plants cannot adjust their physiology to warming, high temperatures do not have an adverse effect on plant performance. Finally, in the third chapter, the effect of warmer temperatures on germination traits was evaluated and it was found that seeds of few páramos plants will be affected by higher temperatures, so it appears that there is a wide range of temperature functioning for germination processes. All results together seem indicate that although páramo plants are more capable of migrating than adapting, they would survive because other mechanisms as decoupling leaf temperature from ambient temperature may operate ensuring their survival.

## **Resumen**

En las montañas del norte de los Andes, por encima del límite arbóreo, existe un ecosistema de distribución restringida denominado localmente como páramo. Los páramos son ecosistemas tropicales de gran altitud en los que existen condiciones especiales que los hacen únicos a ellos y a su flora, como son las condiciones de nubosidad muy dinámicas, la excesiva cantidad de radiación UV, los rápidos

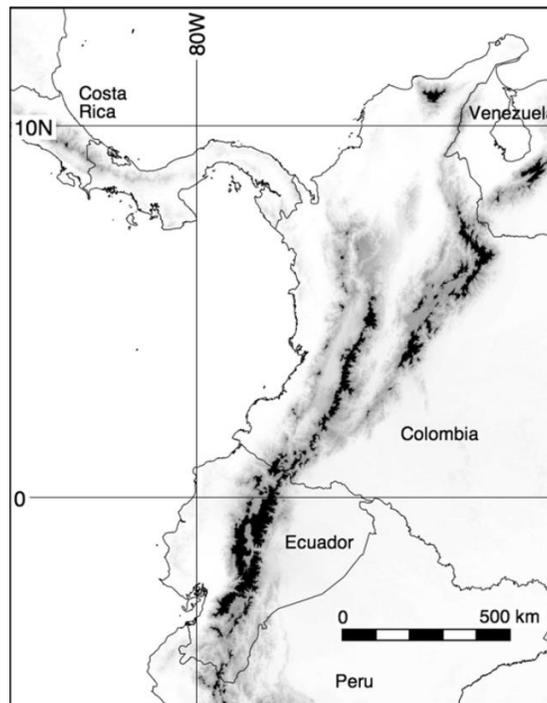
cambios de la luz solar incidente y las pronunciadas fluctuaciones de temperatura durante cada día, entre otras. Los páramos juegan un papel importante como fuente de agua en la región, son significativos sumideros de carbono en los suelos, albergan una vasta flora endémica y poseen una de las tasas de diversificación más rápidas del mundo. Aunque los servicios ecosistémicos de los páramos dependen de su elevada heterogeneidad espacial abiótica y biótica, que genera complejos mosaicos de asociaciones de plantas, lamentablemente se ha trabajado poco sobre la susceptibilidad de las plantas de los páramos al cambio climático. El cambio climático inducido por el hombre está afectando a todos los biomas de la Tierra, incluidos los páramos, donde los modelos predicen que las temperaturas aumentarán, mientras que el volumen y la frecuencia de las precipitaciones disminuirán hasta finales de este siglo. Todas las investigaciones indican que los ambientes más cálidos y secos impulsarán la vegetación hacia límites superiores, como lo hicieron las plantas en el pasado, durante los períodos interglaciares, antes que adaptarse a las nuevas condiciones ambientales. Las plantas tienen tres opciones para responder al cambio climático: la adaptación, la migración o la extinción local; la adaptación implica que las plantas puedan aclimatar su fisiología a las nuevas condiciones ambientales, la migración requiere rasgos reproductivos que favorezcan la dispersión y colonización de nuevas áreas, y está bien establecido, que las especies con rangos de distribución limitados, como las restringidas al páramo, tienen un mayor riesgo de extinción. Sin embargo, las capacidades de adaptación y migración no están suficientemente estudiadas frente al calentamiento global en las plantas del páramo húmedo colombiano. Por ello, esta tesis pretende 1) clasificar funcionalmente un grupo importante de especies vegetales del páramo para seleccionar algunas de ellas para el trabajo en los siguientes capítulos, 2) explorar la aclimatación térmica de la fotosíntesis y la respiración de las plantas (capacidad de adaptación) y 3) evaluar el efecto del calentamiento sobre los rasgos de germinación (capacidad de migración). Esta información podría utilizarse para entender el comportamiento de las especies en futuros escenarios de cambio global. En la sección de introducción se definen los ecosistemas de páramo y su singularidad climática y biológica, así como las principales amenazas actuales para el ecosistema, especialmente el efecto del cambio climático. En el primer capítulo se presentan nuevos hallazgos en la clasificación funcional de las plantas que permiten concluir que aunque las duras condiciones de los páramos promueven una enorme variedad de adaptaciones morfológicas, también es la causa de un número menor de respuestas funcionales, y dado que las respuestas funcionales están estrechamente ligadas a las formas de crecimiento, en los páramos podría ser útil utilizar las formas de crecimiento como proxy de los tipos funcionales de las plantas (TFP) en todos los casos y con precaución en el caso de los arbustos. En el segundo capítulo, se evaluó la aclimatación térmica de la fotosíntesis y la respiración y, además de la respuesta funcional limitada, se encontró que las plantas de páramo tienen una capacidad limitada de aclimatación térmica, pero, aunque las plantas de páramo no puedan ajustar su fisiología al calentamiento, las altas temperaturas no tendrán un efecto adverso en el funcionamiento de las plantas. Finalmente, en el tercer capítulo, se evaluó el efecto de las temperaturas más cálidas sobre los rasgos de germinación y se encontró que las semillas de pocas plantas de páramo se verán afectadas por temperaturas más altas, por lo que parece que existe un amplio rango de funcionamiento de la temperatura para los procesos de germinación. Todos los resultados en conjunto parecen indicar que, aunque las plantas de páramo son más capaces de migrar que de adaptarse, sobrevivirían porque otros mecanismos como el desacoplamiento de la temperatura foliar de la temperatura ambiental pueden operar asegurando su supervivencia.

## INTRODUCTION

### Páramo ecosystems

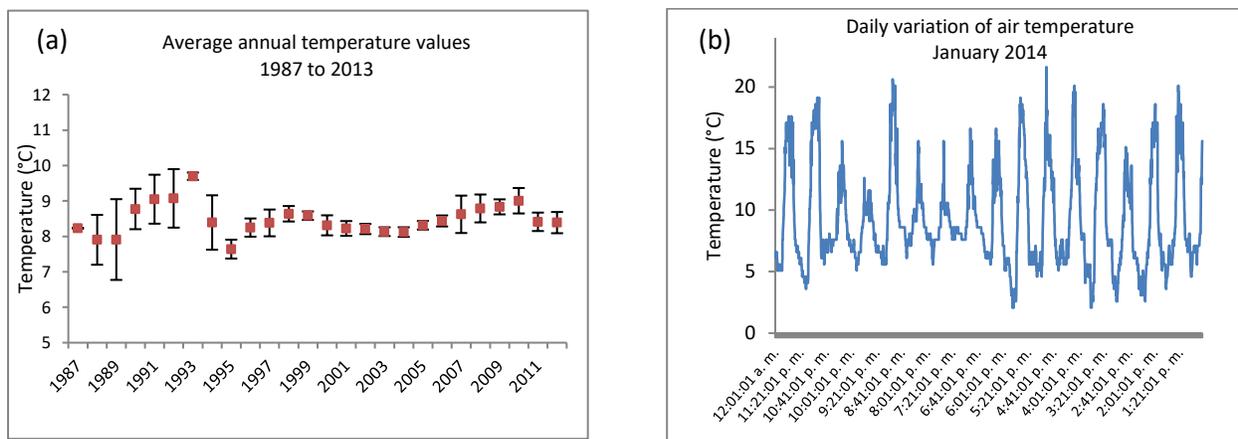
The páramo is a threatened geo-ecosystemic complex (Cresso et al., 2020; Cuesta et al., 2019; Peyre et al., 2020; Sklenář et al., 2021; Tovar et al., 2013; Van der Hammen et al., 2002) located in few mountain tops of Central America and the northern part of South America. Páramos in the Andes are delimited altitudinally between 3000 and 5000 m.a.s.l., formed on volcanic soils derived from the latest uplift of the Andes with successive climatic oscillations of the Pleistocene (Hofstede, 2002; Sklenář & Balslev, 2005). At these elevations, partial pressure of gases such as O<sub>2</sub> and CO<sub>2</sub> are low, evapotranspiration rates (Díazgranados, 2015) and nighttime temperatures are low, while ultraviolet radiation is high (Ramsay, 2001). Páramo plants are also exposed to rapid insolation changes (Sánchez et al., 2014), freezing events (Azócar, 2006; Ramsay, 2001; Sklenář et al., 2010), and sometimes snow (Luteyn, 1999; Sklenář & Balslev, 2005). These young ecosystems of restricted distribution have very varied and restrictive climatic characteristics that have given rise to a high proportion of rare, endemic, and poorly known plant species (Cortés et al., 2018; Llambí & Rada, 2019; Rada et al., 2019), especially vulnerable to rapid environmental changes (Flantua et al., 2019; Madriñán et al., 2013).

Páramo's current distribution and extension (**Figure 1**) make up a significant part of its unique conditions. First, it is located in the tropics, where solar radiation is constant and intense during the day with little seasonal variation throughout the year, which means that growing season in the páramo have 365 days a year (Buytaert et al., 2007, Sánchez et al., 2014). Second, its location above 3000 m.a.s.l. makes it an ecosystem characterized by low temperatures due to the negative thermal gradient that operates in most of the world's mountains, whereby 0.6 to 0.7°C are lost for every 100 m of altitude (Körner, 2003; Urrutia & Vuille, 2009; van der Hammen & Hooghiemstra, 2000).



**Figure 1.** Distribution of the páramo ecosystem (Buytaert, Célleri, et al., 2006). Páramos areas are shown in black.

In general, temperature variations in the páramo are determined by two drivers, the altitudinal gradient, and the air humidity, which depends on cloud formation (Buytaert, Célleri, et al., 2006; Helmer et al., 2019). Páramos are considered cold ecosystems because the mean annual temperature ranges around 8°C (Llambí & Rada, 2019; Mora-Osejo, 2001; **Figure 2a**); however, daily temperature deltas can exceed 20-30K during the dry season (Rada et al., 2019, Sandoval et al., 2019, **Figure 2b**), to such an extent that this ecosystem is considered "summer in the day and winter in the night" (Hedberg, 1964). Although these conditions could be considered remarkably stressful, organisms living there are highly adapted to them (Baruch, 1979; Billings, 1973; Llambí & Rada, 2019; Nürk et al., 2018; Peyre et al., 2020; Rada, 2016), and this suggests that páramo species have physiological mechanisms to respond to these substantial diurnal variations every day of the year (Llambí & Rada, 2019). Furthermore, it has also suggested that this intense daily fluctuation of temperature may be related to this ecosystem's high rates of evolution, the fastest on the planet (Cortés et al., 2018; Madriñán et al., 2013).

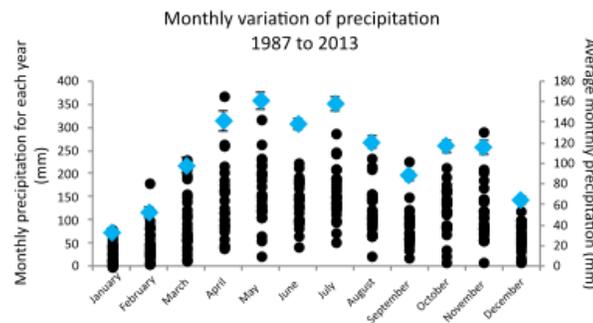


**Figure 2.** Temperature variation at Páramo el Verjón. (a) Mean annual variation for the period from 1987 to 2013. **Source:** IGAC, La Bolsa meteorological station (Latitude: 4° 33' N, Longitude: 74° 00' W; and Altitude: 3195 m.a.s.l.) (b) Daily variation during the month of January 2014 taken by the Ecology and Plant Physiology Group of the Universidad de los Andes, ECOFIV, with Hygrochron Ibutton DS1923 during January 2014.

Páramos are strategic ecosystems that capture and regulate water, generating a complex hydrological network that provides water for agriculture, farming, power generation and human consumption in the large cities of northern South America while serving as buffer for dry seasons (Buytaert, Célleri, et al., 2006; Rodríguez-Morales et al., 2019; Urrutia & Vuille, 2009). Water supply is one of the most important environmental services provided by the páramo, and it is linked to climate regulation (Pabón-Caicedo, 2020; Sandoval et al., 2019), with four key components influencing the hydroecological processes of these ecosystems: precipitation, cloud cover, soils and vegetation (Buytaert, Deckers, et al., 2006; Cresso et al., 2020; Pabón-Caicedo et al., 2020; Rodríguez-Morales et al., 2019).

Precipitation in páramos is frequent but scarce in volume, ranging between 700 and 3000 mm per year (Luteyn, 1992), distributed in two distinct seasons that depend on the influence of each place's winds and air masses. In the páramos of Ecuador and Colombia, influenced by the Intertropical Convergence Zone (ITCZ), it rains most of the year. However, from December to February, precipitation decrease, and this period is considered the dry season (Baruch & Smith, 1979; Buytaert, Célleri, et al., 2006; **Figure 3**). In

contrast, the Venezuelan páramos are considered dryer because of the effect of the Caribbean trade winds in the Andes' northeastern and because the influence of the ITCZ is more seasonal (Llambí & Rada, 2019). The marked precipitation regime in páramos depends both on the geographic and altitudinal location, as well as the slope orientation of the páramo (Buytaert, Célleri, et al., 2006), and it is the rainfall regime what differentiates the dry season from the wet season. In the wet season there is abundant cloudiness accompanied by permanent, low-volume drizzle, low temperatures, and low daily thermal variability, while in the dry season, cloudiness is considerably reduced and clear skies make nights become cooler and days warmer, increasing the risk of frost exposure at dawn and high evaporative demand in the early hours of the day (Rada, 2016), thus numerous plant and foliar adaptations to cope with the harsh environmental conditions can be found (Azócar & Rada, 2006; Rada et al., 2019).



**Figure 3.** Monthly precipitation data from 1987 to 2013 (black circles) and average monthly values for the same period (blue diamonds). Precipitation data from one weather station located near the study site in páramo Matarredonda (IGAC, La Bolsa Meteorological Station. Latitude: 4° 34' N; Longitude: 74° 00' W and Altitude: 3195 m.a.s.l.).

Soils in páramos are dark and humic with high organic matter accumulation and high infiltration and retention capacity, with carbon contents values over 30% and water storage capacity of over 0.4 cm<sup>3</sup> (Buytaert et al., 2007; Llambí & Rada, 2019). The low-temperature regime and the high levels of water saturation in the interstitial spaces of the soil are responsible for the low rates of organic matter decomposition in these environments (Buytaert et al., 2005; Buytaert, Deckers, et al., 2006). Because of these factors, páramos are large reservoirs of soil carbon, containing six times more carbon per area than tropical forests, thus becoming a critical ecosystem when assessing the future carbon balance under climate change conditions (Hofstede, 1999; Curiel-Yuste et al., 2017). However, these conditions also make the soils very acidic, to the degree of preventing adequate root absorption of water and nutrients by plants, making them unproductive soils in which only plant species with low nutritional requirements and species with slow growth rates can naturally grow (Hofstede, 1995).

The socioeconomic importance and the ecosystem services that páramos provide are notably relevant to the region for carbon sequestration and storage, water supply, and climate regulation (Buytaert et al., 2007, Carey et al. 2017, Sandoval et al., 2019). In addition, these places provide ecosystemic and landscape services appropriate for tourism and recreation and, in some cases, relevant to the cosmovision of ancestral cultures (Castaño-Urbe, 2002; Carey et al. 2017, Rodríguez-Morales et al., 2019). Its diverse environmental services are intimately linked to its rich plant biodiversity (Cortés et al., 2018; Sandoval et al., 2019), which are now threatened by climate change, human changes in land use and mining (Cresso et al., 2020; Tovar et al., 2013).

## **Páramo flora**

Vegetation in the páramo has abundantly diversified after occasional migration processes during glaciation periods and subsequent evolutionary processes in the Glacial-Interglacial cycles (Llambí & Rada, 2019, Sklenář et al., 2011), which has resulted in a strong speciation over the last five million years (Madriñán et al., 2013). The tropical alpine flora plays a vital role in the ecosystem's structure and has a significant influence on the ecosystem services it provides (Hofstede et al., 2014, Tovar et al., 2013). High elevation tropical alpine ecosystems are particularly rich in species (more than 5000 different species recorded), highly endemic (more than 60% of vascular plant species are endemic to this ecosystem), and plants have unique morphological and physiological adaptations to face the challenging environment in which they grow (Buytaert, Deckers, et al., 2006; Luteyn, 1999; Madriñán et al., 2013; Peyre et al., 2020; Smith & Young, 1987). Páramo vegetation is grouped in 127 families and 509 genera, where the most representative families are Asteracea, Poacea and Orchidaceae (Peyre, 2015; Sklenář et al., 2011). Low-stature vegetation characterizes this landscape, in which the predominant forms of growth are giant (caulescent) and ground (acaulescent) rosettes, dwarf or sclerophyllous shrubs, cushion plants, tussock grasses and forbs (Hofstede, 1995; Luteyn et al., 1992; Peyre, 2015; Rada et al., 2019).

In páramo plants, most studies on architectural, morphological, and physiological adaptations in the neotropical region come mainly from research conducted in the dryer Venezuelan páramos, while the same type of studies are scarce for the humid Colombian páramos, but physiological responses may be comparable since some of the adaptations found in páramo vegetation have been observed in other tropical high mountain ecosystems in Papua New Guinea, Africa and Hawaii, indicating some evolutionary convergence in response to similar selective forces (Monasterio & Sarmiento, 1991; Rada et al., 2019; Smith, 1994). Here some of these adaptations are highlighted:

- **Adaptations to low temperatures:**

- (a) Retention of dead leaves that remain attached to the stem for long periods of time to maintain water reservoirs in a liquid state for the time of greatest transpiration, which is generally in the early morning hours (Baruch & Smith, 1979; Goldstein et al., 1984a, 1984b; Goldstein & Meinzer, 1983; Rada et al., 1998; Rada, 2016; Smith, 1994).

- (b) Supercooling mechanisms in adult leaves to avoid the harmful effect of freezing temperatures at night (Rada et al., 1985; Rada et al., 1987; Smith, 1994).

- (c) Nyctinastic movements and mucilaginous fluids at the base of leaves to protect the apical meristems from freezing temperatures at night (Smith, 1974).

- (d) Large stems to avoid lower temperatures at ground level (Smith, 1980).

- (e) Densely pubescent leaves to increase leaf temperature, to increase boundary layer resistance and decrease transpiration, to reduce predation by herbivores, and to increase the vapor pressure gradient between the leaf and the atmosphere (Baruch & Smith, 1979; Goldstein & Meinzer, 1983; Monasterio & Sarmiento, 1991; Smith, 1979; Rada et al., 1998; Smith, 1994).

- (f) Predominant growth forms such as rosettes, dwarf shrubs, cushions, and clumps, to reduce exposition to the strong wind and low temperatures (Buytaert et al., 2007; Körner, 2003).

- **Adaptations to water limitation:**

- (a) Water reservoirs in the pith of the stems, specifically studied in giant caulescent rosettes, where a positive correlation between plant height and water storage capacity has been observed (Goldstein et al., 1984; Goldstein & Meinzer, 1983).

- (b) To prevent desiccation due to excessive transpiration, plants have either dense, thick, coriaceous leaves, or soft but very tiny leaves (Baruch & Smith, 1979; Luteyn, 1999; Meinzer & Goldstein, 1985)
  - (c) Growth form as rosettes to better intercept and redirect water from rainfall directly to the roots (Baruch & Smith, 1979; Monasterio & Sarmiento, 1991).
  - (d) Highly pubescent leaves, small cells, and protected stomata to increase resistance and to reduce transpiration rates (Monasterio & Sarmiento, 1991; Smith, 1994).
  - (e) Superficial and fine root systems to take advantage of the water intercepted in the rosette growth form (Monasterio & Sarmiento, 1991).
- **Adaptations to nutrient limitation:**
    - (a) Increased investment in photosynthetic tissue to optimize the distribution of resources (Azócar et al., 2000; Rada et al., 1992; Smith & Young, 1987).
    - (b) Abundant and diverse communities of decomposers in the above-ground necromass that facilitate nutrient cycling (Azócar et al., 2000; Goldstein & Meinzer, 1983; Smith, 1979).
    - (c) Leaching of nutrients from decaying leaves in rosette growth forms (Smith & Young, 1987).
  - **Adaptations to high fluctuations of incident radiation:**
    - (a) Leaves arranged parallel to the branches in order to reduce the impact of high solar radiation (Baruch & Smith, 1979; Castaño-Urbe, 2002).
    - (b) Evergreen leaves to maintain assimilation rates permanently throughout the year (Monasterio & Sarmiento, 1991).
    - (c) Rosettes to ensure different insertion angles and to take advantage of incident light at any time of the day (Medina, 1974).
    - (d) Pubescent leaves to regulate leaf temperature and keep them close to their photosynthetic optimum (Meinzer & Goldstein, 1985; Rada et al., 1992; Rada et al., 1998).
  - **Reproductive adaptations:**

Most páramo species present adaptation to be dispersed by wind like seeds with air chambers, low weight, or specialized structures. In shrubs it is common to find dispersal mechanisms by zoochory and by hydrochory, as seeds with floating capacity to take advantage of the humid conditions of the environment (Melcher et al., 2004). In addition, given the high competition generated by the nutrient-poor soils in which they must germinate and the limitations in seedling recruitment, it is common to find species with asexual reproduction strategies, synchronization of flowering, production of numerous seeds, and also latent seeds, in order to establish persistent seed banks even for periods longer than one year for some species (Monasterio & Sarmiento, 1991; Vargas et al., 2014).

## **Climate change, its impact on the tropics and specifically on the páramo**

The evidence of global climate change at different scales in recent years is undeniable and is highly correlated with human activity, generating significant consequences in natural systems. The latest report of the International Panel on Climate Change (IPCC) shows that temperature is increasing in all parts of the globe and that extreme weather events as heatwaves, droughts, and floods are already pushing the biodiversity to their limits. Unfortunately, we are breaking records in most of the planet's vital signs, we have recently experienced almost a decade of the warmest years on record and the global mean temperature was around 1.2°C higher than it was before the industrial revolution (1850-1900). Ocean levels, temperature and acidification continue to rise and for hundreds to millions of years are irreversible. Carbon dioxide, the most significant greenhouse gas, reached 413.2 parts per million (ppm) in 2020,

which is 149% more than pre-industrial levels while methane levels are now 262% higher than they were in 1750 (IPPC, 2021).

The most severe and rapid impacts of climate change, both ecological and social, are predicted to occur in tropical areas (Mora et al., 2013). For the tropics, model projections estimate a temperature increase between 2°C and 3.4°C and an average decrease in precipitation of 4% (Buytaert, Célleri, et al., 2006). For Colombia, based on current records showing average air temperature increases of 0.1 to 0.2°C per decade over the last 30 years, it is expected a rise in temperature of  $1.5 \pm 3.5^\circ\text{C}$  and an increase in precipitation up to 300 mm (Anderson et al., 2011; Buytaert et al., 2011; Cuesta et al., 2019; Urrutia and Vuille, 2009). For the Colombian páramos, one of the most threatened tropical high mountain ecosystems by climate change (Buytaert et al., 2011; Cresso et al., 2020), already an increase of 0.1°C per decade in the average temperature has been recorded over the past fifty years (Sklenář et al., 2021). For this ecosystem the temperature is expected to increase by 1.5 to 4°C over the next 100 years (Anderson et al., 2012; Buytaert et al., 2011; Cresso et al., 2020; Pabón-Caicedo et al., 2020; Sklenář et al., 2021). In terms of precipitation, several studies agree that the trends are more geographically and temporally variable in the region, and the intensity and frequency of precipitation will increase, but that rainfall and drizzle will decrease significantly in the Colombian páramos (Buytaert et al., 2011; Cuesta et al., 2019; IPCC, 2014; Pabón-Caicedo et al., 2020; Sklenář et al., 2021; Tovar et al., 2013; Urrutia & Vuille, 2009). As a result, cloudiness is expected to be drastically reduced, increasing, in turn, the intensity of solar radiation during the day and decreasing the temperature during the night (Helmer et al., 2019; Sklenář & Balslev, 2005). At the same time, páramo species will be exposed to increased competition with other species climbing the mountain following their thermal niche (Dirnböck et al., 2003; Graae et al., 2018). Additionally, the synergy between warming and land-use change, resulting from agriculture activities also shifting their boundaries altitudinally, can alter hydrological cycles (Pabón-Caicedo et al., 2020; Rodríguez-Morales et al., 2019; Urrutia & Vuille, 2009) and the rise of crop pests and diseases (IPCC, 2021).

### **Páramo threats**

Land-use change, invasion of exotic species, and mining are important factors that threaten the sustainability of the páramo. However, the increase in global temperature is one of the main risks for vegetation in the páramo (Cresso et al., 2020; Cuesta et al., 2019; León et al., 2015; Peyre et al., 2020; Sklenář et al., 2021; Tovar et al., 2013; Van der Hammen et al., 2002). The páramo flora has resisted a long sequence of climatic events that produced the expansion and contraction of its boundaries over millions of years, but today faces a tremendous challenge given the velocity and intensity of these alterations (Anderson et al., 2012; Peyre et al., 2020; Sklenář et al., 2021). Due to these new conditions, there is increasing evidence that páramo species will migrate to higher altitudes (Peyre et al., 2020; Rada et al., 2019; Tovar, et al., 2013), as they did in previous geological times, rather than adapt to the new circumstances (Madriñán et al., 2013; Moret et al. 2019; Morueta-Holme et al. 2015; Sklenář et al., 2021). Moreover, migration of other plant species, parasites and pathogens from lower altitudes will also play an important role in shaping the future of this unique ecosystem (Anderson et al., 2012). The elevational shift of the páramo's borders and their consequent area lost are expected for 2010-2069, under two future emission scenarios A1B (depicts a future world with extremely rapid economic growth, a peak in global population in the mid-century and subsequent decrease, and the quick adoption of new, more efficient, and balanced technologies) and A2 (describes a world that is extremely diverse; IPCC, 2007) (Tovar et al., 2013).

In summary, there is ample evidence that the ecosystem of the tropical alpine Andes presents a unique combination of environmental circumstances that determines different types of stresses to which plants are adapted. This fact has two direct consequences: first, it makes páramos strategic ecosystems for their high biodiversity, but also highly vulnerable to climatic change; and second, it raises a serious question about the capacity of páramo plants to respond to climate change. In general, adaptation, migration, or local extinction are the three ways in which plants can respond to climate change (Holt, 1990; Peterson et al., 2001; Thuiller et al. 2008). Adaptation requires that the plant has the capacity to acclimate to the new environmental conditions, while migration requires that the plant has good colonization capacity, based on its reproductive characteristics (Graae et al., 2018). Since páramos are hotspots of biodiversity, assessing the response of the rich páramo flora to climate change is an enormous challenge that can be address with functional classification. In this context, my thesis aims to functionally classify the páramo flora and understand the response of vegetation to global change, since at present it is not clearly known what its capacity and speed of adjustment to warming will be, thus predictions of the effects on the diversity, dynamics, or resilience of humid Colombian páramo ecosystems are limited (Cuesta et al., 2012; Hofstede et al., 2014).

Considering the concern generated by this uncertain panorama, in this thesis I covered some of the topics that have scarcely explored for the Colombian páramo. In the **first chapter** I classified 42 of the most common species of the páramo on functional groups, and ecological strategies using 21 physiological, morphological, and reproductive traits. In the **second chapter**, I worked with some of these species from different functional groups to evaluate their ability to acclimate to higher temperatures in plants growing under controlled conditions, on growth chambers, at current páramo temperatures and at temperatures 4.8°C warmer. Finally, in the **third chapter**, I evaluated the temperature effect on seed germination in a group of common páramo plants to evaluate their germination requirements and understand how they could be affected in a future warmer world.

## **Chapter 1: Functional groups**

Even though the use of functional traits to answer ecological questions has been applied for less than three decades (Adler et al., 2014), thanks to their extensive versatility, they have had an important impact on different areas of plant and animal biology. Functional traits have been defined as morphological, anatomical, biochemical, physiological or phenological characteristics that can be measured at the individual level (Violle et al., 2007). Its significance is that they can be used to scale questions to different levels of organization, from the individual to the community (Lanta et al., 2011). The measurement of functional traits has been used in the definition of functional groups, which are understood as groups of species that, although unrelated, reflect a similar physiological response and ecological role, thus allowing the understanding of ecosystem stability, since it is considered that an ecosystem with greater stability has multiple functional groups with different responses to environmental factors (Ives & Carpenter, 2007).

Historically, different methods have been used to classify species within a plant community. Phylogenetic or structural approaches are widely used; however, classification into functional groups has recently received increasing attention. The phylogenetic approach groups species according to a common evolutionary history shared by the most closely related species. The structural classification is a more intuitive approach and is based on similar plant architectures; whereas the functional classification considers the role each species plays in the ecosystem to form plant functional types (PFT) (Laporte et al., 2012). This classification determines the basic units in which vegetation dynamics can be observed

in the context of environmental changes, particularly useful in the scenario of global warming to predict the individual and population response of species to environmental constraints (Díaz et al., 2013; Dormann, 2002). The success of this type of methodology depends not only on knowing the ecophysiological traits of the species that conform the groups, but also on considering the types of stresses that act as filters determining the adaptive responses of the plants in each environment (Azócar et al., 2000).

There are few previously published studies on tropical alpine plants classification in PFT where growth forms have been used as proxy of PFT. For example, in the Venezuelan páramos Azócar et al., (2000) considered the five predominant growth forms of the ecosystem (acaulescent rosettes, giant caulescent rosettes, cushions, macollas and sclerophyllous shrubs) as five functional groups. In that study, the response to water and thermal stress was identical in all groups, which indicates that the vulnerability of this ecosystem is very high because unfavorable climatic changes will affect most of the components in the same way (Azócar et al., 2000). Other studies in Venezuelan páramos have classified different growth forms according to their ability to avoid or tolerate a variety of stresses (Azócar et al., 1988; Rada, 2016; Rada et al., 2019; Squeo et al., 1991). In Colombia, Cárdenas-Arévalo and Vargas-Ríos (2008) obtained three PFT after evaluating 11 functional traits in 70 páramo plant species, mostly grasses and forbs (Cárdenas-Arévalo & Vargas-Ríos, 2008). These previous studies suggest that páramos are particularly vulnerable given their limited number of strategies; however, there is a lack of widely accepted PFTs in páramo plants (Zimmer et al., 2018), and specially for humid páramos, as in the Eastern cordillera range in Colombia, thus it was decided to evaluate larger number of growth forms and traits compared to the work of Cárdenas-Arévalo and Vargas-Ríos (2008) and to define PFTs based on Hierarchical Clustering on Principal Components (HCPC), instead of growth forms as a proxy of PFT, as in the case of the Venezuelan research.

Understanding páramo plant responses to climatic change in the vast number of species of a humid Colombian páramo is an arduous task, that can be reduced using the functional classification, thus I started my thesis evaluating the functional originality, the ecological strategies to each species belong and the number of PFTs in a Colombian páramo of the eastern range of Los Andes using a series of physiological, morphological and reproductive traits in plants from different growth forms to answer the following research questions:

- (a) How many ecological strategies (CSR) and functional types (PFT) can be identified in the páramos and how they correspond to growth forms,
- (b) Which valuable traits can be useful for species classification in the páramos, and
- (c) How species originality varies among growth forms and PFTs.

## **Chapter 2: Effect of warming on photosynthetic and respiratory rates in páramo plants**

Temperature is one of the most influential environmental factors in the distribution of plants on the planet, as well as in the control of their growth, since the rates of photosynthesis and respiration are strongly affected by temperature, in the short term (minutes to hours) and in the long term (months to years) (Hughes, 2000). Recent studies have reported mechanisms of acclimation for both metabolic processes; photosynthesis acclimates to a new temperature when CO<sub>2</sub> assimilation rates change favorably in response to the new temperature leaf, which is known as thermal acclimation (Dusenge et al., 2021; Slot & Winter, 2017; Ziegler et al., 2020). Thermal acclimation of photosynthesis can be caused by temperature-dependent changes in photosynthetic biochemistry, including the maximum rate of Rubisco carboxylation ( $V_{\text{cmax}}$ ) and the maximum rate of electron transport ( $J_{\text{max}}$ ) (Farquhar et al., 1980; Kattge &

Knorr, 2007; Yamori et al., 2005), but in addition can also be regulated by changes in chlorophyll content, fatty acid composition, or by changes in the allocation of nitrogen inside the leaf to produce photosynthetic proteins, such as the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco (Ristic et al., 2007; Scafaro et al., 2017; Yamori et al., 2014). Respiration also acclimates to new temperatures and responds faster and stronger than photosynthesis, it has been reported that this acclimation process can occur within one to two days after plant exposure to a new growth temperature (Atkin, Holly, et al., 2000; Bolstad et al., 1999; Campbell et al., 2007).

It is widely accepted that tropical species are operating close to their physiological temperature optimum and have narrow ranges of thermal variability, since these species are not exposed to seasonal fluctuations as in temperate or boreal zones, consequently it is expected that even small increases in temperature could adversely affect tropical species, since they are growing under an annual thermal stability (Doughty & Goulden, 2008; Janzen, 1967; Way & Oren, 2010); however, páramo plants face a very different situation, despite being in the tropics, since diurnal temperature fluctuations far exceed annual mean temperature variations in páramos (Llambí & Rada, 2019; Mora-Osejo, 2001; Rada et al., 2019, Sandoval et al., 2019). On the other hand, it has been accepted that plant growth of high-altitude species is limited by temperature, given the low temperatures in which they grow, thus it is expected that an increase caused by global warming could increase the productivity of these species, just as boreal species (Körner, 2003; Way & Oren, 2010). In páramos, ambient temperature tends to vary significantly over short time periods and distances, and is determined more by attributes such as topography, orographic precipitation, or substrate, than by macroclimatic conditions (Körner, 2003; Sklenář et al., 2016), whereas temperature experienced by plants is strongly influenced by growth form, plant height and foliar morphology (Sklenář et al., 2016), thus decoupling leaf temperature from environmental temperature (Rada et al., 1992). In most plant species, environmental temperature has a strong influence on leaf temperature; however, this is not the case in the páramos, which indicates that the acclimation capacity of the metabolic processes of the species in this ecosystem may be even lower, making this research more relevant.

The photosynthetic capacity of páramo plants is directly affected by temperature and its effect on plant processes will depend on the morphological, physiological and phenological characteristics of the species (Dullhoste, 2010; Körner, 2003; Rada et al., 1992). The key thermal filter driving plant species adaptations is generally related to the need to maintain a favorable heat balance against the risk of freezing damage at night and rapid temperature variation during the day, with these events being the best studied (Baruch & Smith, 1979; Goldstein et al., 1984; Rada et al., 1985; Rada et al., 1987; Rada et al., 1992; Sklenář et al., 2016). Previous research in the Venezuelan Andean páramo provided evidence that physiological responses in most cases are species-specific and related with the microclimate in which they were grown (Monasterio & Sarmiento, 1991). These investigations showed that temperature, caused by variations in altitude, is the most influential factor on assimilation rates, finding species whose optimum temperature increases with altitude, others in which it decreases and others in which there is no altitudinal effect (Rada et al., 1987, 1998; Rada et al., 1992).

Unfortunately, the mechanisms of acclimation of photosynthesis and respiration in páramo species are currently poorly understood, and the effect of warming on the carbon balance in these ecosystems requires more attention, as this will contribute to more accurate predictions about the role of this ecosystem in mitigating or intensifying global climate change. Furthermore, extrapolation of responses from other species and/or biomes requires caution, as photosynthesis responses are strongly related to the thermal regimes to which the vegetation is subjected in its original habitat (Berry & Bjorkman, 1980). Therefore, this thesis points to explore photosynthesis and respiration acclimation to temperature in páramo species

belonging to different functional groups, as they have been insufficiently studied and to increase our understanding of the role that this ecosystem could play in carbon fluxes.

We want to answer two research questions:

- (a) Is there any photosynthetic and respiratory acclimation to temperature in páramo plant species?
- (b) Is this acclimation particular to each species or is there a trend across PFTs?

### **Chapter 3: Effect of temperature on seed germination**

A seed is a miracle. It is the result of a complex series of molecular, biochemical and structural plant changes, which contains all the information and reserves necessary to generate a new individual. This dispersal unit fulfills important functions in time and space, since it is the method for colonization of new environments, as well as the mechanism of renewal and persistence of the species generation after generation (Baskin & Baskin, 1998; Debouza et al., 2021). For the seed to achieve its goal, it is necessary that the embryo is transformed into a seedling through the process of germination. Germination consists mainly in the activation of metabolic processes by increases in humidity and respiratory activity of the seed. At this stage, the seed is vulnerable to environmental conditions, especially temperature, since many enzymatic reactions that occur during imbibition are temperature dependent.

Regarding the ecology of páramo seeds, many aspects important for their success have been studied. For example, on dispersal forms there is enough information collected both for individual species (Cardenas et al., 2002; Posada, 2013; Velasco-Linares & Vargas, 2008), as well as for 154 species (Frantzen & Bouman, 1989) and 89 plant genera of the ecosystem (Melcher et al., 2004). There are also studies on seed banks in the páramo (Cardenas et al., 2002; Guariguata & Azocar, 1988; Mora et al., 2007), and on the recruitment of juveniles (Estrada & Monasterio, 1988; Fagua & González, 2007; Mora et al., 2007). But much less is known about the effect of temperature on the germination of páramo seeds (Vargas et al., 2014).

Today, because atmospheric conditions are changing more rapidly than in the past, there is a marked need to understand better and earlier the impact of high temperatures on the response of plants, not only in their vegetative stage, but also in the germination processes. Although global models predict moderate effects of global warming change in tropical environments, despite this the high mountain tropical ecosystem, locally called páramo, are considered highly threatened by rising temperatures.

In the aseasonal tropical alpine ecosystems there are special conditions that make them and their flora unique (Cortés et al., 2018; Llambí & Rada, 2019; Rada et al., 2019). Despite the fact that these environmental circumstances are extremely harsh, species living there are well adapted to them (Baruch, 1979; Billings, 1973; Llambí & Rada, 2019; Nürk et al., 2018; Peyre et al., 2020; Rada, 2016), and numerous plant and foliar adaptations to cope with the extreme environmental conditions are found, but most of them are described in the vegetative stage, and the response to warming in seed of plants of high-altitude tropical ecosystems is scarce. Therefore, in this chapter we want to answer:

- (a) Is there an effect of warming temperatures on the time and speed of germination?
- (b) Whether the current distribution range may function as an indicator of species vulnerability at the germination stage?
- (c) Is vulnerability to warming at the germination stage a species or PFT pattern?

## References

- Anderson, E. P., Marengo, J. A., Villalba, R., Halloy, S. R. P., Young, B. E., Cordero, D., Gast, F., Jaimes, E., & Ruiz, D. (2012). Consecuencias del cambio climático en los ecosistemas y servicios ecosistémicos de los Andes Tropicales. In S. K. Herzog, R. Martínez, P. M. Jørgensen, & H. Tiessen (Eds.), *Climate change and Biodiversity in the Tropical Andes* (p. 410). Inter-American Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE).
- Atkin, O. K., Holly, C., & Ball, M. C. (2000). Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature: The importance of changes in the capacity and temperature sensitivity of respiration. *Plant, Cell and Environment*, *23*(1), 15–26. <https://doi.org/10.1046/j.1365-3040.2000.00511.x>
- Atkin, O. K., & Tjoelker, M. G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. In *Trends in Plant Science* (Vol. 8, Issue 7, pp. 343–351). Elsevier Current Trends. [https://doi.org/10.1016/S1360-1385\(03\)00136-5](https://doi.org/10.1016/S1360-1385(03)00136-5)
- Azócar, A., Rada, F., & García-Núñez, C. (2000). Aspectos ecofisiológicos para la conservación de ecosistemas tropicales contrastantes. *Boletín de La Sociedad Botánica de México*, *65*, 6.
- Baruch, Z. (1979). Elevation Differentiation in *Espeletia Schultzii* (Compositae), A Giant Rosette Plant of the Venezuelan Paramos. *Ecology*, *60*(1), 85–98. <https://doi.org/10.2307/1936471>
- Baruch, Z., & Smith, A. P. (1979). Morphological and physiological correlates of niche breadth in two species of *Espeletia* (Compositae) in the Venezuelan Andes. *Oecologia*, *38*(1), 71–82. <https://doi.org/10.1007/BF00347825>
- Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and, evolution of dormancy and germination*. Elsevier.
- Berry, J., & Bjorkman, O. (1980). Photosynthetic Response and Adaptation to Temperature in Higher Plants. *Annual Review of Plant Physiology*, *31*(1), 491–543. <https://doi.org/10.1146/annurev.pp.31.060180.002423>
- Billings, W. D. (1973). Arctic and Alpine Vegetations: Similarities, Differences, and Susceptibility to Disturbance. *BioScience*, *23*(12), 697–704. <https://doi.org/10.2307/1296827>
- Bolstad, Mitchell, & Vose. (1999). Foliar temperature-respiration response functions for broad-leaved tree species in the southern Appalachians. *Tree Physiology*, *19*(13), 871–878.
- Buytaert, W., Célleri, R., De Bièvre, B., Cisneros, F., Wyseure, G., Deckers, J., & Hofstede, R. (2006). Human impact on the hydrology of the Andean páramos. *Earth-Science Reviews*, *79*(1–2), 53–72. <http://dx.doi.org/10.1016/j.earscirev.2006.06.002>
- Buytaert, W., Deckers, J., & Wyseure, G. (2006). Description and classification of nonallophanic Andosols in south Ecuadorian alpine grasslands (páramo). *Geomorphology*, *73*(3–4), 207–221. <http://dx.doi.org/10.1016/j.geomorph.2005.06.012>
- Buytaert, W., Iñiguez, V., & Bièvre, B. De. (2007). The effects of afforestation and cultivation on water yield in the Andean páramo. *Forest Ecology and Management*, *251*(1–2), 22–30. <http://dx.doi.org/10.1016/j.foreco.2007.06.035>
- Buytaert, W., Sevink, J., De Leeuw, B., & Deckers, J. (2005). Clay mineralogy of the soils in the south Ecuadorian páramo region. *Geoderma*, *127*(1–2), 114–129. <https://doi.org/10.1016/j.geoderma.2004.11.021>
- Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O., & Hurry, V. (2007). Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytologist*, *176*(2), 375–389. <https://doi.org/10.1111/j.1469-8137.2007.02183.x>

- Cardenas, C., Posada, C., & Vargas, O. (2002). Banco de semillas germinable de una comunidad vegetal de paramo humedo sometida a quema y pastoreo (Parque Nacional Natural Chingaza, Colombia)cardenas2002.pdf. *Ecotropicos*, 15(1), 51–60.
- Castaño-Urbe, C. (2002). Colombia alto andina y la significancia ambiental del bioma páramo en el contexto de los andes tropicales: Una aproximación a los efectos futuros por el cambio climático global (Global Climatic Tensor). In C. Castaño-Urbe (Ed.), *Aproximación al efecto del Global Climatic Tensor en el Bioma Páramo* (p. 387). IDEAM.
- Cortés, A. J., Garzón, L. N., Valencia, J. B., & Madriñán, S. (2018). On the Causes of Rapid Diversification in the Páramos: Isolation by Ecology and Genomic Divergence in Espeletia. *Frontiers in Plant Science*, 9, 1700. <https://doi.org/10.3389/fpls.2018.01700>
- Cuesta Francisco, Muriel P., Beck S., Meneses R.I., Halloy S., Salgado S, Ortiz E, B. M. T. (2012). *Biodiversidad y Cambio Climático en los Andes Tropicales—Conformación de una red de investigación para monitorear sus impactos y delinear acciones de adaptación* (B. M. T. Cuesta Francisco, Muriel P., Beck S., Meneses R.I., Halloy S., Salgado S, Ortiz E, Ed.). Red Gloria-Andes.
- Curiel-Yuste, J., Hereş, A. M., Ojeda, G., Paz, A., Pizano, C., García-Angulo, D., & Lasso, E. (2017). Soil heterotrophic CO<sub>2</sub> emissions from tropical high-elevation ecosystems (Páramos) and their sensitivity to temperature and moisture fluctuations. *Soil Biology and Biochemistry*. <https://doi.org/10.1016/j.soilbio.2017.02.016>
- Debouza, N. E., Babu Thruppoyil, S., Gopi, K., Zain, S., & Ksiksi, T. (2021). Plant and seed germination responses to global change, with a focus on CO<sub>2</sub>: A review. *One Ecosystem*, 6, e74260. <https://doi.org/10.3897/oneeco.6.e74260>
- Díaz, S., Purvis, A., Cornelissen, J. H. C., Mace, G. M., Donoghue, M. J., Ewers, R. M., Jordano, P., & Pearse, W. D. (2013). Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecology and Evolution*, 3(9), 2958–2975. <https://doi.org/10.1002/ece3.601>
- Diazgranados, M. (2015). *Una mirada biológica a los páramos circundantes a la Sabana de Bogotá*.
- Dirnböck, T., Dullinger, S., & Grabherr, G. (2003). A regional impact assessment of climate and land-use change on alpine vegetation. *Journal of Biogeography*, 30(3), 401–417. <https://doi.org/10.1046/j.1365-2699.2003.00839.x>
- Dormann S. J. Woodin, C. F. (2002). Climate change in the Arctic: Using plant functional types in a meta-analysis of field experiments. *Functional Ecology*, 16(1), 4–17.
- Doughty, C. E., & Goulden, M. L. (2008). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences*, 113(G1), n/a-n/a. <https://doi.org/10.1029/2007JG000632>
- Dullhoste, R. (2010). *Respuestas eco-fisiológicas de plantas del límite arbóreo (Selva nublada—Páramo) al estrés térmico, hídrico y lumínico en los Andes Venezolanos*.
- Estrada, C., & Monasterio, M. (1988). Ecología poblacional de una roseta gigante, *Espeletia spicata* Sch. Bip. (Compositae), del páramo desértico. *Ecotropicos*, 1, 25–39.
- Fagua, J. C., & González, V. H. (2007). Growth rates, reproductive phenology, and pollination ecology of *Espeletia grandiflora* (Asteraceae), a giant andean caulescent rosette. *Plant Biology*, 9(1), 127–135.
- Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta*, 149(1), 78–90. <https://doi.org/10.1007/BF00386231>
- Frantzen, N. M. L. H. F., & Bouman, F. (1989). *Dispersal and growth form patterns of some zonal páramo vegetation types*. 38(4), 449–465.

- Funk, J. L., Larson, J. E., Ames, G. M., Butterfield, B. J., Cavender-Bares, J., Firn, J., Laughlin, D. C., Sutton-Grier, A. E., Williams, L., & Wright, J. (2017). Revisiting the Holy Grail: Using plant functional traits to understand ecological processes. *Biological Reviews*, 92(2), 1156–1173. <https://doi.org/10.1111/brv.12275>
- Gauthier, P. P. G., Crous, K. Y., Ayub, G., Duan, H., Weerasinghe, L. K., Ellsworth, D. S., Tjoelker, M. G., Evans, J. R., Tissue, D. T., & Atkin, O. K. (2014). Drought increases heat tolerance of leaf respiration in *Eucalyptus globulus* saplings grown under both ambient and elevated atmospheric [CO<sub>2</sub>] and temperature. *Journal of Experimental Botany*, 65(22), 6471–6485. <https://doi.org/10.1093/jxb/eru367>
- Goldstein, G., & Meinzer, F. (1983). Influence of insulating dead leaves and low temperatures on water balance in an Andean giant rosette plant. *Plant, Cell and Environment*, 6(8), 649–656. <https://doi.org/10.1111/1365-3040.ep11589230>
- Goldstein, G., Meinzer, F., & Monasterio, M. (1984a). The role of capacitance in the water balance of Andean giant rosette species. *Plant, Cell and Environment*, 7(3), 179–186. <https://doi.org/10.1111/1365-3040.ep11614612>
- Goldstein, G., Meinzer, F., & Monasterio, M. (1984b). The role of capacitance in the water balance of Andean giant rosette species. *Plant, Cell and Environment*, 7(3), 179–186. <https://doi.org/10.1111/1365-3040.ep11614612>
- Greenpeace. (2009). El cambio climático: Futuro negro para los páramos. *Distrito Federal, México. Consejo Nacional Para La ...*, 18.
- Guariguata, M. R., & Azocar, A. (1988). Seed Bank Dynamics and Germination Ecology in *Espeletia timotensis* (Compositae), an Andean Giant Rosette. *Biotropica*, 20(1), 54–59. JSTOR. <https://doi.org/10.2307/2388426>
- Gunderson, C. A., O'hara, K. H., Campion, C. M., Walker, A. V., & Edwards, N. T. (2010). Thermal plasticity of photosynthesis: The role of acclimation in forest responses to a warming climate. *Global Change Biology*, 16(8), 2272–2286. <https://doi.org/10.1111/j.1365-2486.2009.02090.x>
- Hedberg, O. (1964). Features of Afroalpine Plant Ecology. *Acta Phytogeographica Suecica* 49, 150.
- Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., & Onoda, Y. (2006). Temperature acclimation of photosynthesis: Mechanisms involved in the changes in temperature dependence of photosynthetic rate. *Journal of Experimental Botany*, 57(2), 291–302.
- Hofstede, R. (2002). El manejo del páramo como ecosistema estratégico. In *Los Páramos Andinos: Los desafíos del Siglo XXI* (Vol. 74, Issue 17 2, pp. 5–41).
- Hofstede, R., Calles, J., López, V., Polanco, R., Torres, F., Ulloa, J., Vásquez, A., & Cerra, M. (2014). *Los páramos andinos ¿Qué sabemos? Estado de conocimiento sobre el impacto del cambio climático en el ecosistema páramo*. UICN.
- Hofstede, R. G. M. (1995). The effects of grazing and burning on soil and plant nutrient concentrations in Colombian páramo grasslands. *Plant and Soil*, 173(1), 111–132. <https://doi.org/10.1007/BF00155524>
- Hughes, L. (2000). Biological consequences of global warming: Is the signal already apparent? In *Trends in Ecology and Evolution*. [https://doi.org/10.1016/S0169-5347\(99\)01764-4](https://doi.org/10.1016/S0169-5347(99)01764-4)
- IPCC. (2021). *Climate Change 2021: Impacts, Adaptation, and Vulnerability. A Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*.
- Ives, A. R., & Carpenter, S. R. (2007). Stability and Diversity of Ecosystems. *Science*, 317(5834), 58–62. <https://doi.org/10.1126/science.1133258>
- Janzen, D. H. (1967). Why Mountain Passes are Higher in the Tropics. In *The American Naturalist* (Vol. 101, Issue 919, pp. 233–249). <https://doi.org/10.1086/282487>

- Kappelle, M., Lovejoy, T. E., & Gámez Lobo, R. (2016). *Costa Rican Ecosystems*. University of Chicago Press. <https://doi.org/10.7208/chicago/9780226121642.001.0001>
- Kattge, J., & Knorr, W. (2007). Temperature acclimation in a biochemical model of photosynthesis: A reanalysis of data from 36 species. *Plant, Cell and Environment*, 30(9), 1176–1190. <https://doi.org/10.1111/j.1365-3040.2007.01690.x>
- Körner, C. (2003). Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. In *Alpine Plant Life, 2nd Edn*. <https://doi.org/10.1007/978-3-642-18970-8>
- Kroner, Y., & Way, D. A. (2016). Carbon fluxes acclimate more strongly to elevated growth temperatures than to elevated CO<sub>2</sub> concentrations in a northern conifer. *Global Change Biology*, 22(8), 2913–2928. <https://doi.org/10.1111/gcb.13215>
- Llambí, L., & Rada, F. (2019). Ecological research in the tropical alpine ecosystems of the Venezuelan páramo: Past, present and future. *Plant Ecology & Diversity*, 1–20. <https://doi.org/10.1080/17550874.2019.1680762>
- Laporte, M. A., Mougenot, I., & Garnier, E. (2012). ThesauForm-Traits: A web based collaborative tool to develop a thesaurus for plant functional diversity research. *Ecological Informatics*, 11, 34–44. <https://doi.org/10.1016/j.ecoinf.2012.04.004>
- Laughlin, D. C. (2014). The intrinsic dimensionality of plant traits and its relevance to community assembly. *Journal of Ecology*, 102(1), 186–193. <https://doi.org/10.1111/1365-2745.12187>
- León, O., Jiménez, D., & Marín, C. (2015). Marco conceptual para la identificación de la zona de transición entre el bosque altoandino y páramo. In C. Sarmiento & O. León (Eds.), *Transición bosque– páramo. Bases conceptuales y métodos para su identificación en los Andes colombianos*. (Primera, p. 156). Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Luteyn, J. (1999). Páramos: A checklist of plant diversity, geographical distribution, and botanical literature. Costa Rica and Panama. *Memoirs of the New York Botanical Garden*, 84, 138–141.
- Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. In *Frontiers in Genetics* (Vol. 4).
- Marín, C., Medina-Rangel, G., Jiménez, D., Sarmiento, M., León, O., Díaz-Triana, J., & Paiba, J. (2015). Protocolos metodológicos para la caracterización de las comunidades bióticas a lo largo del gradiente altitudinal bosque-páramo. In C. E. Sarmiento Pinzón & O. A. León Moya (Eds.), *Transición bosque– páramo. Bases conceptuales y métodos para su identificación en los Andes colombianos* (Primera Ed, p. 156). Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Medina, E. (1974). Dark CO<sub>2</sub> fixation, habitat preference and evolution within the bromeliaceae. *Evolution*, 28(4), 677–686. <https://doi.org/10.1111/j.1558-5646.1974.tb00799.x>
- Meinzer, F., & Goldstein, G. (1985). Some Consequences of Leaf Pubescence in the Andean Giant Rosette Plant *Espeletia Timotensis*. *Ecology*, 66(2), 512–520. <https://doi.org/10.2307/1940399>
- Melcher, I. M., Bouman, F., & Cleef, A. M. (2004). Seed atlas of the monocotyledonous genera of the páramo. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 199(4), 286–308. <https://doi.org/10.1078/0367-2530-00157>
- Monasterio, M., & Sarmiento, L. (1991). Adaptive radiation of *Espeletia* in the cold andean tropics. *Trends in Ecology & Evolution*, 6(12), 387–391. [https://doi.org/10.1016/0169-5347\(91\)90159-U](https://doi.org/10.1016/0169-5347(91)90159-U)
- Mora, F., Chaparro, H. A., & Vargas, O. (2007). Dinámica de la germinación, latencia de semillas y reclutamiento de plántulas en *Puya cryptantha* y *P. trianae*, dos rosetas gigantes de los páramos colombianos. *Ecotropicos*, 20(1), 31–40.
- Mora, C., Frazier, A. G., Longman, R. J., Dacks, R. S., Walton, M. M., Tong, E. J., Sanchez, J. J., Kaiser, L. R., Stender, Y. O., Anderson, J. M., Ambrosino, C. M., Fernandez-Silva, I., Giuseffi,

- L. M., & Giambelluca, T. W. (2013). The projected timing of climate departure from recent variability. *Nature*, 502(7470), 183–187. <https://doi.org/10.1038/nature12540>
- Mora-Osejo, L. E. (2001). *Contribuciones al estudio comparativo de la conductancia y de la transpiración foliar de especies de plantas del páramo*.
- Nürk, N. M., Michling, F., & Linder, H. P. (2018). Are the radiations of temperate lineages in tropical alpine ecosystems pre-adapted? *Global Ecology and Biogeography*, 27(3), 334–345. <https://doi.org/10.1111/geb.12699>
- O’Sullivan, O. S., Weerasinghe, K. W. L. K., Evans, J. R., Egerton, J. J. G., Tjoelker, M. G., & Atkin, O. K. (2013). High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal high-temperature limits to respiratory function. *Plant, Cell and Environment*, 36(7), 1268–1284. <https://doi.org/10.1111/pce.12057>
- Peyre, G., Lenoir, J., Karger, D. N., Gomez, M., Gonzalez, A., Broennimann, O., & Guisan, A. (2020). The fate of páramo plant assemblages in the sky islands of the northern Andes. *Journal of Vegetation Science*, 31(6), 967–980. <https://doi.org/10.1111/jvs.12898>
- Posada, J. (2013). *El rol de la dispersión de semillas en la regeneración de la vegetación de páramo en un paisaje fragmentado (Tesis de Maestría)*. [Tesis de maestría]. Universidad de los Andes.
- Rada, F. (2016). Functional Diversity in Tropical High Elevation Giant Rosettes. In G. Goldstein & L. S. Santiago (Eds.), *Tropical Tree Physiology: Adaptations and Responses in a Changing Environment* (pp. 181–202). Springer International Publishing. [https://doi.org/10.1007/978-3-319-27422-5\\_8](https://doi.org/10.1007/978-3-319-27422-5_8)
- Rada, F., Azócar, A., Gonzalez, J., & Briceño, B. (1998). Leaf gas exchange in *Espeletia schultzii* Wedd, a giant caulescent rosette species, along an altitudinal gradient in the Venezuelan Andes. *Acta Oecologica*, 19(1), 73–79. [https://doi.org/10.1016/S1146-609X\(98\)80010-6](https://doi.org/10.1016/S1146-609X(98)80010-6)
- Rada, F., Azócar, A., & García-Núñez, C. (2019). Plant functional diversity in tropical Andean páramos. *Plant Ecology & Diversity*, 1–15. <https://doi.org/10.1080/17550874.2019.1674396>
- Rada, F., Goldstein, G., Azócar, A., & Meinzer, F. (1985). Freezing avoidance in Andean giant rosette plants. *Plant, Cell & Environment*, 8(7), 501–507. <https://doi.org/10.1111/j.1365-3040.1985.tb01685.x>
- Rada, F., Goldstein, G., Azócar, A., & Torres, F. (1987). Supercooling along an Altitudinal Gradient in *Espeletia schultzii*, a Caulescent Giant Rosette Species. In *Journal of Experimental Botany* (Vol. 38). <https://doi.org/10.1093/jxb/38.3.491>
- Rada, F., Gonzalez, J., Azócar, A., Briceño, B., & Jaimez, R. (1992). Net photosynthesis-leaf temperature relations in plant species with height along an altitudinal gradient. *Acta Oecologica*, 13(5), 535–542.
- Rada, F., Gonzalez, J., Azócar, A., Briceño, B., & Jaimez, R. (1992). Net Photosynthesis-Leaf Temperature Relations in Plant-Species with Different Height Along an Altitudinal Gradient. *Acta Oecologica-International Journal of Ecology*.
- Ristic, Z., Bukovnik, U., & Prasad, P. V. V. (2007). Correlation between Heat Stability of Thylakoid Membranes and Loss of Chlorophyll in Winter Wheat under Heat Stress. In *Crop Science* (Vol. 47). <https://doi.org/10.2135/cropsci2006.10.0674>
- Scafaro, A. P., Xiang, S., Long, B. M., Bahar, N. H. A., Weerasinghe, L. K., Creek, D., Evans, J. R., Reich, P. B., & Atkin, O. K. (2017). Strong thermal acclimation of photosynthesis in tropical and temperate wet-forest tree species: The importance of altered Rubisco content. *Global Change Biology*, 23(7), 2783–2800. <https://doi.org/10.1111/gcb.13566>
- Sklenář, P., & Balslev, H. (2005). Superpáramo plant species diversity and phytogeography in Ecuador. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 200(5), 416–433. <https://doi.org/10.1016/j.flora.2004.12.006>

- Sklenář, P., Kučerová, A., Macková, J., & Romoleroux, K. (2016). Temperature Microclimates of Plants in a Tropical Alpine Environment: How Much does Growth Form Matter? *Arctic, Antarctic, and Alpine Research*, 48(1), 61–78. <https://doi.org/10.1657/AAAR0014-084>
- Smith, A. P. (1974). Bud Temperature in Relation to Nyctinastic Leaf Movement in an Andean Giant Rosette Plant. *Biotropica*, 6(4), 263–266. <https://doi.org/10.2307/2989670>
- Smith, A. (1979). Function of Dead Leaves in *Espeletia schultzii* (Compositae), and Andean Caulescent Rosette Species. In *Biotropica* (Vol. 11). <https://doi.org/10.2307/2388171>
- Smith, A. P. (1980). The Paradox of Plant Height in an Andean Giant Rosette Species. *Journal of Ecology*, 68(1), 63–73. <https://doi.org/10.2307/2259244>
- Smith, A. P. (1994). Introduction to tropical alpine vegetation. In *Tropical alpine environments: Plant form and function* (pp. 1–17).
- Smith, A. P., & Young, T. P. (1987). Tropical Alpine Plant Ecology. *Annual Review of Ecology and Systematics*, 18(1), 137–158. <https://doi.org/10.1146/annurev.es.18.110187.001033>
- Suter, M., & Edwards, P. J. (2013). Convergent succession of plant communities is linked to species' functional traits. *Perspectives in Plant Ecology, Evolution and Systematics*, 15(4), 217–225. <https://doi.org/10.1016/j.ppees.2013.05.001>
- Van der Hammen, T., Pabón Caicedo, J. D., Gutiérrez, H., & Alarcón, J. C. (2002). El Cambio Global y los Ecosistemas de Alta Montaña de Colombia. In *Páramos y Ecosistemas Alto Andinos de Colombia en Condición HotSpot & Global Climatic Tensor* (pp. 162–209).
- Vargas, O., Pérez-Martínez, L., Insuasty, J., Rodríguez Castillo, N. A., & Melgarejo, L. M. (2014). *Semillas de plantas de páramo: Ecología y métodos de germinación aplicados a la restauración ecológica*.
- Baruch, Z. (1979). Elevation Differentiation in *Espeletia Schultzii* (Compositae), A Giant Rosette Plant of the Venezuelan Paramos. *Ecology*, 60(1), 85–98. <https://doi.org/10.2307/1936471>
- Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and, evolution of dormancy and germination*. Elsevier.
- Billings, W. D. (1973). Arctic and Alpine Vegetations: Similarities, Differences, and Susceptibility to Disturbance. *BioScience*, 23(12), 697–704. <https://doi.org/10.2307/1296827>
- Cardenas, C., Posada, C., & Vargas, O. (2002). Banco de semillas germinable de una comunidad vegetal de paramo humedo sometida a quema y pastoreo (Parque Nacional Natural Chingaza, Colombia)cardenas2002.pdf. *Ecotropicos*, 15(1), 51–60.
- Cortés, A. J., Garzón, L. N., Valencia, J. B., & Madriñán, S. (2018). On the Causes of Rapid Diversification in the Páramos: Isolation by Ecology and Genomic Divergence in *Espeletia*. *Frontiers in Plant Science*, 9, 1700. <https://doi.org/10.3389/fpls.2018.01700>
- Debouza, N. E., Babu Thruppoyil, S., Gopi, K., Zain, S., & Ksiksi, T. (2021). Plant and seed germination responses to global change, with a focus on CO<sub>2</sub>: A review. *One Ecosystem*, 6, e74260. <https://doi.org/10.3897/oneeco.6.e74260>
- Estrada, C., & Monasterio, M. (1988). Ecología poblacional de una roseta gigante, *Espeletia spicata* Sch. Bip. (Compositae), del páramo desértico. *Ecotropicos*, 1, 25–39.
- Fagua, J. C., & González, V. H. (2007). Growth rates, reproductive phenology, and pollination ecology of *Espeletia grandiflora* (Asteraceae), a giant andean caulescent rosette. *Plant Biology*, 9(1), 127–135.
- Frantzen, N. M. L. H. F., & Bouman, F. (1989). *Dispersal and growth form patterns of some zonal páramo vegetation types*. 38(4), 449–465.
- Guariguata, M. R., & Azocar, A. (1988). Seed Bank Dynamics and Germination Ecology in *Espeletia timotensis* (Compositae), an Andean Giant Rosette. *Biotropica*, 20(1), 54–59. JSTOR. <https://doi.org/10.2307/2388426>

- Llambí, L., & Rada, F. (2019). Ecological research in the tropical alpine ecosystems of the Venezuelan páramo: Past, present and future. *Plant Ecology & Diversity*, 1–20. <https://doi.org/10.1080/17550874.2019.1680762>
- Melcher, I. M., Bouman, F., & Cleef, A. M. (2004). Seed atlas of the monocotyledonous genera of the páramo. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 199(4), 286–308. <https://doi.org/10.1078/0367-2530-00157>
- Mora, F., Chaparro, H. A., & Vargas, O. (2007). Dinámica de la germinación, latencia de semillas y reclutamiento de plántulas en *Puya cryptantha* y *P. trianae*, dos rosetas gigantes de los páramos colombianos. *Ecotropicos*, 20(1), 31–40.
- Nürk, N. M., Michling, F., & Linder, H. P. (2018). Are the radiations of temperate lineages in tropical alpine ecosystems pre-adapted? *Global Ecology and Biogeography*, 27(3), 334–345. <https://doi.org/10.1111/geb.12699>
- Peyre, G., Lenoir, J., Karger, D. N., Gomez, M., Gonzalez, A., Broennimann, O., & Guisan, A. (2020). The fate of páramo plant assemblages in the sky islands of the northern Andes. *Journal of Vegetation Science*, 31(6), 967–980. <https://doi.org/10.1111/jvs.12898>
- Posada, J. (2013). *El rol de la dispersión de semillas en la regeneración de la vegetación de páramo en un paisaje fragmentado (Tesis de Maestría)*. [Tesis de maestría]. Universidad de los Andes.
- Rada, F. (2016). Functional Diversity in Tropical High Elevation Giant Rosettes. In G. Goldstein & L. S. Santiago (Eds.), *Tropical Tree Physiology: Adaptations and Responses in a Changing Environment* (pp. 181–202). Springer International Publishing. [https://doi.org/10.1007/978-3-319-27422-5\\_8](https://doi.org/10.1007/978-3-319-27422-5_8)
- Rada, F., Azócar, A., & García-Núñez, C. (2019). Plant functional diversity in tropical Andean páramos. *Plant Ecology & Diversity*, 1–15. <https://doi.org/10.1080/17550874.2019.1674396>
- Vargas, O., Pérez-Martínez, L., Insuasty, J., Rodríguez Castillo, N. A., & Melgarejo, L. M. (2014). *Semillas de plantas de páramo: Ecología y métodos de germinación aplicados a la restauración ecológica*.
- Velasco-Linares, P., & Vargas, O. (2008). La dispersión por aves y la restauración de los ecosistemas altoandinos. *Restauración ecológica del bosque altoandino. Estudios diagnósticos y experimentales en los alrededores del Embalse de Chisacá*, 217–228.
- von Caemmerer, S., & Evans, J. R. (2015). Temperature responses of mesophyll conductance differ greatly between species. *Plant, Cell and Environment*, 38(4), 629–637. <https://doi.org/10.1111/pce.12449>
- Way, D. A., & Oren, R. (2010). Differential responses to changes in growth temperature between trees from different functional groups and biomes: A review and synthesis of data. *Tree Physiology*, 30(6), 669–688. <https://doi.org/10.1093/treephys/tpq015>
- Way, D. A., & Sage, R. F. (2008). Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) B.S.P.]. *Plant, Cell and Environment*, 31(9), 1250–1262. <https://doi.org/10.1111/j.1365-3040.2008.01842.x>
- Yamori, W., Hikosaka, K., & Way, D. A. (2014). Temperature response of photosynthesis in C3, C4, and CAM plants: Temperature acclimation and temperature adaptation. *Photosynthesis Research*, 119(1–2), 101–117. <https://doi.org/10.1007/s11120-013-9874-6>
- Yamori, W., Noguchi, K., & Terashima, I. (2005). Temperature acclimation of photosynthesis in spinach leaves: Analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell and Environment*, 28(4), 536–547. <https://doi.org/10.1111/j.1365-3040.2004.01299.x>

# 1. CHAPTER I

## Insights into the functional ecology of páramo plants in Colombia

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### Abstract

Páramos are exceptionally species-rich tropical alpine ecosystems that provide several important ecosystem services, including carbon storage and water provision. The responses of páramo plant species to climatic change are largely unknown but classifying species by functional type and ecological strategy can help reduce complexity for modeling and conservation efforts. We measured 22 traits in 42 plant species with various growth forms in the Colombian Andes. We classified plant ecological strategies using CSR (C: competitor, S: stress tolerator, R: ruderal) analysis and identified plant functional types (PFTs) using hierarchical clustering of principal components. Additionally, we calculated the functional originality of each species to identify species with unique trait combinations. We identified a high degree of convergence among traits and strategies, with most species characterized by the S and SC strategies. Considering the full set of traits, only three PFTs were identified: one composed of forbs and shrubs with tender leaves (PFT1), another composed only of species with rosettes form (PFT2), and a third composed of shrubs with tough leaves (PFT3). Values of functional originality were generally low, suggesting high redundancy in plant traits. PFT2 species, mostly rosettes, exhibited the highest functional originality of all the groups. Our results imply the existence of a limited set of ecophysiological strategies in the páramos, likely driven by the extremely stressful conditions in these systems. This functional redundancy may indicate heightened vulnerability to environmental changes; however, the presence of multiple species with shared functional traits could provide some resilience to disturbance.

### 1.1 INTRODUCTION

Predicting the responses of mountain ecosystems to climate change has become a major priority, since high mountain regions are warming faster than lowlands and could be more sensitive to climate change than other ecosystems at the same latitudes (Díaz & Bradley, 1997; Liu & Chen, 2000; Ning & Bradley, 2014; Rangwala et al., 2013). Over the next century, projections suggest a  $3.0 \pm 1.5^{\circ}\text{C}$  rise in average temperature in the high tropical Andes (Buytaert et al., 2011) and a decline in precipitation in Northern Colombia and Venezuela (Buytaert et al., 2011; Urrutia & Vuille, 2009). In the Andes, a unique type of tropical alpine ecosystem locally known as the páramos exists at elevations above 3,200 m.a.s.l. The vegetation in the páramos has evolved under a unique set of stressful conditions including low atmospheric pressure, large daily fluctuations in temperature, frequent freezing temperatures, intense UV radiation, and high wind speeds (Buytaert, Deckers, et al., 2006; Llambí & Rada, 2019; Rada et al., 2019; Sarmiento, 1986). These conditions, in combination with geographical isolation related to the island-like distribution of mountainous communities, have promoted a high rate of speciation (Flantua et al., 2019; Madriñán et al., 2013; Sklenář & Ramsay, 2001) and have resulted in exceptionally high endemism. Thus, the páramos host an estimated 3,595 species of vascular plants, 60% of which are endemic (Luteyn, 1999b). Predicting which of these thousands of species are most vulnerable to the warming and drying trends expected for the Andes (Buytaert et al., 2011; Urrutia & Vuille, 2009) is an overwhelming but critical challenge.

Functional ecology tools can help address the complexity of this challenge by classifying species by ecological strategy and plant functional type (PFT). Functional classification of species in the páramos is fundamental for reducing the complexity of their floristic composition and necessary for including these communities in future global vegetation models (Woodward & Cramer, 1996). Moreover, effective management, conservation, and restoration plans for the páramos depend on a deeper understanding of species functionality and originality (Kondratyeva et al., 2019; Pavoine et al., 2017).

Classification of species by PFT is not straightforward. One fast and relatively easy approach is to assign species to functional types based on discrete traits such as growth form, leaf habit, or ability to fix nitrogen (Powers & Tiffin, 2010; Ramsay et al., 2000; Reich et al., 2007; Wright et al., 2004). However, the utility and ecological meaning of functional type classifications based on these discrete traits must be evaluated on a case-by-case basis (Chapin et al., 1996; Powers & Tiffin, 2010). Another approach is to categorize species based on the CSR triangle ecological scheme developed by Grime (Grime, 1977; Matos et al., 2020; Pierce et al., 2017). This approach might be a relevant way to infer plants' functional response to climate change in alpine zones, mainly because of the lack of general patterns in plant functional traits related to climate change in alpine plants (Zimmer et al., 2018). Under this scheme, species are classified as either competitor (C), stress tolerator (S), or ruderal (R) based on three morphological traits representing the extremes of leaf economics and size spectra (specific leaf area, leaf area, and leaf dry mass content). The CSR theory proposes that C species have traits that allow them to obtain resources quickly and develop large organ and body sizes that improve resource provisioning. These traits allow the species to survive in relatively stable, productive habitats. S species, on the other hand, have traits that allow them to protect, repair, and retain their resources, given that they occupy variable, often resource-poor, and stressful environments. Thus, these species grow slowly but may gradually reach a large size. R species are short-lived, small, and invest their resources in reproduction to regenerate elsewhere, since they live in unstable environments where disturbances are frequent (Grime, 1977; Pierce et al., 2017). A strength of the CSR approach is that it requires few easy-to-measure parameters and has been widely used in studies across distinct habitats, including alpine zones (Zimmer et al., 2018).

A major challenge of simplistic approaches such as CSR is that similar ecological strategies may be achieved by species with different combinations of traits (Rosado & de Mattos, 2017). In the páramos, stressful conditions can result in a narrow range of ecological strategies, necessitating the use of a more extensive set of traits for characterizing plant strategies and classifying species by PFT. Species may be classified using continuous, quantitative traits that are assumed to reflect life-history trade-offs, stress adaptations, and ecosystem roles (de Bello et al., 2010; Lavorel & Garnier, 2002; Matos et al., 2020). For example, traits such as leaf area, foliar nitrogen, and stem height are positively related to species' ability to acquire resources, maximize photosynthetic capacity, and sequester carbon (Díaz et al., 2004; Wright et al., 2004). Traits such as water use efficiency, leaf dry matter content (LDCM), and midday leaf water potential are likewise related to the ability to tolerate water deficit (Markestijn et al., 2011; Martínez-Garza et al., 2013). Moreover, traits such as seed mass and seed dispersal syndromes are related to reproductive investment, mobile capabilities, and establishment success (Leishman et al., 2000; Levin et al., 2003; Thomson et al., 2011). Some of these traits may affect biogeochemical cycles, invasion resistance, resilience to disturbance, and net primary productivity (NPP) and therefore can also be linked to species' roles in the ecosystem (Dormann, 2002; Lavorel & Garnier, 2002). As sampling of these traits often requires time-consuming fieldwork, these traits have been underused in the functional classification of páramo species.

Previous studies of PFTs in the páramos have yielded differing classifications of species groups (Cárdenas-Arévalo & Vargas-Ríos, 2008; Rada et al., 2019). In the drier Venezuelan páramos, rosette species have been considered stress ‘avoiders’, possessing traits to avoid or reduce the effect of stresses such as water deficit or freezing (Azócar et al., 1988; Squeo et al., 1991). For example, rosettes may have insulating structures for preventing ice formation and may store water in the pith to sustain a favorable water balance in dry conditions (Rada, 2016). Grasses, cushion plants, and forbs have conversely been categorized as stress tolerators, possessing traits and mechanisms to cope with the stress (Rada et al., 2019). For example, tolerators may resist ice-crystal formation in tissues during freezing (Rada et al., 2019) or accumulate solutes to adjust osmotic potential and keep their cell turgid during drought (Rada, Goldstein, Azócar, et al., 1985). In the Venezuelan páramos, shrubs have been classified either as tolerators or avoiders (Rada et al., 2019). In the Colombian páramos, 11 traits were used to classify grasses and bamboos into one group, forbs and cushion plants into a second group, and rosettes into a third group, with shrubs distributed across groups (Cárdenas-Arévalo & Vargas-Ríos, 2008).

While the páramos are known to be species rich (Luteyn, 1999; Madriñán et al., 2013), the functional diversity and functional originality of species in these systems have not been assessed. Classical metrics of biological diversity focus on the number of species (i.e. species richness or diversity) and disregard species differences in traits and functionality (Cousins, 1991). Quantifying this source of diversity is important because the functional traits of a species might reflect its role in the ecosystem (Lavorel & Garnier, 2002; Matos et al., 2020). Ecologists have proposed new indices to characterize this diversity (Kondratyeva et al., 2019; Pavoine et al., 2017) and evaluate a species functional originality, that is, how original or redundant a species is according to its functional traits (Pavoine et al., 2005, 2017). Given the need to protect biodiversity with limited resources in many cases, priority systems have been proposed based on protecting phylogenetically original (Faith, 1992; Isaac et al., 2007) and functionally original species (Mouillot et al., 2008; Pavoine et al., 2017). To our knowledge, no estimates of functional originality for páramo plants have been published, although preliminary data could be used to identify species with a unique set of traits and develop strategies to protect páramo biodiversity.

In this study, we measured 22 traits from 42 páramo species to understand how growth form relates to functional classification. We also used this data set to explore how functional originality in the páramos is distributed among different growth forms. We used several functional classification schemes, including Grimes’ (1977) CSR triangle, and a classification based on 22 traits related to water relations, gas exchange, nutrient status, acquisitive strategies, or seed dispersal. Our main objectives were to (a) establish how many ecological strategies (CSR) and functional types (PFT) can be identified in the páramos and how they correspond with growth forms, (b) identify valuable traits for species classification in the páramos, and (c) assess species originality among growth forms. Based on previous work (Chapin et al., 1996; Rada et al., 2019), our ‘null hypothesis’ was that growth form would be sufficiently predictive of PFT and ecological strategy. An ‘alternate’ hypothesis was that due to the stressful conditions of the páramos, species will show a narrow range of CSR strategies in which stress tolerance would be the most common strategy regardless of growth form and that PFTs would similarly show a narrow range, except for rosettes, which would form a distinct group, as reported in previous studies in the Venezuelan Andes. In this case, we would expect that most species would share traits that allow them to withstand the harsh conditions of the páramos and exhibit low functional originality.

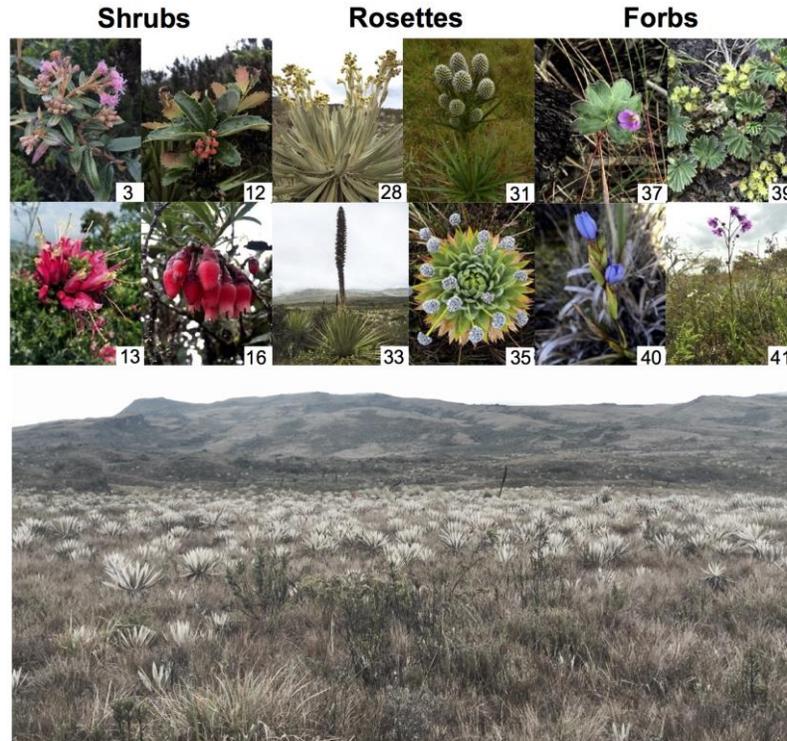
## 1.2 MATERIALS AND METHODS

### 1.2.1 Study site and species

All measurements were taken in the Páramo el Verjón-Matarredonda, located in the Eastern Andes of Colombia (4° 33' N, 74° 00' W), between 3290 and 3400 m.a.s.l. This páramo is part of the Cruz-Verde-Sumapaz páramo complex and occupies an area of 690 ha (**Figure 1**). The páramo is part of a private reserve known as the Matarredonda Ecological Park (*Parque Ecológico Matarredonda*). Like many of the Andes' páramos, it has a long history of human use, which has not been thoroughly studied. The park contains stone trails built by Muisca communities and the Spaniards and based on conversations with the owners of the reserve, some sections of the páramo were cultivated at least 40 years ago.

Current disturbances are limited to tourist impacts and are mostly confined to trail systems. The páramo is surrounded by a matrix of forest, roads, and agriculture. Vegetation is characterized by a mixed community of rosette, shrub, graminoid, forb, and moss species. The mean annual air temperature is 8.3°C, but daily temperature varies from a maximum of 23.2°C to a minimum of -2.6°C, sometimes in a single day (Leon-García & Lasso, 2019). Annual precipitation ranges between 1000 and 1500 mm yr<sup>-1</sup>, with a dry season from December through February.

Surveys were targeted for 42 species most representative of páramo communities. Species were grouped into three growth form categories using a simplified classification system: all erect and prostrate herbs were considered forbs (N = 6); all basal, stem, and acaulescent rosettes were considered rosettes (N = 9); and all prostrate and upright shrubs were considered shrubs (N = 27) (**Figure 1**; **Table 1**). Collection sites were limited to areas with no signs of recent disturbance.



**Figure 1.** A view of the páramo at El Verjón-Matarredonda, located in the eastern Andean range of Colombia between 3290 and 3400 m.a.s.l. Pictured at the top are some representative species of each

growth form studied. Shrub species are *Ageratina gynoxoides* (3), *Berberis goudotii* (12) *Bejaria resinosa* (13) and *Macleania rupestris* (16); rosettes species are *Espeletia grandiflora* (28), *Eryngium humboldtii* (31), *Puya goudotiana* (33), *Paepalanthus alpinus* (35); and forbs are *Geranium multiceps* (37), *Lachemilla orbiculata* (39), *Orthrosanthus chimboracensis* (40) and *Senecio formosoides* (41). Photos by Marisol Cruz.

**Table 1.** List of studied species organized by family, growth forms (GF), and area of occurrence. Occurrences were obtained from GBIF (<https://www.gbif.org/en/>). We considered as a valid country of occurrence if there were more than one specimen recorded in that country. If a species was listed in more than four South American countries is registered as South America (SA) and more than three central American countries as Central America (CA). Countries are as follow: Bo = Bolivia, Co = Colombia, Cr = Costa Rica, Ec = Ecuador, Gu = Guatemala, Me = México, Pa = Panamá, Pe = Perú, Ve = Venezuela. Growth forms are as follow: US = Upright shrub; PS = Prostrate shrubs, SR = Stem rosette; BR= Basal rosette, AR= Acaulescent rosette, PH= Reptant and prostrate herbs, and EH= Erect herbs

ID	Scientific name	Family	GF	Occurrences
<b>Shrubs</b>				
1	<i>Oreopanax mutisianus</i> (Kunth) Decne. & Planch.	Araliaceae	US	Co & Ec
2	<i>Ageratina asclepiadea</i> (L.f.) R.M.King & H.Rob.	Asteraceae	US	Co & Ve
3	<i>Ageratina gynoxoides</i> (Wedd.) R.M.King & H.Rob.	Asteraceae	US	Co
4	<i>Baccharis bogotensis</i> Kunth	Asteraceae	US	Co & Me
5	<i>Baccharis tricuneata</i> (L.f.) Pers.	Asteraceae	US	SA
6	<i>Pentacalia firmipes</i> (Greenm.) Cuatrec.	Asteraceae	US	Cr & Pa
7	<i>Pentacalia ledifolia</i> (Kunth) Cuatrec.	Asteraceae	US	Co & Ve
8	<i>Pentacalia nitida</i> (Kunth) Cuatrec.	Asteraceae	US	Co & Ec
9	<i>Pentacalia vaccinioides</i> (Kunth) Cuatrec.	Asteraceae	US	Co, Ve & Ec
10	<i>Diplostegium phyllicoides</i> (Kunth) Wedd.	Asteraceae	US	Me, Co & Ec
11	<i>Diplostegium rosmarinifolium</i> (Benth.) Wedd.	Asteraceae	US	Co & Ve
12	<i>Berberis goudotii</i> Triana & Planch.	Berberidaceae	US	Co & Bo
13	<i>Bejaria resinosa</i> Mutis ex L.f.	Ericacea	US	SA
14	<i>Gaultheria anastomosans</i> (Mutis ex L.f.) Kunth	Ericacea	US	Co, Ve, Ec, Bo & Pa
15	<i>Gaultheria hapalotricha</i> A.C.Sm.	Ericacea	PS	Co, Ve & Bo
16	<i>Macleania rupestris</i> (Kunth) A.C.Sm.	Ericaceae	US	USA, CA & SA
17	<i>Gaultheria myrsinoides</i> Kunth (syn <i>Pernettya prostrate</i> )	Ericacea	PS	Me, Cr & SA
18	<i>Ribes andicola</i> Jancz.	Grossulariaceae	US	SA
19	<i>Brachyotum strigosum</i> (L. f.) Triana	Melastomataceae	US	Co, Pe, USA
20	<i>Bucquetia glutinosa</i> (L. f.) DC.	Melastomataceae	US	Co
21	<i>Miconia summa</i> Cuatrec.	Melastomataceae	US	Co
22	<i>Tibouchina grossa</i> (L. f.) Cogn.	Melastomataceae	US	Co, Ve, Ec, Bo

23	<i>Monnina salicifolia</i> Ruiz & Pav.	Polygalaceae	US	SA
24	<i>Myrsine dependens</i> (Ruiz & Pav.) Spreng.	Primulaceae	US	Cr, Pa & SA
25	<i>Hesperomeles obtusifolia</i> (DC.) Lindl.*	Rosaceae	US	Cr, Pa & SA
26	<i>Rubus acanthophyllos</i> Focke*	Rosaceae	US	Co, Ve, Ec & Pe
27	<i>Symplocos theiformis</i> (L. f.) Oken	Symplocaceae	US	Co
<b>Rosettes</b>				
28	<i>Espeletia grandiflora</i> Humb. & Bonpl.	Asteraceae	SR	Co
29	<i>Espeletiopsis corymbosa</i> (Humb. & Bonpl.) Cuatrec.	Asteraceae	SR	Co
30	<i>Espeletia argentea</i> Humb. & Bonpl.	Asteraceae	B	Co
31	<i>Eryngium humboldtii</i> F.Delaroche	Apiaceae	R	SA
32	<i>Greigia stenolepis</i> L.B.Sm.	Bromeliaceae	B	Co
33	<i>Puya goudotiana</i> Mez	Bromeliaceae	R	Co
34	<i>Puya santosii</i> Cuatrec.	Bromeliaceae	B	Co
35	<i>Paepalanthus alpinus</i> Körn. ( <i>Syn</i> <i>Paepalanthus columbiensis</i> )	Eriocaulaceae	R	Co
36	<i>Acaena cylindristachya</i> Ruiz & Pav*	Rosaceae	R	Cr, Co, Ve, Pe & Bo
<b>Forbs</b>				
37	<i>Geranium multiceps</i> Turcz.	Geraniaceae	PH	Col & Ve
38	<i>Geranium sibbaldioides</i> Benth.	Geraniaceae	PH	SA
39	<i>Lachemilla orbiculata</i> (Ruiz & Pav.) Rydb*	Rosaceae	PH	Me & SA
40	<i>Orthrosanthus chimboracensis</i> (Kunth) Baker	Iridaceae	E	CE & SA
41	<i>Senecio formosoides</i> Cuatrec.	Asteraceae	H	Co, Ve & Ec
42	<i>Valeriana pilosa</i> Ruiz & Pav.	Caprifoliaceae	E	Cr, Co, Ve & Pe

### 1.2.2 Functional traits

For each species, we measured 22 traits, listed in **Table 2**. Leaf morphological and chemical data and plant height were collected in October–November 2013. We sampled leaf area, thickness, toughness, SLA (specific leaf area), LDMC (leaf dry matter content), and carbon and nitrogen content from the same leaves in each species, limiting destructive sampling. In 2014, we collected physiological data. Traits related to drought tolerance were collected at the end of the dry season (February–March 2014), and carbon assimilation was measured during sunny days in the wet season (April–October 2014). Seed traits were collected opportunistically during reproductive phases until 2015. Each set of physiological traits was measured during the same week (i.e., the height of all plants in one week and the leaf area in one week, etc.) to reduce the effect of the variation due to seasonal effects.

**Table 2.** Functional traits measured in the páramo plants and their probable relation with processes and functions.

Functional trait	Abbrev.	Related with
<b>Continuous traits and units</b>		
Plant height (m)	H	Competitiveness, robustness, potential strength, fecundity, carbon storage, light acquisition
Seed mass (mg)	SM	Dispersal, establishment, survival, competitiveness
Leaf area (mm <sup>2</sup> )	LA	Resource acquisition, allometric factors and environmental stresses
Specific leaf area (mm <sup>2</sup> mg <sup>-1</sup> )	SLA	Relative Growth Rate (RGR) and environmental conditions
Leaf dry matter content (mg g <sup>-1</sup> )	LDMC	Leaf density, decomposition rates and plant defense.
Leaf thickness (mm)	LT	Defense, carbon balance, resource acquisition and use, longevity
Leaf toughness (N mm <sup>-1</sup> )	LS	Defense, mechanical damage, anatomical variation, longer leaf lifespans, carbon investment.
Leaf water potential at pre-dawn (MPa)	$\Psi_{\text{pre-dawn}}$	Water status and hydraulic strategies
Leaf water potential at noon (MPa)	$\Psi_{\text{noon}}$	Water status and hydraulic strategies
$\Delta \Psi_{\text{noon}} - \Psi_{\text{pre-dawn}}$		Hydraulic strategies
Light-saturated photosynthetic rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	$A_{\text{max}}$	Metabolic capacity and leaf economic spectrum
Leaf transpiration rate (mol m <sup>-2</sup> s <sup>-1</sup> )	E	Photosynthetic rates, physical leaf characteristics and hydraulic strategies.
Intercellular CO <sub>2</sub> /Ambient CO <sub>2</sub>	$C_i/C_a$	Related to stomatal limitations of gas exchange when it is compared with stomatal conductance.
Water use efficiency	WUE	Carbon balance, water use efficiency, resource use and acquisition
Leaf phosphorus content (mg g <sup>-1</sup> )	LPC	Leaf quality, nutritional quality of the soil
Leaf carbon content (mg g <sup>-1</sup> )	LCC	Leaf construction, resource use
Leaf nitrogen content (mg g <sup>-1</sup> )	LNC	Concentration of RuBisCO, photosynthesis and fast-to-slow strategy, nutritional quality of the soil

Traits were measured for three healthy reproductive individuals per species. Leaf measurements were performed on three mature leaves exposed to full light, using standard protocols developed by Cornelissen et al. (2003). Plant height was measured as the distance from the ground to the top of the main photosynthetic tissues. Leaf area was measured with a flatbed scanner (Hewlett Packard G3010). All weight measurements were obtained using a 0.01 mg precision balance (Sartorius MSE 125p). Leaf dry mass was obtained after drying samples in an oven at 70°C for 72 h. Leaf toughness was assessed with a punch test using a modified Pesola (Schindellegi Switzerland; (Kitajima & Poorter, 2010)). Leaf thickness was measured using a 0–25-mm external micrometer (Redline mechanics). Leaf nitrogen concentration

and leaf carbon concentration were determined using an elemental analyzer, and leaf phosphorus concentration was determined by colorimetric analysis. All nutrient analyses were performed in the Soil and Water laboratory at the Universidad Nacional de Colombia, Bogota.

We also recorded qualitative traits including pubescence, spinescence, and dispersal syndrome. Pubescence is a fundamental characteristic of plants in this ecosystem that regulates leaf temperature, increases reflectance, and may act as the first line of defense against herbivores (Hanley et al., 2007; Seelmann et al., 2007; Wang et al., 2015). It may also facilitate fog capture and slow release of water. We defined three categories of pubescence: (a) non-pubescent for glabrous foliar laminae, (b) low pubescence when <30% of the lamina was covered with trichomes, and (c) high pubescence when >30% of the lamina was covered with trichomes. For spinescence, we defined two categories: with and without spines. Data on the dispersal syndrome for each species were obtained from published literature or personal observations following Pérez-Harguindeguy et al. (2013). Categories for these data included anemochory (wind dispersal), endozoochory (internal animal transport), exozoochory (external animal transport), hydrochory (dispersal by water), and barochory (dispersion by gravity).

We measured the following traits related to leaf gas exchange and water balance using an open infrared gas exchange analyzer Li-Cor 6400XT (Li-Cor, Lincoln Nebraska): light-saturated photosynthetic rate, leaf transpiration rate, the ratio between intercellular CO<sub>2</sub>/ambient CO<sub>2</sub>, and water use efficiency (how much water is lost for each CO<sub>2</sub> molecule assimilated). These values were obtained on sunny mornings during the wet season, between 10:00 and 12:00, with a light intensity of 1,800 μmol photons m<sup>-2</sup> s<sup>-1</sup>, CO<sub>2</sub> concentration of 400 μmol mol<sup>-1</sup>, and relative humidity of 60%.

To evaluate the species' drought tolerance, we measured leaf water potentials at pre-dawn and midday during the end of the dry season, following standard protocols using a pressure chamber (PMS, Corvallis, Oregon). Pre-dawn water potential indicates the water potential of the soil when transpiration is at its minimum, and midday water potential captures the integrated effects of plant traits and the environment on the minimum water potential that a plant reaches in natural conditions (Bhaskar & Ackerly, 2006). This value is known to be correlated to the turgor loss point and other drought tolerance traits (Bartlett et al., 2016). Additionally, we assessed cuticular conductance following Scoffoni et al. (2018), measured as the rate of change in mass for hydrated leaves drying in the laboratory over 6–8 hours after stomatal closure.

### 1.2.3 Data analysis

We omitted two variables from the analysis that were highly correlated with other variables (>0.8): the ratio between intercellular CO<sub>2</sub>/ambient CO<sub>2</sub>, which was highly correlated with WUE, and the delta of pre-dawn and midday water potential, which was highly correlated with midday water potential (**Figure S1**).

We used the globally calibrated CSR strategy calculator StrateFy (Pierce et al., 2017) to identify the relative proportion of each ecological CSR strategy associated with each species based on their SLA, LDMC, and LA values. StrateFy computes CSR membership by regressing leaf trait values against principal component analysis (PCA) axes derived from a multivariate analysis based on leaf trait data from 3,068 tracheophytes representing 198 families, six continents, and 14 biomes (Pierce et al., 2017). The StrateFy calculator thus compared páramo target species against the multivariate functional space of a global data set.

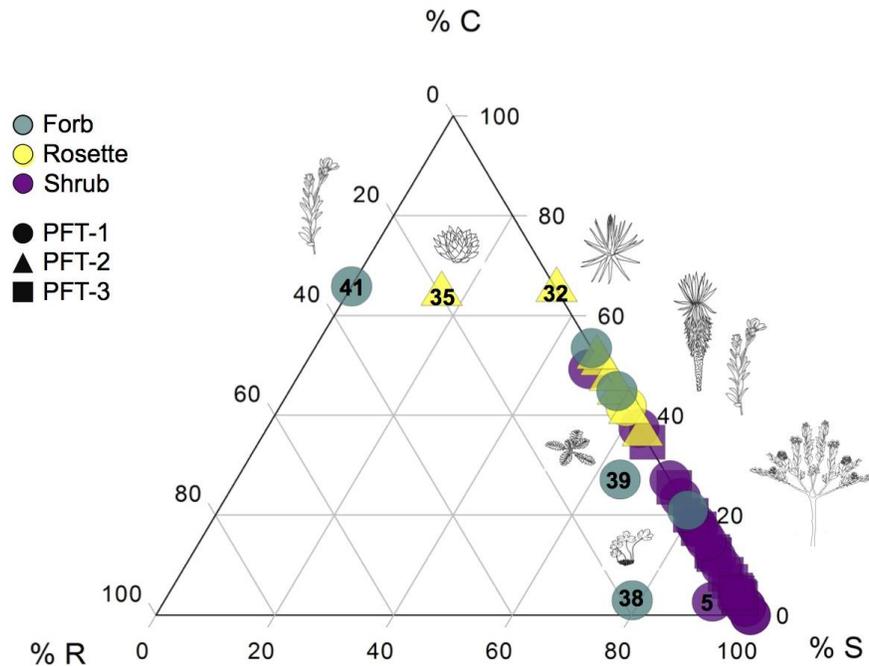
We used the Hierarchical Clustering on Principal Components (HCPC) method with trait data to identify PFTs, evaluate the degree to which individual traits explained the observed variation, and examine the distribution of páramo species and growth forms in the trait space. We performed the HCPC method on the 10 first dimensions retained from a factor analysis of mixed data (FAMD) (metric, Euclidean; linkage, Ward), implemented in the FactoMineR package (Version 1.41) (Kassambara, 2017). Following the approach described by Palacio et al. (2020), we used the Kaiser and Guttman criterion as the factor retention criterion; that is, we retained the first nine dimensions with eigenvalues greater than 1 and retained the 10th dimension, since all 10 together explained 81% of the data's variability (Palacio et al., 2020). FAMD is a principal component method that balances the influence of quantitative and qualitative variables by scaling (Pagès, 2014). After FAMD, we implemented hierarchical clustering using Ward's criterion, setting the optimal number of clusters between 3 and 10. We plotted all figures using the Factoextra package (Version 1.0.7) and ggplot2 (Version 3.1.0).

To assess species functional originality, we used the `distinctDis` function in the R package `adiv` (Pavoine, 2020). We calculated the average distance index (AV) for each species, defined as the average dissimilarity between a focal species and all others in a set. The more dissimilar a species is from all others, the larger its originality (maximum value = 1), and the more similar it is to other species in the set, the lower its originality (minimum value = 0). This index is considered appropriate for evaluating functional trait-based originality and is highly correlated with other functional and phylogenetic indexes (Kondratyeva et al., 2020; Pavoine et al., 2017). A drawback of this index is that it provides very similar values for all species (Pavoine et al., 2005) and is sensitive to changes in the pool of species included in the analysis.

To examine the variation in quantitative traits associated with PFTs and growth forms, we performed ANOVAs with Tukey's tests for variables with normal distributions and Kruskal-Wallis and Wilcox tests for variables with non-normal distributions. For categorical variables, we assessed differences between PFTs and growth forms with a Fisher's exact test. All analyses were performed in R (Version 1.3.1093 - R Development Core Team, 2009–2016).

### 1.3. RESULTS

Based on `StrateFy` estimates, most páramo species were classified either as stress tolerators (S) or a combination of stress tolerators/competitors (S/CS) (**Figure 2; Table 3**). Only four species had ruderal (R) qualities: two forbs (*Geranium sibbaldioides* with 18.4% R and *Senecio formosoides* with 34.2% R), one rosette (*Paepalanthus alpinus* with 19.8% R), and one shrub *Rubus acanthophyllos* (2.5% R). Most species classified as S were shrubs, while rosettes frequently contained C qualities. *Greigia stenolopis*, a terrestrial bromeliad, and *Paepalanthus alpinus* were the rosette species with the highest competitor scores (65.1% C and 64.3% C, respectively; **Figure 2; Table 3**).



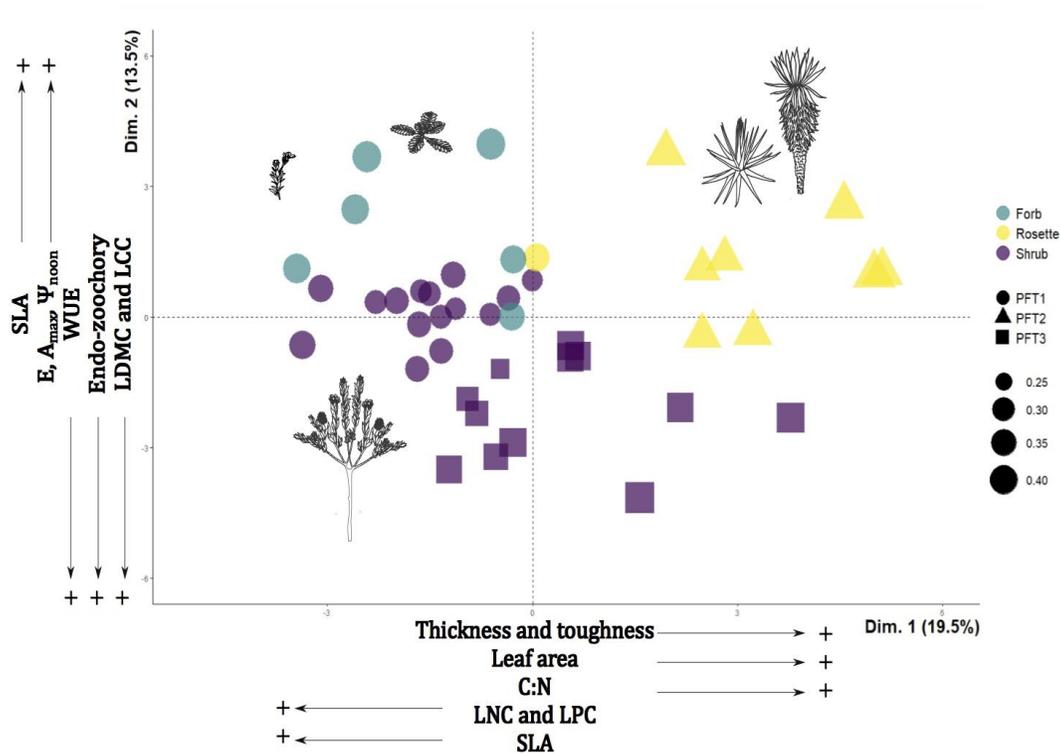
**Figure 2.** Ecological strategies in the páramos according to the CSR (competitor, stress-tolerator, ruderal) theory using the globally calibrated analysis tool ‘Stratefy’ based on 3,068 tracheophytes from 14 biomes (Pierce et al., 2017). Strategy convergence was evident for most páramo species independent of their growth forms, with most species falling in the S, C, CS, and CR classes suggested by Grime (2001). Species with distinctive strategies are identified with numbers inside symbols: (5) *B. tricuneata*; (32) *E. humboldtii*; (35) *P. alpinus*; (38) *G. siboldioides* and (39) *L. orbiculata*.

**Table 3.** Classification of the páramo species on CSR ecological strategies following Pierce et al., (2017), the plant functional types (PFTs) to which the species belong after our cluster analysis using all variables, and their originality index (AV).

Scientific name	PFTs	% C	% S	% R	Strategy	AV
<b>Shrubs</b>						
<i>Oreopanax mutisianus</i>	PFT-3	25.6	74.4	0.0	S/CS	0.31
<i>Ageratina asclepiadea</i>	PFT-1	37.4	62.6	0.0	S/CS	0.29
<i>Ageratina gynoxoides</i>	PFT-3	12.2	87.8	0.0	S	0.32
<i>Baccharis bogotensis</i>	PFT-1	18.4	81.6	0.0	S/CS	0.31
<i>Baccharis tricuneata</i>	PFT-1	2.7	92.3	5.0	S	0.26
<i>Pentacalia firmipes</i>	PFT-1	11.6	88.4	0.0	S	0.28
<i>Pentacalia ledifolia</i>	PFT-1	1.6	98.4	0.0	S	0.28
<i>Pentacalia nitida</i>	PFT-1	9.7	90.3	0.0	S	0.29
<i>Pentacalia vaccinioides</i>	PFT-1	27.0	73.0	0.0	S/CS	0.27
<i>Diplostephium phlycoides</i>	PFT-1	0.0	100.0	0.0	S	0.27
<i>Diplostephium rosmarinifolium</i>	PFT-1	0.0	100.0	0.0	S	0.27
<i>Berberis goudotii</i>	PFT-3	12.8	87.2	0.0	S	0.36
<i>Bejaria resinosa</i>	PFT-3	8.2	91.8	0.0	S	0.30
<i>Gaultheria anastomosans</i>	PFT-3	4.5	95.5	0.0	S	0.29
<i>Gaultheria hapalotricha</i>	PFT-3	17.3	82.7	0.0	S/CS	0.32

<i>Macleania rupestris</i>	PFT-3	34.6	65.4	0.0	S/CS	0.33
<i>Gaultheria myrsinoides</i> (syn <i>Pernettya prostrata</i> )	PFT-3	3.4	96.6	0.0	S	0.25
<i>Ribes andicola</i>	PFT-1	15.9	84.1	0.0	S	0.33
<i>Brachyotum strigosum</i>	PFT-1	1.8	98.2	0.0	S	0.30
<i>Bucquetia glutinosa</i>	PFT-1	17.5	82.5	0.0	S/CS	0.26
<i>Miconia summa</i>	PFT-3	6.7	93.3	0.0	S	0.27
<i>Tibouchina grossa</i>	PFT-1	14.8	85.2	0.0	S	0.30
<i>Monnina salicifolia</i>	PFT-1	23.5	76.5	0.0	S/CS	0.30
<i>Myrsine dependens</i>	PFT-3	3.8	96.2	0.0	S	0.26
<i>Hesperomeles obtusifolia</i>	PFT-3	5.0	95.0	0.0	S	0.32
<i>Rubus acanthophyllos</i>	PFT-1	49.3	48.2	2.5	CS	0.30
<i>Symplocos theiformis</i>	PFT-3	20.0	80.0	0.0	S/CS	0.30
<b>Rosettes</b>						
<i>Espeletia grandiflora</i>	PFT-2	50.3	49.7	0.0	CS	0.39
<i>Espeletiopsis corymbosa</i>	PFT-2	44.5	55.5	0.0	CS	0.34
<i>Espeletia argentea</i>	PFT-2	36.2	63.8	0.0	S/CS	0.34
<i>Eryngium humboldtii</i>	PFT-2	51.6	48.4	0.0	CS	0.36
<i>Greigia stenolepis</i>	PFT-2	65.1	34.9	0.0	C/CS	0.42
<i>Puya goudotiana</i>	PFT-2	40.7	59.3	0.0	CS	0.40
<i>Puya santosii</i>	PFT-2	47.3	52.7	0.0	CS	0.36
<i>Paepalanthus alpinus</i> (Syn <i>Paepalanthus columbiensis</i> )	PFT-2	64.3	15.9	19.8	C/CSR	0.38
<i>Acaena cylindristachya</i>	PFT-1	41.7	58.3	0.0	CS	0.32
<b>Forbs</b>						
<i>Geranium multiceps</i>	PFT-1	21.0	79.0	0.0	S/CS	0.32
<i>Geranium sibbaldioides</i>	PFT-1	3.0	78.7	18.4	S/SR	0.36
<i>Lachemilla orbiculata</i>	PFT-1	27.1	64.6	8.3	S/CS	0.35
<i>Orthrosanthus chimboracensis</i>	PFT-1	44.9	55.1	0.0	CS	0.33
<i>Senecio formosoides</i>	PFT-1	65.8	0.0	34.2	C/CR	0.38
<i>Valeriana pilosa</i>	PFT-1	53.4	46.6	0.0	CS	0.37

In the analysis of all traits, we identified three PFTs (**Figure 3**) that broadly corresponded to growth forms, although shrubs were split among two PFTs (**Figure 3; Table 3**). In the FAMD, 42.7% of the variability was explained by the three first principal dimensions (**Table S1**). The first axis was correlated with traits for the allocation of carbon and light capture (leaf area, SLA, C:N), traits related to defense (thickness and toughness), and traits related to plant nutritional status (leaf N and P content) (**Figure S2**). The second axis was correlated with water deficit strategy traits (LDMC, WUE, water potential at noon, and transpiration rate), seed dispersal traits, and traits associated with structural support and photosynthetic tissue (LDMC, LCC, SLA, and  $A_{max}$ ) (**Table S1; Figure S2**).



**Figure 3.** Clusters generated by HCPC of 42 plant species from Páramo el Verjón-Matarredonda based on 20 functional traits. Labels display the key traits defining each cluster. Symbols show the three PFTs identified, and colors show growth forms using a simplified classification system in which all erect and prostrate herbs are depicted as forbs; all basal, stem, and acaulescent rosettes are depicted as rosettes; and all prostrate and upright shrubs as shrubs. The symbols' size indicates the functional originality of the species, where larger symbols depict higher originality. PFT1 denoted with circles includes all forbs, shrubs with tender leaves, and one rosette. PFT2 denoted with triangles includes all but one rosette, and PFT3 denoted with squares includes only hard-leaved shrubs.

The first and largest functional group (PFT1) included forbs and shrubs with thin and soft leaves, high SLA, high nitrogen and phosphorus contents, all traits associated with high growth rates, and an acquisitive growth strategy (**Figure 3**). Most PFT1 species belonged to the Asteraceae and Melastomataceae families (**Table 1**). PFT1 species were also somewhat tolerant of water deficit, with intermediate values of midday leaf water potential (**Figure 3; Table S2**).

The second functional group (PFT2) included all the rosette species except for one acaulescent rosette species, *Acaena cylindristachya* (**Table 1**). Plants in this group had the largest, thickest, and toughest leaves of all the groups, with low SLAs, low nitrogen and phosphorus contents, traits associated with slower growth rates and longer foliar life spans (**Figure 3; Table S2**). The leaves of plants in this group had high C:N ratios, making them likely to decompose slowly. The leaves of plants in this group were also densely pubescent, which reduces water loss and freezing. Species in this group had low WUE values and the highest leaf water potentials (less negative), suggesting that they avoid water deficit.

The third functional group (PFT3) was composed exclusively of prostrate and upright shrubs, including species from the Ericaceae, Melastomataceae, and Asteraceae families, among others (**Table 1 and Table 3**). PFT3 plants were the tallest of all the groups and had tough leaves, high carbon contents, low phosphorus contents, and high leaf dry matter contents (**Figure 3; Table S2**). These shrubs had fruits with heavier seeds dispersed by endo-zoochory, low transpiration rates, low rates of photosynthesis, and the most negative values of leaf water potential at noon, an indication that they could be more tolerant of water deficit than the other groups (**Figure 3**). This group included species with the lowest originality values (**Table 3**).

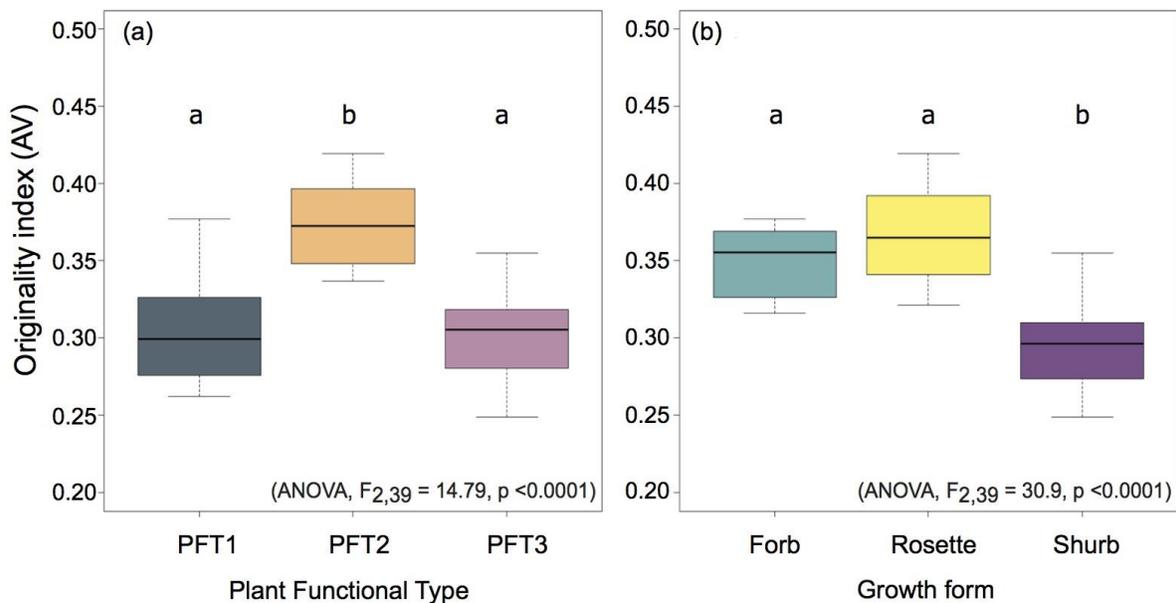
Traits that did not differ between growth forms included seed mass, cuticular conductance, light-saturated photosynthetic rate, transpiration rate, and leaf water potential at pre-dawn (**Table 4**). Only leaf area was significantly different between the growth forms. Shrubs tended to be taller, with lower leaf areas, higher leaf carbon contents, and higher water use efficiencies. Rosette species tended to have larger, thicker, and tougher leaves, with lower leaf phosphorus and nitrogen contents and larger C/N ratios. Forb species were smaller and had larger SLA, thinner leaves rich in phosphorous and nitrogen, intermediate values of water use efficiency, and lower water potential values at noon (**Table 4**).

**Table 4.** Functional trait variation between growth forms identified in the Páramo el Verjón. Statistical parameters from the ANOVAs and Kruskal-Wallis test are shown in the last column. Plant functional traits that do not share a lowercase letter are statistically different from one another as determined by a post-hoc Tukey's and Wilcox test.

Functional Trait	Forbs	Rosettes	Shrubs	Statistical analysis and <i>p</i> -value (df = 2 for all)
Plant height	0.3 ± 0.1 <b>a</b>	0.6 ± 0.1 <b>a</b>	1.4 ± 0.1 <b>b</b>	Chi <sup>2</sup> = 19.6; <i>p</i> < 0.001
Seed mass	0.26 ± 0.14	0.41 ± 0.09	8.2 ± 5.5	Chi <sup>2</sup> = 2.9; <i>p</i> = 0.23
Leaf area	1726.1 ± 570.1 <b>a</b>	8745.7 ± 2081.8 <b>b</b>	794.5 ± 323.3 <b>c</b>	Chi <sup>2</sup> = 21.2; <i>p</i> < 0.001
SLA	11 ± 1.7 <b>a</b>	5.4 ± 1.1 <b>b</b>	6.45 ± 0.4 <b>b</b>	Chi <sup>2</sup> = 10.9; <i>p</i> < 0.05
LDMC	321.1 ± 44.0 <b>a</b>	324.4 ± 23.3 <b>a</b>	424.3 ± 15.4 <b>b</b>	F = 7.3; <i>p</i> < 0.05
Leaf thickness	0.25 ± 0.06 <b>a</b>	0.65 ± 0.1 <b>b</b>	0.36 ± 0.02 <b>a</b>	Chi <sup>2</sup> = 10.9; <i>p</i> < 0.05
Leaf toughness	1.35 ± 0.73 <b>a</b>	4.23 ± 0.87 <b>b</b>	1.24 ± 0.19 <b>a</b>	Chi <sup>2</sup> = 10.3; <i>p</i> < 0.05
Leaf water potential at pre-dawn	-0.29 ± 0.06	-0.31 ± 0.03	-0.29 ± 0.02	Chi <sup>2</sup> = 1.1; <i>p</i> = 0.57
Leaf water potential at noon	-1.6 ± 0.1 <b>a</b>	-1.1 ± 0.1 <b>b</b>	-1.5 ± 0.1 <b>ab</b>	Chi <sup>2</sup> = 9.4; <i>p</i> < 0.05
Light-saturated photosynthetic rate	8.4 ± 1.7	7.5 ± 1.4	6.7 ± 0.5	F = 0.9; <i>p</i> = 0.49
Leaf transpiration rate	0.005 ± 0.001	0.003 ± 0.0003	0.003 ± 0.0002	Chi <sup>2</sup> = 4.7; <i>p</i> = 0.09
Water use efficiency	0.6 ± 0.1 <b>ab</b>	0.5 ± 0.1 <b>a</b>	0.8 ± 0.04 <b>b</b>	Chi <sup>2</sup> = 7.6; <i>p</i> < 0.05
Leaf phosphorus content	1.5 ± 0.2 <b>a</b>	0.9 ± 0.1 <b>b</b>	1.1 ± 0.1 <b>a</b>	Chi <sup>2</sup> = 6.9; <i>p</i> < 0.05
Leaf carbon content	433.9 ± 7.9 <b>a</b>	444.7 ± 3.7 <b>a</b>	473.0 ± 4.2 <b>b</b>	F = 13.9; <i>p</i> < 0.001

Leaf nitrogen content	16.7 ± 1.3a	11.4 ± 0.6b	15.7 ± 0.7a	F = 6.8; p < 0.05
C/N ratio	27.6 ± 2.2a	40.3 ± 2.3b	32.8 ± 2.0a	Chi <sup>2</sup> = 10.7; p < 0.05
Cuticular conductance	6.5 ± 1.2	5.9 ± 1.3	7.6 ± 0.7	F = 0.9; p = 0.39

Values of functional originality ranged between 0.25 and 0.42, with shrubs having the lowest values of originality (**Figure 3 and Figure 4**) and rosettes presenting the highest values of originality (**Figure 3 and Figure 4; Table 3**). The species with the highest originality were two terrestrial bromeliads, *Greigia stenolepis* (AV = 0.42) and *Puya goudotiana* (AV = 0.40), followed by *Espeletia grandiflora* (AV = 0.39), *Paepalanthus alpinus* (AV = 0.38), and one forb, *Senecio formosoides* (AV = 0.38; **Table 3**).



**Figure 4.** Values of functional originality across plant functional types (a) and growth forms (b). Letters indicate statistical differences as determined by a post-hoc Tukey's test and Wilcox test. The horizontal line inside the box plot indicates the median; the ends of the box are the upper and lower quartiles, and the lines outside the box represent the highest and lowest values recorded for each trait.

## 1.4 DISCUSSION

### 1.4.1 Reconciling growth forms, plant functional types, and functionality of páramo species

There is substantial spatial variation in the physiognomy of páramo vegetation. However, similar growth forms, including graminoids, shrubs, forbs, bamboos, giant rosettes, cushion plants, and acaulescent rosettes, are found in communities along the elevation gradient (Cleef, 1978; Ramsay, 2001; Smith & Young, 1987). This consistency in growth forms across páramo systems has led to the informal use of growth form as a proxy for functional type (Azócar & Rada, 2006; Hedberg & Hedberg, 1979; Rada et al., 2019; Sklenář et al., 2016). In this study, we examined whether functional traits and ecological strategies corresponded with typical growth forms found in the páramo or whether there are functionally distinct groups within growth forms. We established that growth forms generally corresponded with PFT groups and thus could be suitable for grouping species with similar ecological and physiological traits, as was found for the Venezuelan páramos (Rada et al., 2019) and the tundra (Chapin et al., 1996). This is

consistent with the idea that growth forms are related to functional adaptations (Kattge et al., 2011; Reich et al., 2003; Westoby et al., 2002).

As with previous studies of functional traits in the páramos (Cárdenas-Arévalo & Vargas-Ríos, 2008; Rada et al., 2019), we established that rosettes and forbs comprised distinct functional groups and that shrubs belonged to multiple groups. Classifying species into PFTs based exclusively on growth form should be performed with caution, especially for shrubs. One limitation of our study is that we did not include grasses or cushion plants in our analysis. Including these groups would potentially increase the number of PFTs. However, in the Venezuelan páramos, all nine species of grasses studied shared similar water and temperature resistance strategies (Rada et al., 2019) and thus comprised only one additional group. Similarly, in Chingaza, another Colombian páramo, Cárdenas-Arévalo & Vargas-Ríos (2008) identified only three PFTs after analyzing 70 species with many growth forms, including rosettes, shrubs, forbs, grasses, and cushion plants.

The apparent split between ecological and functional strategies for páramo shrubs (Cárdenas-Arévalo & Vargas-Ríos, 2008; Rada et al., 2019) merits further study. Our data suggest that the differences among shrub groupings are primarily associated with shrub woodiness, rather than growth form (all prostrate shrubs are lumped together with the upright shrub in PFT3). Shrubs in PFT3 had small, tough, thick leaves and are considered sclerophyllous shrubs. Woodiness in this group could facilitate the maintenance of turgor during periods of water deficit (Rada et al., 2019), enabling tolerance to more negative water potential values. Shrubs associated with PFT1 had tender, somewhat larger, and thinner leaves. This clustering could be due to phylogenetic relatedness; however, *Ageratina gynoxoides*, a shrub in the Asteraceae, was part of PFT3, while the remaining Asteraceae shrubs were in PFT1. Similarly, shrubs from the Melastomataceae family were split between PFT groups (*Miconia summa* in PFT3 and the other three in PFT1).

While some of the resulting patterns among species may be driven by taxonomy-related traits, phylogenetic signals are clearly not the only factors influencing the observed groupings. For example, there are species from the Asteraceae in all three PFTs, including rosettes in PFT2, one forb species (*Senecio formosoides*) and most of the Asteraceae shrubs in PFT1, and one shrub in PFT3. Another reason for the divergent classification of shrubs among PFTs could be related to the type of biotic interactions they have, such as association with N-fixing bacteria or with specific Mycorrhizas. We do not currently have information on those associations, and this hypothesis would need to be tested.

Diurnal temperature variation in tropical alpine systems is a dominant factor driving species adaptive responses; in a single day, differences between absolute minimum and absolute maximum temperatures may exceed 20 to 30K (Leon-Garcia & Lasso, 2019; Rada et al., 2019; Sklenář et al., 2016), including freezing temperatures. Adaptation strategies for freezing temperatures tend to vary by growth form (Rada et al., 2019), with rosettes avoiding freezing, and forbs, cushion plants, and grasses tolerating freezing (Azócar et al., 1988; Squeo et al., 1991). However, shrubs are hard to place in a single strategy; some species avoid freezing (Squeo et al., 1991), while others tolerate freezing (Azócar, 2006). Likewise, species' tolerance to high temperatures appears to be associated with growth forms as well (Leon-Garcia & Lasso, 2019), with rosettes being more tolerant than other forms.

Water availability, influenced by daily changes in temperature, is another important environmental driver in the páramos (Rada et al., 2019). If temperatures drop below 0°C, water freezes and becomes unavailable, and when temperatures increase on clear days, evaporative demand increases (Ramirez et al., 2014). Therefore, water deficit can occur even in relatively mesic páramo communities, especially in the dry season. The adaptive strategies of páramo plants to water deficit also seem to be somewhat related to growth form (Rada et al., 2019). In the Colombian páramos studied here, and the more xeric Venezuelan páramos, midday water potentials of many shrub species can be quite low, suggesting a tolerance strategy. Furthermore, rosettes maintain higher (less negative) water potentials during the day, even in the dry season, which indicates that they rely on avoidance mechanisms (Rada et al., 2019). Many of these stem rosettes are known to store water in their stem pith, allowing them to sustain higher water potentials throughout the day (Goldstein et al., 1984b; Meinzer et al., 1985). In this study, forbs and shrubs that grouped together in PFT1 had midday leaf water potentials intermediate between those of rosettes in PFT2 and shrubs in PFT3. These species also had thinner and more tender leaves than species in the other PFT groups, suggesting that they would be more likely to lose turgor during periods of water deficit. Thus, most species at our study site exhibited traits consistent with an avoidance strategy, having relatively high (less negative) water potential values at noon. PFT3 shrubs were an exception, having tough leaves and the lowest midday water potentials, slightly higher WUEs, and higher LDMCs than species in other PFTs. Higher LDMC values are associated with a higher modulus of elasticity (Niinemets, 2001) and more rigid lignified cell walls (Niinemets & Kull, 1998), enabling the maintenance of turgor with lower leaf water potentials. High LDMC is also associated with higher cavitation resistance (Markesteijn et al., 2011) and is a typical trait associated with survival in dry conditions (Martínez-Garza et al., 2013).

Species in the PFT3 group may be more tolerant of water deficit than species in the other groups and thus may have a higher probability of surviving in drier conditions to come. Furthermore, PFT3 shrubs are predominantly dispersed by endozoochory and thus have greater dispersal ranges than herbs and aerial dispersal species (Tamme et al., 2014). This could make these species more successful as climate niches move (Tamme et al., 2014; Tovar et al., 2020). Shrubs dispersed by endozoochory in the high tropical alpine communities across the Andes also have higher thermal optima than species with other dispersal modes and could eventually outcompete cold-adapted species such as the barochorous herbaceous species under warming scenarios (Tovar et al., 2020). By contrast, forb and rosette species in the PFT1 and PFT2 groups may be less tolerant of drier conditions and less successful at migrating, since their seeds are dispersed mainly by wind, gravity, or water (Tovar et al., 2020).

#### 1.4.2 Functional strategies and originality in the páramos

The results of the CSR analysis (Pierce et al., 2017) and PFT classification support our hypothesis that the stressful conditions of the páramos limit the number of ecological strategies available and constrain trait originality for most species. The convergence of traits and the reduced number of strategies found in the páramos are consistent with the results of previous studies, which have shown that functional diversity in the highlands lags behind species richness, resulting in trait similarity and functional clustering for bees (Sydenham et al., 2015), woody plant species (Xu et al., 2017), and passerine birds (He et al., 2018). Two recent global analyses of the functional structure of avian assemblages across elevations and latitudes established that bird's assemblages in the tropical highlands are strongly functionally clustered and redundant (Jarzyna et al., 2019; Montaña-Centellas et al., 2020). In the páramos, we identified relatively low values of trait originality, suggesting high functional redundancy among plants. This may be due to

a combination of factors; on the one hand, under the harsher environmental conditions of the páramos, environmental filtering processes may be driving convergence in traits (Cornwell & Ackerly, 2009; Díaz et al., 1998; Xu et al., 2017). On the other hand, the rapid speciation that has occurred in the páramos (Madriñán et al., 2013) may have driven the diversification of many related species with similar characteristics, which would reduce the average trait originality as well.

With respect to traits, species are considered original if they possess rare trait values in comparison to other species in the community. Species with the most distinct functional traits could be supporting key functions, and their removal might result in a reduction of ecosystem functionality (Leitão et al., 2016; Mouillot et al., 2013). Here, we use those indices to identify potentially key species with unique set of traits. In the páramos, we found that values of functional originality ranged between 0.25 and 0.42, with shrubs presenting the lowest values of originality and rosettes the highest originality. In this community, rosettes are the most original species contributing the most to the trait diversity of the páramo community and should be a key group to include in conservation and restoration programs in the páramo (Mouillot et al., 2008; Pavoine et al., 2005).

#### 1.4.3 Future environmental changes and possible responses

Climatic projections suggest a  $3.0 \pm 1.5^\circ\text{C}$  temperature rise for the highlands of the Andes in the next century (Buytaert et al., 2011) and decreasing precipitation in Northern Colombia and Venezuela (Buytaert et al., 2011; Urrutia & Vuille, 2009). While most páramo plants can tolerate higher temperatures (Körner, 2003; Leon-Garcia & Lasso, 2019; Rada et al., 2008; Rada et al., 1992), responses to water deficit differ greatly among functional groups. The combination of lower precipitation and higher temperatures may increase air evaporative demand and reduce soil water content, exacerbating water stress in these regions. For the drier Venezuelan páramo, where many grasses and shrubs are relatively tolerant of water deficits, the shrub–herbaceous plant community is likely to be resilient (Llambí & Rada, 2019; Rada et al., 2019). However, the iconic giant rosettes, common to many of the páramos of the Northern Andes, could suffer in a drier climate given their low capacity to tolerate water deficit (Azócar et al., 2000). Giant rosettes had the highest trait originality scores in our study and are also known to serve as nurse plants, integral for maintaining páramo plant diversity (Mora et al., 2019). Additionally, the rosette-like arrangement of heavily pubescent leaves form a basket that intercepts rain and fog water. This water is slowly released to the soil, supporting the system-wide regulation and provision of water, one of the páramo's key ecosystem services. A páramo community that lacks rosettes (PFT2) would certainly be deficient in functionality.

Shrub species in the PFT3 group are most likely to withstand future drier conditions given their higher tolerance to water deficit and high LDMC values. However, the leaf water potentials measured in this group ( $-1.72 \pm 0.17$  MPa) were significantly higher than the lowest water potential reported by Pérez-Harguindeguy et al., (2016) in the worldwide database ( $-7$  MPa) or the lowest values reported for woody bamboos ( $-3.8$  Mpa) and grasses ( $-4.0$  MPa) from the Venezuelan páramos (Ely et al., 2019; Rada et al., 2019). Thus, even the more drought-tolerant páramo species studied here may be living on the edge of their safety margin. More physiological studies on the vulnerability of páramo plants to water deficit are urgently needed, given that water deficit is likely to be a key environmental driver of adaptation in the páramos as the effects of climate change are felt. The PFT classification developed here can also guide drought experiments to evaluate PFT responses to water deficit and vulnerability to future changes.

#### 1.4.4 Species traits and ecosystem services

In addition to supplying major South America cities with water (Carrillo-Rojas et al., 2016), the páramos are important carbon reservoirs in tropical mountains (Hofstede, 1999). There is a growing consensus that the effects of species on ecosystem function are partially mediated by the traits of their component species (Chapin et al., 2000; Díaz & Cabido, 2001; Kazakou et al., 2006). Assessing the functional traits of species can help identify and quantify links between páramo species and ecosystem functions (Hubbell, 2005). For example, LDMC and C:N ratios appear to be crucial traits that influence litter quality and biogeochemical cycling (Kazakou et al., 2006), since litter decomposition is related to its physical and chemical properties (Cornelissen et al., 1999; Pérez-Harguindeguy et al., 2000; Wardle et al., 2002). Species belonging to PFT1 with tender leaves, high N and P content, and low LDMC are probably fast-growing species whose dead leaves will decompose rapidly, increasing the turnover of carbon and nutrients. Species in PFT3 with high LDMC and species in PFT2 with thicker and tougher leaves and higher C:N ratios decompose slowly, decreasing the availability of carbon and nutrients in the system. These are two important processes affected by traits that ultimately control how páramos function as carbon reservoirs.

To evaluate species' putative roles in sequestering carbon, we would need several parameters related to carboxylation efficiency (e.g.,  $V_{\text{cmax}}$ ), which are unknown for plants growing at this high elevation. Here, we only measured the light-saturated photosynthetic rate and the  $C_i/C_a$  ratio. Neither of these parameters differed among growth forms or PFTs, supporting the optimization of photosynthetic traits hypothesis (Wang et al., 2016), which suggests that incorporating the páramo's taxa into global vegetation models (Woodward & Cramer, 1996) could be simplified despite the immense species diversity of the páramo.

Our results suggest that the páramo, despite being highly diverse, has few functional types and strategies. At this high elevation, with the stressful and unique abiotic conditions of the páramo, environmental filtering might be an important force driving trait convergence (Gastauer et al., 2020). Biotic interactions could also be an important driving force in the convergence of traits (Kraft et al., 2015), and further studies should explore whether these interactions drive convergence in the páramo. Moreover, careful examinations of shrub species and other growth forms such as grasses, sedges, and cushion plants are necessary for fully understanding the diversity of functional strategies in the páramo.

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## REFERENCES

- Azócar, A., & Rada, F. (2006). *Ecofisiología de Plantas de Páramo*. Centro Editorial Litorama.
- Azócar, A., Rada, F., & García-Núñez, C. (2000). Aspectos ecofisiológicos para la conservación de ecosistemas tropicales contrastantes. *Boletín de La Sociedad Botánica de México*, 65, 89–94.
- Azócar, A., Rada, F., & Goldstein, G. (1988). Freezing tolerance in *Draba chionophila*, a miniature caulescent rosette species. *Oecologia*, 75, 156–160. <https://doi.org/10.1007/BF00378830>
- Azócar, J. (2006). *Relación entre anatomía foliar, forma de vida y mecanismos de resistencia a temperaturas congelantes en diferentes especies en el Páramo de Piedras Blancas*. (p. 74). <http://www.saber.ula.ve/handle/123456789/40113>
- Bartlett, M. K., Klein, T., Jansen, S., Choat, B., & Sack, L. (2016). The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *Proceedings of the National Academy of Sciences*, 113(46), 13098–13103. <https://doi.org/10.1073/pnas.1604088113>
- Bhaskar, R., & Ackerly, D. D. (2006). Ecological relevance of minimum seasonal water potentials. *Physiologia Plantarum*, 127(3), 353–359. <https://doi.org/10.1111/j.1399-3054.2006.00718.x>
- Buytaert, W., Cuesta, F., & Tobon, C. (2011). Potential Impacts of Climate Change on the Environmental Services of Humid Tropical Alpine Regions. *Global Ecology and Biogeography*, 20, 19–33. <https://doi.org/10.1111/j.1466-8238.2010.00585.x>
- Buytaert, W., Deckers, J., & Wyseure, G. (2006). Description and classification of nonallophanic Andosols in south Ecuadorian alpine grasslands (páramo). *Geomorphology*, 73(3–4), 207–221. <https://doi.org/10.1016/j.geomorph.2005.06.012>
- Cárdenas-Arévalo, G., & Vargas-Ríos, O. (2008). Rasgos de historia de vida de especies en una comunidad vegetal alterada en un páramo húmedo (Parque Natural Chingaza). *Caldasia*, 30, 245–264.
- Carrillo-Rojas, G., Silva, B., Córdova, M., Célleri, R., & Bendix, J. (2016). Dynamic Mapping of Evapotranspiration Using an Energy Balance-Based Model over an Andean Páramo Catchment of Southern Ecuador. *Remote Sensing*, 8(2), 160. <https://doi.org/10.3390/rs8020160>
- Chapin, F. S., Bret-Harte, M. S., Hobbie, S. E., & Zhong, H. (1996). Plant functional types as predictors of transient responses of arctic vegetation to global change. *Journal of Vegetation Science*, 7(3), 347–358. <https://doi.org/10.2307/3236278>
- Chapin, F. S., Zavaleta, E., Eviner, V., Naylor, R., Vitousek, P., Reynolds, H., Hooper, D., Lavorel, S., Sala, O., Hobbie, S., Mack, M., & Díaz, S. (2000). Consequences of changing biodiversity. *Nature*, 405, 234–242. <https://doi.org/10.1038/35012241>
- Cleef, A. M. (1978). Characteristics of neotropical páramo vegetation and its subantarctic relations. *Erdwiss. Forsch.*, 11, 365–390.
- Cornelissen, J. H. C., Lavorel, S., Garnier, E., Az, S., Buchmann, N., Gurvich, D. E., Reich, P. B., Steege, H. ter, Morgan, H. D., Heijden, M. G. A. van der, Pausas, J. G., & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), 335–380. <https://doi.org/10.1071/BT02124>
- Cornelissen, J. H. C., Perez-Harguindeguy, N., Díaz, S., Grime, J. P., Marzano, B., Cabido, M., Vendramini, F., & Cerabolini, B. (1999). Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytologist*, 143(1), 191–200. <https://doi.org/10.1046/j.1469-8137.1999.00430.x>

- Cornwell, W. K., & Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs*, 79(1), 109–126. <https://doi.org/10.1890/07-1134.1>
- Cousins, S. H. (1991). Species diversity measurement: Choosing the right index. *Trends in Ecology & Evolution*, 6(6), 190–192. [https://doi.org/10.1016/0169-5347\(91\)90212-G](https://doi.org/10.1016/0169-5347(91)90212-G)
- de Bello, F., Lavorel, S., Díaz, S., Harrington, R., Cornelissen, J. H. C., Bardgett, R. D., Berg, M. P., Cipriotti, P., Feld, C. K., Hering, D., Martins da Silva, P., Potts, S. G., Sandin, L., Sousa, J. P., Storkey, J., Wardle, D. A., & Harrison, P. A. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, 19(10), 2873–2893. <https://doi.org/10.1007/s10531-010-9850-9>
- Díaz, H., & Bradley, R. (1997). Temperature variations during the last century at High elevation sites. *Climatic Change*, 36, 253–279. <https://doi.org/10.1023/A:1005335731187>
- Díaz, S., & Cabido, M. (2001). Vive la différence: Plant functional diversity matters to ecosystem processes. *Trends in Ecology & Evolution*, 16(11), 646–655. [https://doi.org/10.1016/S0169-5347\(01\)02283-2](https://doi.org/10.1016/S0169-5347(01)02283-2)
- Díaz, S., Cabido, M., & Casanoves, F. (1998). Plant functional traits and environmental filters at a regional scale. *Journal of Vegetation Science*, 9(1), 113–122. <https://doi.org/10.2307/3237229>
- Díaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., Montserrat-Martí, G., Grime, J. P., Zarrinkamar, F., Asri, Y., Band, S. R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M. C., Shirvany, F. A., ... Zak, M. R. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, 15(3), 295–304. <https://doi.org/10.1111/j.1654-1103.2004.tb02266.x>
- Dormann, S. J. W. (2002). Climate change in the Arctic: Using plant functional types in a meta-analysis of field experiments. *Functional Ecology*, 16(1), 4–17.
- Ely, F., Rada, F., Fermin, G., & Clark, L. G. (2019). Ecophysiology and genetic diversity in species of the bamboo *Chusquea* in the high Andes, Venezuela. *Plant Ecology & Diversity*, 12(6), 555–572. <https://doi.org/10.1080/17550874.2019.1673847>
- Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation*, 61(1), 1–10. [https://doi.org/10.1016/0006-3207\(92\)91201-3](https://doi.org/10.1016/0006-3207(92)91201-3)
- Flantua, S. G. A., O’Dea, A., Onstein, R. E., & Hooghiemstra, H. (2019). The flickering connectivity system of the north Andean páramos. *Journal of Biogeography*, 46(8), 1808–1825. <https://doi.org/10.1101/569681>
- Gastauer, M., Thiele, J., Porembski, S., & Neri, A. V. (2020). How do altitude and soil properties influence the taxonomic and phylogenetic structure and diversity of Brazilian páramo vegetation? *Journal of Mountain Science*, 17(5), 1045–1057. <https://doi.org/10.1007/s11629-019-5403-1>
- Goldstein, G., Meinzer, F., & Monasterio, M. (1984). The role of capacitance in the water balance of Andean giant rosette species. *Plant, Cell and Environment*, 7(3), 179–186. <https://doi.org/10.1111/1365-3040.ep11614612>
- Grime, J. P. (1977). Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *The American Naturalist*, 111(982), 1169–1194. JSTOR.
- Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, 8(4), 157–178. <https://doi.org/10.1016/j.ppees.2007.01.001>

- He, X., Luo, K., Brown, C., & Lin, L. (2018). A taxonomic, functional, and phylogenetic perspective on the community assembly of passerine birds along an elevational gradient in southwest China. *Ecology and Evolution*, 8(5), 2712–2720. <https://doi.org/10.1002/ece3.3910>
- Hedberg, I., & Hedberg, O. (1979). Tropical-Alpine Life-Forms of Vascular Plants. *Oikos*, 33(2), 297–297. <https://doi.org/10.2307/3544006>
- Hofstede, R. (1999). El páramo como espacio para la fijación de carbono atmosférico. In G. Medina, P. Mena, & C. Josse (Eds.), *El páramo como espacio de mitigación de carbono atmosférico* (1st ed., pp. 57–57). Abya Yala.
- Hubbell, S. (2005). Neutral theory in community ecology and the hypothesis of functional equivalence. *Functional Ecology*, 19, 166–172. <https://doi.org/10.1111/j.0269-8463.2005.00965.x>
- Isaac, N. J. B., Turvey, S. T., Collen, B., Waterman, C., & Baillie, J. E. M. (2007). Mammals on the EDGE: Conservation Priorities Based on Threat and Phylogeny. *PLoS ONE*, 2(3), e296. <https://doi.org/10.1371/journal.pone.0000296>
- Jarzyna, M. A., Quintero, I., & Jetz, W. (2019). *Functional community assembly and turnover along elevation and latitude*. *Ecology*. <https://doi.org/10.1101/706523>
- Kassambara, A. (2017). *Practical Guide to Cluster Analysis in R: Unsupervised Machine Learning*. STHDA. <https://books.google.com.co/books?id=plEyDwAAQBAJ>
- Kattge, J., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Bönsch, G., Garnier, E., Westoby, M., Reich, P. B., Wright, I. J., Cornelissen, J. H. C., Violle, C., Harrison, S. P., Van Bodegom, P. M., Reichstein, M., Enquist, B. J., Soudzilovskaia, N. A., Ackerly, D. D., Anand, M., ... Wirth, C. (2011). TRY – a global database of plant traits. *Global Change Biology*, 17(9), 2905–2935. <https://doi.org/10.1111/j.1365-2486.2011.02451.x>
- Kazakou, E., Vile, D., Shipley, B., Gallet, C., & Garnier, E. (2006). Co-variations in litter decomposition, leaf traits and plant growth in species from a Mediterranean old-field succession. *Functional Ecology*, 20, 21–30. <https://doi.org/10.1111/j.1365-2435.2006.01080.x>
- Kitajima, K., & Poorter, L. (2010). Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist*, 186(3), 708–721. <https://doi.org/10.1111/j.1469-8137.2010.03212.x>
- Kondratyeva, A., Grandcolas, P., & Pavoine, S. (2019). Reconciling the concepts and measures of diversity, rarity and originality in ecology and evolution. *Biological Reviews*, 94(4), 1317–1337. <https://doi.org/10.1111/brv.12504>
- Kondratyeva, A., Knapp, S., Durka, W., Kühn, I., Vallet, J., Machon, N., Martin, G., Motard, E., Grandcolas, P., & Pavoine, S. (2020). Urbanization Effects on Biodiversity Revealed by a Two-Scale Analysis of Species Functional Uniqueness vs. Redundancy. *Frontiers in Ecology and Evolution*, 8, 73. <https://doi.org/10.3389/fevo.2020.00073>
- Körner, C. (2003). Alpine Plant Life: Functional Plant Ecology Of High Mountain Ecosystems. In *Alpine Plant Life, 2nd Edn*. <https://doi.org/10.1007/978-3-642-18970-8>
- Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., & Levine, J. M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology*, 29(5), 592–599. <https://doi.org/10.1111/1365-2435.12345>
- Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: Revisiting the Holy Grail. *Functional Ecology*, 16(5), 545–556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>
- Leishman, M., Wright, I. J., Moles, A., & Westoby, M. (2000). The evolutionary ecology of seed size. Seed: Ecology of regeneration in plant communities. *Univ. Southampton, UK*, 416, 31–59.
- Leitão, R. P., Zuanon, J., Villéger, S., Williams, S. E., Baraloto, C., Fortunel, C., Mendonça, F. P., & Moullot, D. (2016). Rare species contribute disproportionately to the functional structure of

- species assemblages. *Proceedings of the Royal Society B: Biological Sciences*, 283(1828), 20160084. <https://doi.org/10.1098/rspb.2016.0084>
- Leon-Garcia, I. V., & Lasso, E. (2019). High heat tolerance in plants from the Andean highlands: Implications for páramos in a warmer world. *PLoS ONE*, 14(11), 1–14. <https://doi.org/10.1371/journal.pone.0224218>
- Levin, S. A., Muller-Landau, H. C., Nathan, R., & Chave, J. (2003). The Ecology and Evolution of Seed Dispersal: A Theoretical Perspective. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), 575–604. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132428>
- Liu, X., & Chen, B. (2000). Climatic Warming in the Tibetan Plateau during recent decades. *International Journal of Climatology*, 20, 1729–1742. <https://doi.org/10.1002/1097-0088>
- Llambí, L., & Rada, F. (2019). Ecological research in the tropical alpine ecosystems of the Venezuelan páramo: Past, present and future. *Plant Ecology & Diversity*, 1–20. <https://doi.org/10.1080/17550874.2019.1680762>
- Luteyn, J. (1999). Páramos: A checklist of plant diversity, geographical distribution, and botanical literature. Costa Rica and Panama. *Memoirs of the New York Botanical Garden*, 84, 138–141.
- Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, 4(192). <https://doi.org/10.3389/fgene.2013.00192>
- Markesteyn, L., Poorter, L., Bongers, F., Paz, H., & Sack, L. (2011). Hydraulics and life history of tropical dry forest tree species: Coordination of species' drought and shade tolerance. *New Phytologist*, 191(2), 480–495. <https://doi.org/10.1111/j.1469-8137.2011.03708.x>
- Martínez-Garza, C., Bongers, F., & Poorter, L. (2013). Are functional traits good predictors of species performance in restoration plantings in tropical abandoned pastures? *Forest Ecology and Management*, 303, 35–45. <https://doi.org/10.1016/j.foreco.2013.03.046>
- Matos, I. S., Eller, C. B., Oliveras, I., Mantuano, D., & Rosado, B. H. P. (2020). Three eco-physiological strategies of response to drought maintain the form and function of a tropical montane grassland. *Journal of Ecology*, 1365–2745.13481. <https://doi.org/10.1111/1365-2745.13481>
- Meinzer, F. C., Goldstein, G. H., & Rundel, P. W. (1985). Morphological changes along an altitude gradient and their consequences for an andean giant rosette plant. *Oecologia*, 65(2), 278–283. <https://doi.org/10.1007/BF00379230>
- Montaño-Centellas, F. A., McCain, C., & Loiselle, B. A. (2020). Using functional and phylogenetic diversity to infer avian community assembly along elevational gradients. *Global Ecology and Biogeography*, 29(2), 232–245. <https://doi.org/10.1111/geb.13021>
- Mora, M. A., Llambí, L. D., & Ramírez, L. (2019). Giant stem rosettes have strong facilitation effects on alpine plant communities in the tropical Andes. *Plant Ecology & Diversity*, 12(6), 593–606. <https://doi.org/10.1080/17550874.2018.1507055>
- Mouillot, D., Bellwood, D. R., Baraloto, C., Chave, J., Galzin, R., Harmelin-Vivien, M., Kulbicki, M., Lavergne, S., Lavorel, S., Mouquet, N., Paine, C. E. T., Renaud, J., & Thuiller, W. (2013). Rare Species Support Vulnerable Functions in High-Diversity Ecosystems. *PLoS Biology*, 11(5). <https://doi.org/10.1371/journal.pbio.1001569>
- Mouillot, D., Culioli, J. M., Pelletier, D., & Tomasini, J. A. (2008). Do we protect biological originality in protected areas? A new index and an application to the Bonifacio Strait Natural Reserve. *Biological Conservation*, 141(6), 1569–1580. <https://doi.org/10.1016/j.biocon.2008.04.002>
- Niinemets, Ü. (2001). Global-Scale Climatic Controls of Leaf Dry Mass Per Area, Density, and Thickness in Trees and Shrubs. *Ecology*, 82(2), 453–469. [https://doi.org/10.1890/0012-9658\(2001\)082\[0453:GSCCOL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0453:GSCCOL]2.0.CO;2)

- Niinemets, Ü., & Kull, O. (1998). Stoichiometry of foliar carbon constituents varies along light gradients in temperate woody canopies: Implications for foliage morphological plasticity. *Tree Physiology*, 18(7), 467–479. <https://doi.org/10.1093/treephys/18.7.467>
- Ning, L., & Bradley, R. (2014). Winter Precipitation Variability and Corresponding Teleconnections over the Northeastern United States. *Journal of Geophysical Research: Atmospheres*, 119. <https://doi.org/10.1002/2014JD021591>
- Pagès, J. (2014). *Multiple Factor Analysis by Example Using R*. Chapman and Hall/CRC.
- Palacio, F., Apodaca, M., & Crisci, J. (2020). *Análisis Multivariado para Datos Biológicos Teoría y su aplicación utilizando el lenguaje R*.
- Pavoine, S. (2020). adiv: An r package to analyse biodiversity in ecology. *Methods in Ecology and Evolution*, 11(9), 1106–1112. <https://doi.org/10.1111/2041-210X.13430>
- Pavoine, S., Bonsall, M. B., Dupaix, A., Jacob, U., & Ricotta, C. (2017). From phylogenetic to functional originality: Guide through indices and new developments. *Ecological Indicators*, 82, 196–205. <https://doi.org/10.1016/j.ecolind.2017.06.056>
- Pavoine, S., Ollier, S., & Dufour, A.-B. (2005). Is the originality of a species measurable? *Ecology Letters*, 8(6), 579–586. <https://doi.org/10.1111/j.1461-0248.2005.00752.x>
- Pérez-Harguindeguy, N., Díaz, S., Cornelissen, J. H. C., Vendramini, F., Cabido, M., & Castellanos, A. (2000). Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. *Plant and Soil*, 218(1), 21–30. <https://doi.org/10.1023/A:1014981715532>
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., & Gurvich, D. E. (2016). Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 64(8), 715–716.
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., De Vos, A. C., ... Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), 167–234. <https://doi.org/10.1071/BT12225>
- Pierce, S., Negreiros, D., Cerabolini, B. E. L., Kattge, J., Díaz, S., Kleyer, M., Shipley, B., Wright, S. J., Soudzilovskaia, N. A., Onipchenko, V. G., van Bodegom, P. M., Frenette-Dussault, C., Weiher, E., Pinho, B. X., Cornelissen, J. H. C., Grime, J. P., Thompson, K., Hunt, R., Wilson, P. J., ... Tampucci, D. (2017). A global method for calculating plant CSR ecological strategies applied across biomes world-wide. *Functional Ecology*, 31(2), 444–457. <https://doi.org/10.1111/1365-2435.12722>
- Powers, J. S., & Tiffin, P. (2010). Plant functional type classifications in tropical dry forests in Costa Rica: Leaf habit versus taxonomic approaches. *Functional Ecology*, 24(4), 927–936. <https://doi.org/10.1111/j.1365-2435.2010.01701.x>
- Rada, F. (2016). Functional Diversity in Tropical High Elevation Giant Rosettes. In G. Goldstein & L. S. Santiago (Eds.), *Tropical Tree Physiology: Adaptations and Responses in a Changing Environment* (pp. 181–202). Springer International Publishing. [https://doi.org/10.1007/978-3-319-27422-5\\_8](https://doi.org/10.1007/978-3-319-27422-5_8)
- Rada, F., Azócar, A., & García-Núñez, C. (2019). Plant functional diversity in tropical Andean páramos. *Plant Ecology & Diversity*, 1–15. <https://doi.org/10.1080/17550874.2019.1674396>
- Rada, F., Briceño, B., & Azócar, A. (2008). How do two *Lupinus* species respond to temperature along an altitudinal gradient in the Venezuelan Andes? *Revista Chilena De Historia Natural*, 81(3). <https://doi.org/10.4067/S0716-078X2008000300003>

- Rada, F., Goldstein, G., Azócar, A., & Meinzer, F. (1985). Freezing avoidance in Andean giant rosette plants. *Plant, Cell & Environment*, 8(7), 501–507. <https://doi.org/10.1111/j.1365-3040.1985.tb01685.x>
- Rada, F., Gonzalez, J., Azócar, A., Briceño, B., & Jaimez, R. (1992). Net photosynthesis-leaf temperature relations in plant species with height along an altitudinal gradient. *Acta Oecologica*, 13(5), 535–542.
- Ramirez, L., Rada, F., & Llambí, L. (2014). Linking patterns and processes through ecosystem engineering: Effects of shrubs on microhabitat and water status of associated plants in the high tropical Andes. *Plant Ecology*, 216(2), 213–225. <https://doi.org/10.1007/s11258-014-0429-5>
- Ramsay, P. M. (Ed.). (2001). Diurnal temperature variation in the major growth forms of an Ecuadorian páramo plant community. In *The ecology of Volcán Chiles: High-altitude ecosystems on the Ecuador-Colombia border* (pp. 101–112). Pebble & Shell.
- Ramsay, P. M., Kent, M., & Duckworth, J. (2000). Plant functional types: An alternative to taxonomic plant community description in biogeography? *Progress in Physical Geography*, 24, 515–542. <https://doi.org/10.1177/030913330002400403>
- Rangwala, I., Sinsky, E., & Miller, J. (2013). Amplified warming projections for high altitude regions of the Northern Hemisphere mid-latitudes from CMIP5 models. *Environmental Research Letters*, 8(2), 24–40. <https://doi.org/10.1088/1748-9326/8/2/024040>
- Reich, P. B., Wright, I. J., Cavender-bares, J., Craine, J. M., Oleksyn, J., Westoby, M., Walters, M. B., Cavender-Bares, J., & Walters, M. B. (2003). The Evolution of Plant Functional Variation: Traits, Spectra, and Strategies. *Source: International Journal of Plant Sciences Int. J. Plant Sci*, 1643(164), 143–164. <https://doi.org/10.1086/374368>
- Reich, P. B., Wright, I. J., & Lusk, C. H. (2007). Predicting Leaf Physiology from Simple Plant and Climate Attributes: A Global GLOPNET Analysis. *Ecological Applications*, 17(7), 1982–1988.
- Rosado, B. H. P., & de Mattos, E. A. (2017). On the relative importance of CSR ecological strategies and integrative traits to explain species dominance at local scales. *Functional Ecology*, 31(10), 1969–1974. <https://doi.org/10.1111/1365-2435.12894>
- Sarmiento, G. (1986). Ecologically crucial features of climate in high tropical mountains. In F. Vuilleumier & M. Monasterio (Eds.), *High Altitude Tropical Biogeography* (pp. 11–45). Oxford University Press.
- Scoffoni, C., Albuquerque, C., Cochard, H., Buckley, T. N., Fletcher, L. R., Caringella, M. A., Bartlett, M., Brodersen, C. R., Jansen, S., McElrone, A. J., & Sack, L. (2018). The Causes of Leaf Hydraulic Vulnerability and Its Influence on Gas Exchange in *Arabidopsis thaliana*. *Plant Physiology*, 178(4), 1584 LP – 1601. <https://doi.org/10.1104/pp.18.00743>
- Seelmann, L., Auer, A., Hoffmann, D., & Schausberger, P. (2007). Leaf pubescence mediates intraguild predation between predatory mites. *Oikos*, 116(5), 807–817. <https://doi.org/10.1111/j.2007.0030-1299.15895.x>
- Sklenář, P., Kučerová, A., Macková, J., & Romoleroux, K. (2016). Temperature Microclimates of Plants in a Tropical Alpine Environment: How Much does Growth Form Matter? *Arctic, Antarctic, and Alpine Research*, 48(1), 61–78. <https://doi.org/10.1657/AAAR0014-084>
- Sklenář, P., & Ramsay, P. M. (2001). Diversity of zonal páramo plant communities in Ecuador. *Diversity and Distributions*, 7, 113–124. <https://doi.org/10.1046/j.1472-4642.2001.00101.x>
- Smith, A. P., & Young, T. P. (1987). Tropical Alpine Plant Ecology. *Annual Review of Ecology and Systematics*, 18(1), 137–158. <https://doi.org/10.1146/annurev.es.18.110187.001033>
- Squeo, F. A., Rada, F., Azócar, A., & Goldstein, G. (1991). Freezing Tolerance and Avoidance in High Tropical Andean Plants: Is It Equally Represented in Species with Different Plant Height? *Oecologia*, 86(3), 378–382.

- Sydenham, M. A. K., Moe, S. R., Totland, Ø., & Eldegard, K. (2015). Does multi-level environmental filtering determine the functional and phylogenetic composition of wild bee species assemblages? *Ecography*, *38*(2), 140–153. <https://doi.org/10.1111/ecog.00938>
- Tamme, R., Götzenberger, L., Zobel, M., Bullock, J. M., Hooftman, D. A. P., Kaasik, A., & Pärtel, M. (2014). Predicting species' maximum dispersal distances from simple plant traits. *Ecology*, *95*(2), 505–513. <https://doi.org/10.1890/13-1000.1>
- Thomson, F. J., Moles, A. T., Auld, T. D., & Kingsford, R. T. (2011). Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology*, *99*(6), 1299–1307. <https://doi.org/10.1111/j.1365-2745.2011.01867.x>
- Tovar, C., Melcher, I., Kusumoto, B., Cuesta, F., Cleef, A., Meneses, R. I., Halloy, S., Llambí, L. D., Beck, S., Muriel, P., Jaramillo, R., Jácome, J., & Carilla, J. (2020). Plant dispersal strategies of high tropical alpine communities across the Andes. *Journal of Ecology*, *108*(5), 1910–1922. <https://doi.org/10.1111/1365-2745.13416>
- Urrutia, R., & Vuille, M. (2009). Climate Change Projections for the Tropical Andes Using a Regional Climate Change Model: Temperature and Precipitation Simulations for the 21st Century. *Journal of Geophysical Research*, *114*. <https://doi.org/10.1029/2008JD011021>
- Wang, H., Prentice, I. C., Davis, T. W., Keenan, T. F., Wright, I. J., & Peng, C. (2016). Photosynthetic responses to altitude: An explanation based on optimality principles. *New Phytologist*, *213*(3). <https://doi.org/10.1111/nph.14332>
- Wang, X., Chen, H., Shan, Z., Hao, Q., Zhang, C., Yang, Z., Zhang, X., Yuan, S., Qiu, D., Chen, S., Jiao, Y., & Zhou, X. (2015). Herbivore defense responses and associated herbivore defense mechanism as revealed by comparing a resistant wild soybean with a susceptible cultivar. *Crop Journal*, *3*(6), 451–467. <https://doi.org/10.1016/j.cj.2015.07.001>
- Wardle, D. A., Bonner, K. I., & Barker, G. M. (2002). Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. *Functional Ecology*, *16*(5), 585–595. <https://doi.org/10.1046/j.1365-2435.2002.00659.x>
- Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., & Wright, I. J. (2002). Plant Ecological Strategies: Some Leading Dimensions of Variation Between Species. *Annual Review of Ecology and Systematics*, *33*(1), 125–159. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>
- Woodward, F. I., & Cramer, W. (1996). Plant functional types and climatic change: Introduction. *Journal of Vegetation Science*, *7*(3), 306–308. <https://doi.org/10.1111/j.1654-1103.1996.tb00489.x>
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., ... Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*, 821–821.
- Xu, J., Chen, Y., Zhang, L., Chai, Y., Wang, M., Guo, Y., Li, T., & Yue, M. (2017). Using phylogeny and functional traits for assessing community assembly along environmental gradients: A deterministic process driven by elevation. *Ecology and Evolution*, *7*(14), 5056–5069. <https://doi.org/10.1002/ece3.3068>
- Zimmer, A., Meneses, R. I., Rabatel, A., Soruco, A., Dangles, O., & Anthelme, F. (2018). Time lag between glacial retreat and upward migration alters tropical alpine communities. *Special Issue on Alpine and Arctic Plant Communities: A Worldwide Perspective*, *30*, 89–102. <https://doi.org/10.1016/j.ppees.2017.05.003>

## SUPPORTING INFORMATION

**Table S1.** Contribution of the analyzed traits to the first ten dimensions of the factor analysis of mixed data (FAMD). At the end of the table, we show the eigenvalue, the percentage of the total variance explained by each dimension and the cumulative percentage of variance explained

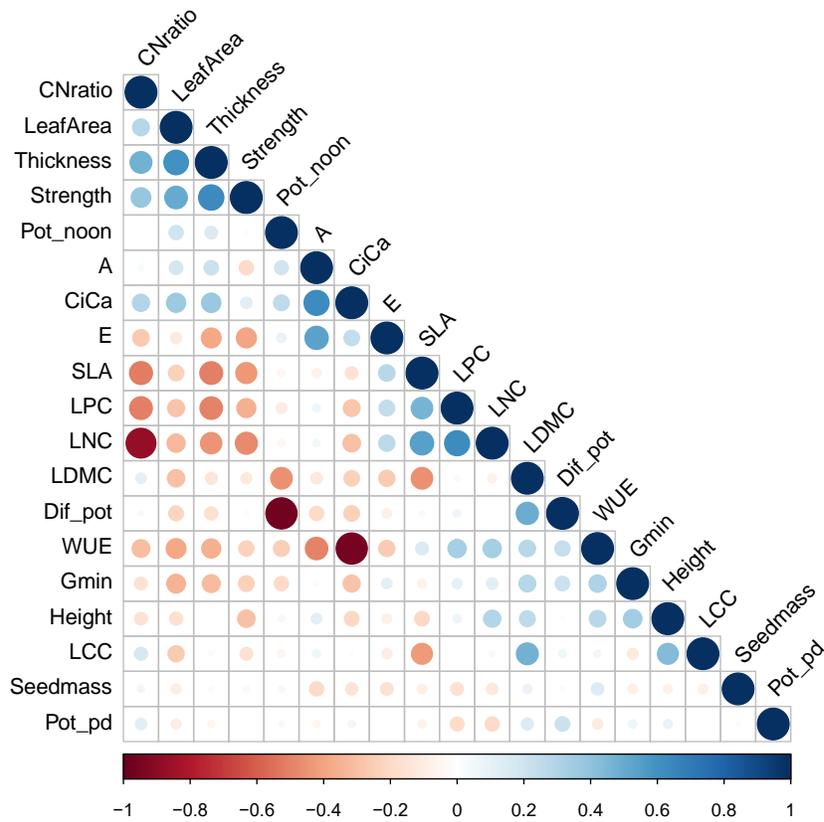
Variables	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Dim. 5	Dim. 6	Dim. 7	Dim. 8	Dim. 9	Dim. 10
Leaf thickness	13.51	0.01	1.35	0.70	1.51	2.07	0.14	2.61	3.60	1.50
Leaf nitrogen content (LNC)	13.09	0.36	0.13	2.83	3.74	5.01	2.11	1.28	0.04	0.34
Leaf toughness	12.02	0.16	5.40	0.05	2.95	1.39	3.65	0.15	0.13	0.10
Leaf C:N	10.91	1.23	0.23	1.11	6.89	2.66	2.08	0.004	0.07	3.42
Leaf phosphorus content (LPC)	9.72	0.42	0.12	0.97	2.37	2.34	1.55	5.54	1.42	6.23
Leaf area	9.29	3.42	0.13	0.78	4.38	3.99	0.15	3.11	0.08	0.30
Specific Leaf Area (SLA)	7.90	7.44	7.93	0.68	0.42	0.01	0.60	1.26	2.60	0.01
Spinescence of leaves	5.718	1.38	7.19	0.38	3.40	3.69	10.70	1.59	0.01	4.50
Water use efficiency (WUE)	5.10	6.06	1.23	0.39	4.80	3.74	10.10	0.01	0.09	0.73
Seed dispersal syndrome	4.55	16.75	18.15	35.60	24.21	21.00	23.78	46.91	19.15	25.65
Leaf transpiration rate (E)	2.66	8.45	3.29	0.66	1.76	1.81	9.83	1.10	5.05	3.11
Cuticular conductance ( $g_{min}$ )	2.38	1.90	0.29	16.08	0.01	0.46	0.42	19.58	7.25	5.32
Plant height	1.34	4.68	12.77	2.04	3.18	0.97	0.01	3.61	15.47	1.39
Pubescence of leaves	0.97	6.01	5.63	21.80	24.44	5.10	4.95	1.48	3.58	1.53
Leaf water potential at noon ( $\Psi_{noon}$ )	0.43	6.75	4.48	2.16	9.72	3.83	5.88	2.93	0.13	14.98
Leaf dry matter content (LDMC)	0.23	17.54	1.39	4.21	1.20	0.28	1.02	0.54	4.15	0.21
Light saturated photosynthetic rate ( $A_{max}$ )	0.06	6.84	17.43	0.08	1.22	0.03	5.95	0.19	0.01	5.87
Seed mass	0.05	0.90	1.18	2.36	0.42	2.91	15.41	5.87	28.35	11.16
Leaf carbon content (LCC)	0.05	9.01	11.65	5.62	2.09	0.13	1.03	2.23	0.39	12.74
Leaf water potential at pre-dawn ( $\Psi_{pre-dawn}$ )	0.04	0.69	0	1.51	1.27	38.58	0.63	0.002	8.42	0.91
Eigenvalue	4.87	3.38	2.43	1.86	1.67	1.63	1.45	1.27	1.01	0.88
Percentage of total variance explained	19.47	13.54	9.71	7.44	6.68	6.50	5.79	5.06	4.03	3.54
Cumulative percentage of variance explained	19.47	33.01	42.72	50.16	56.84	63.34	69.13	74.2	78.22	81.76

**Table S2.** Functional traits variation between the three PFTs identified in the páramo el Verjón. Statistical parameters from the ANOVAS and Kruskal-Wallis test are shown in the last column. Plant functional traits that do not share a lowercase letter are statistically different from one another as determined by post-hoc Tukey's and Wilcox test.

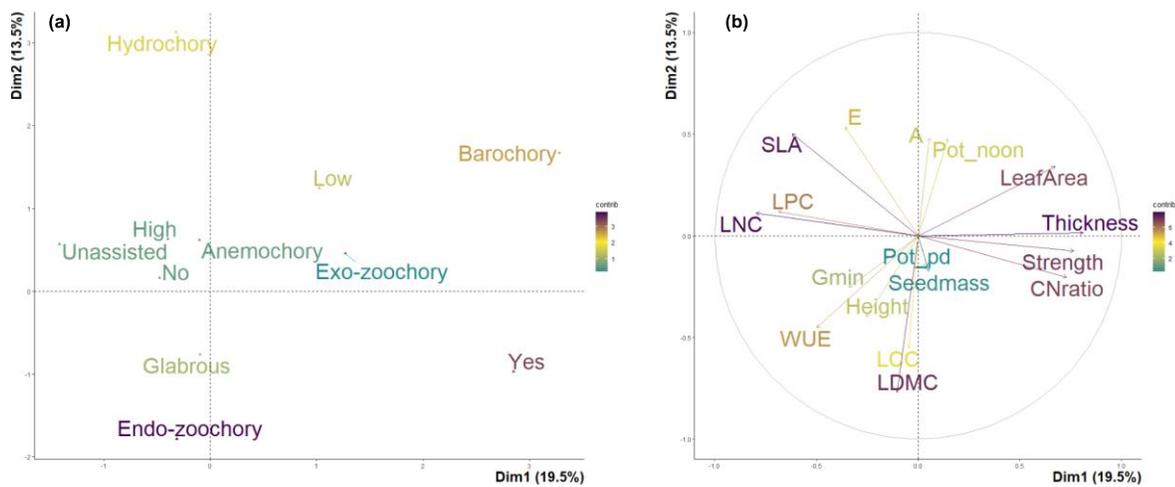
Functional Traits	PFT 1 (Forbs and tender leaves shrubs)	PFT 2 (Only rosettes)	PFT 3 (Hard leaf shrubs)	Statistics and <i>P</i> -value (df= 2 for all)
Plant height	1.2 ± 0.2	0.6 ± 0.1	1.19 ± 0.17	Chi <sup>2</sup> = 4.6; <i>P</i> = 0.103
Seed mass	0.2 ± 0.05 <b>a</b>	0.4 ± 0.1 <b>b</b>	18.3 ± 11.9 <b>b</b>	Chi <sup>2</sup> = 11.2; <i>P</i> = 0.004
Leaf area	1347.7 ± 441.2 <b>a</b>	9251.4 ± 2289.9 <b>b</b>	571.6 ± 218.6 <b>a</b>	Chi <sup>2</sup> = 16.1; <i>P</i> = 0.003
SLA	8.5 ± 0.6 <b>a</b>	5.3 ± 1.2 <b>b</b>	4.9 ± 0.4 <b>b</b>	Chi <sup>2</sup> = 20.6; <i>P</i> < 0.001
LDMC	369.0 ± 16.9 <b>a</b>	311.7 ± 22.1 <b>a</b>	474.1 ± 21.4 <b>b</b>	F = 12.8; <i>P</i> < 0.001
Leaf thickness	0.3 ± 0.02 <b>a</b>	0.70 ± 0.1 <b>b</b>	0.4 ± 0.04 <b>c</b>	Chi <sup>2</sup> = 19.3; <i>P</i> < 0.001
Leaf toughness	0.9 ± 0.2 <b>a</b>	4.7 ± 0.8 <b>b</b>	1.9 ± 0.3 <b>c</b>	Chi <sup>2</sup> = 24.4; <i>P</i> < 0.001
Leaf water potential at pre-dawn	-0.28 ± 0.03	-0.33 ± 0.02	-0.29 ± 0.03	Chi <sup>2</sup> = 3.3; <i>P</i> = 0.192
Leaf water potential at noon	-1.32 ± 0.1 <b>ab</b>	-1.13 ± 0.1 <b>a</b>	-1.72 ± 0.2 <b>b</b>	Chi <sup>2</sup> = 6.5; <i>P</i> = 0.04
Light-saturated photosynthetic rate	7.8 ± 0.5	7.5 ± 1.6	5.6 ± 1.0	F = 1.9; <i>P</i> = 0.163
Intercellular CO <sub>2</sub> /Ambient CO <sub>2</sub>	0.34 ± 0.06	0.48 ± 0.10	0.31 ± 0.06	Chi <sup>2</sup> = 0.7; <i>P</i> = 0.72
Leaf transpiration rate	0.004 ± 0.0003 <b>a</b>	0.003 ± 0.0003 <b>b</b>	0.002 ± 0.0002 <b>b</b>	Chi <sup>2</sup> = 19.8; <i>P</i> < 0.001
Water use efficiency	0.69 ± 0.06 <b>a</b>	0.49 ± 0.09 <b>a</b>	0.75 ± 0.05 <b>a</b>	Chi <sup>2</sup> = 5.40; <i>P</i> = 0.07
Leaf phosphorus content	1.4 ± 0.09 <b>a</b>	0.8 ± 0.08 <b>b</b>	0.9 ± 0.07 <b>b</b>	Chi <sup>2</sup> = 17.2; <i>P</i> < 0.001
Leaf carbon content	457.1 ± 4.7 <b>a</b>	445.4 ± 4.1 <b>a</b>	479.7 ± 7.8 <b>b</b>	F = 6.6; <i>P</i> = 0.003
Leaf nitrogen content	17.0 ± 0.7 <b>a</b>	11.3 ± 0.7 <b>b</b>	13.5 ± 0.8 <b>b</b>	F = 13.8; <i>P</i> < 0.001
C/N ratio	28.4 ± 1.1 <b>a</b>	41.1 ± 2.5 <b>b</b>	38.5 ± 3.6 <b>b</b>	Chi <sup>2</sup> = 10.0; <i>P</i> = 0.007
Cuticular conductance	7.5 ± 0.7	5.5 ± 1.4	7.5 ± 1.1	F = 1.1; <i>P</i> = 0.345

**Table S3.** Categorical functional traits variation between growth forms and the three PFTs identified in the páramo “El Verjón”. Statistical differences were tested using Fisher's exact test. Groups with different letters are statistically different from one another as determined by post-hoc test.

Functional Traits	Plant Functional Types				Growth forms			
	PFT1 <b>a</b>	PFT2 <b>b</b>	PFT3 <b>ab</b>	<i>P</i> -value	Forbs <b>ab</b>	Rosettes <b>a</b>	Shrubs <b>b</b>	<i>P</i> -value
<b>Spinescence</b>								
Yes	0	4	2	0.001	0	4	2	0.025
No	22	4	10		6	5	25	
<b>Pubescence</b>	PFT1 <b>a</b>	PFT2 <b>ab</b>	PFT3 <b>b</b>	<i>P</i> -value	Forbs	Rosettes	Shrubs	<i>P</i> -value
Glabrous	10	3	9	0.027	3	3	16	0.44
High	10	3	0		1	4	8	
Low	2	2	3		2	2	3	
<b>Seed dispersal</b>	PFT1 <b>a</b>	PFT2 <b>b</b>	PFT3 <b>c</b>	<i>P</i> -value	Forbs <b>a</b>	Rosettes <b>a</b>	Shrubs <b>b</b>	<i>P</i> -value
Barochory	0	3	0	< 0.001	0	3	0	< 0.001
Anemochory	12	3	2		2	3	12	
Unassisted	4	0	0		3	0	1	
Endo-zoochory	4	0	10		0	0	14	
Exo-zoochory	1	1	0		0	2	0	
Hydrozoochory	1	1	0		1	1	0	



**Figure S1.** Visualization of the correlation matrix of 19 continuous functional traits. Positive correlations are shown in tone of blues and negative correlations in tones of red.



**Figure S2.** The most important quantitative (a) and qualitative (b) variables explaining the two first dimensions of the factor analysis of mixed data and their direction.

## 2. CHAPTER II

### Photosynthetic acclimation to warming in five species from the Andean tropical alpine ecosystems

#### Abstract

In a warming world, there is increasing concern about the future of Earth system CO<sub>2</sub> fluxes given the uncertainties about thermal acclimation of photosynthesis and respiration for many plant communities, including the high elevation tropical flora. The low temperatures at those elevations have historically maintained low decomposition rates resulting in large organic carbon deposits. One of the biggest concerns with the expected rising temperatures and drying trends for the region is that all the carbon stored in the soils will be released turning the páramo in to a carbon source. However, if the vegetation can acclimate to warmer temperatures and increase CO<sub>2</sub> assimilation this could offset this possible increase in CO<sub>2</sub> release and the páramo will remain a carbon sink. To understand the effect of elevated temperatures on the metabolism of five representative páramo plant species, we conducted a +4.8°C warming experiment in growth chambers. We modeled temperature response curves for photosynthesis and respiration in two rosettes, *Puya goudotiana* and *Espeletia grandiflora*, two forbs *Orthrosanthus chimboracensis* and *Valeriana pilosa*, and one shrub, *Diplostephium phyllicoides*. From those curves we estimated the maximum photosynthetic rate ( $A_{400,opt}$ ), the optimum temperature ( $T_{Opt}$ ), the maximal rates of RuBP (ribulose-1,5- biphosphate) carboxylation ( $V_{cmax}$ ) and electron transport rate ( $J_{max}$ ), and the temperature sensitivity of respiration ( $Q_{10}$ ). Additionally, we evaluated the acclimation of heat tolerance parameters ( $T_{crit}$ ,  $T_{50}$  and  $T_{95}$ ), the relative growth rate (RGR), mass per unit leaf area (LMA) and leaf nitrogen content. We found species-specific behavior for each parameter measured, a homeostatic response and limited acclimation capacity for both photosynthesis and respiration in páramo plant species. Our result suggests that under warmer condition, as predicted for the end of this century, the páramo vegetation might not be able to offset the carbon liberated from soil respiration potentially putting these ecosystems at risk of becoming sources of CO<sub>2</sub> and their carbon sequestration capacity will be reduced.

#### 2.1 INTRODUCTION

The Earth's temperature continues to rise at the highest rates in recent history due to the increased release of greenhouse gases, including CO<sub>2</sub> (IPCC, 2021). Projections of how and at what scale this scenario will continue to worsen are strongly linked to understanding how the net CO<sub>2</sub> exchange of the planet's different ecosystems will change as temperatures rise (Chapin III et al., 2009; Loveys et al., 2003; Wythers et al., 2005). Net ecosystem CO<sub>2</sub> exchange depends on the balance between CO<sub>2</sub> assimilation by photosynthesis and the subsequent carbon storage in aboveground biomass and soil organic matter, and how much CO<sub>2</sub> is released by heterotrophic and autotrophic respiration. These metabolic processes appear to have different levels of sensitivity to temperature (Smith et al., 2020), which may not be the same in all ecosystems. The evidence indicates that soil respiration rates increase in response to warming (Bond-Lamberty & Thomson, 2010). Therefore, as the planet warms, the release of CO<sub>2</sub> from soil respiration will increase (Atkin, Edwards, et al., 2000; Atkin & Tjoelker, 2003; Bond-Lamberty & Thomson, 2010). Plant respiration, on the other hand, may be downregulated in response to warming reducing the release of CO<sub>2</sub> and the decline in net primary productivity (Slot et al., 2014; Slot & Kitajima, 2015; Smith & Dukes, 2013; Wang et al., 2020) (King et al. 2006). Less clear is whether photosynthesis can acclimate, maintain if not enhance its functioning, in response to increased temperature (Berry & Bjorkman, 1980; Kumarathunge et al., 2019) and compensate for CO<sub>2</sub> released by other means (Smith et al., 2020). If that does not occur, one would expect an abrupt decrease in the CO<sub>2</sub> assimilation and the carbon storage

capacity of the vegetation (Jump & Peñuelas, 2005), which, in turn, would feedback into the climate. To accurately calculate the rate and magnitude of changes in future land carbon sinks, we need to understand better the photosynthetic and respiration acclimation capacity of the vegetation in more species of critical ecosystems on the planet.

Some of the planet's critical ecosystems for carbon storage are in the tropics, where a favorable climate allows plants to grow year-round. Large extensions of tropical forests are considered essential carbon sinks that must be protected. However, they might be particularly vulnerable to climate change because their species have been exposed to relatively constant temperatures all their lives and are believed to have little capacity to acclimate (Berry & Bjorkman, 1980; Gunderson et al., 2009; Sendall et al., 2015). The data on photosynthetic acclimation from tropical tree species so far shows an unclear picture. Some species seem to be able to acclimate their respiration, thus reducing CO<sub>2</sub> release, as well as their photosynthesis, increasing assimilation. Other species, on the contrary, have not shown any acclimation capacity. Due to the limited number of studies and the immense diversity of the tropics, it is still unclear how forests will function in a warmer future, whether a more significant proportion of species will be able to acclimatize and thus maintain the role of the forest as an essential carbon sink, or whether, on the contrary, their carbon sequestration capacity will be reduced. However, forests are not the only tropical ecosystems with demonstrated capacity for carbon sequestration. In the high tropical mountains, above approx. 3,000 m.a.s.l., an ecosystem capable of sequestering as much carbon or more than the forest (mainly underground) is located (Chimner & Karberg, 2008; Curiel-Yuste et al., 2017).

Páramos, tropical alpine ecosystems found above the treeline in many mountains of the Americas, are highly diverse ecosystems with 3,595 plant species, of which 60% are endemic (Keating, 1999; Luteyn, 1999b; Madriñán et al., 2013). Apart from their fascinating biological and evolutionary interest, páramos are essential for the survival of human populations in their vicinity to which they provide clean water continuously (Buytaert et al., 2007; Castaño-Urbe, 2002). In addition, they provide another less obvious service; they are carbon reservoirs. The low temperatures at those elevations and the high aluminum content of their soils have historically maintained low decomposition rates resulting in large organic carbon deposits. One of the biggest concerns with the expected rising temperatures and drying trends for the region (Buytaert et al., 2011; Urrutia & Vuille, 2009) is that all the carbon stored in the soils will be released. Similar to what is happening with tundra soils, but on a smaller scale impacting the worldwide carbon cycle (Chimner & Karberg, 2008; Curiel-Yuste et al., 2017). The only thing that could offset this possible increase in CO<sub>2</sub> release is if the vegetation acclimates to warmer temperatures, then increasing its photosynthetic capacity and thus CO<sub>2</sub> assimilation. The few *in situ* warming studies conducted to date in the páramo are not conclusive in this regard. The Colombian páramos show a homeostatic response to warming. After three years of manipulation, neither soil and vegetation respiration nor photosynthesis has been affected by warming (Lasso et al., 2021). However, in Ecuadorian páramos, productivity has increased after nine years of warming, which could indicate that plants are acclimatizing and/or that the plant community is changing (Duchicela et al., 2021). The only páramo site with an Eddy covariance tower indicates that the páramo no longer behaves as a sink but as a source, releasing more carbon than it takes up per day (Carrillo-Rojas et al., 2019). Therefore, there is a need to study the metabolism of páramo plants and their ability to acclimate to temperature under controlled conditions to piece together the puzzle and better understand the carbon flow boxes of these ecosystems.

Plant metabolism and its contribution to the carbon cycle include two key processes, photosynthesis, and respiration. Both processes depend on a series of biochemical reactions catalyzed by temperature-

sensitive enzymes (Bernacchi et al., 2003; Farquhar et al., 1980; Moore et al., 2021; Von Caemmerer, 2000). Two main biochemical processes govern carbon assimilation and photosynthetic rates and can acclimate to increasing temperature. One is carboxylation or the ability of the enzyme Rubisco to carboxylate ribulose-bisphosphate (RuBP). The other is the regeneration of RuBP or the rate at which the Calvin cycle and light reactions can regenerate RuBP (Von Caemmerer, 2000). In addition, plants can enhance the thermal stability of Rubisco activase (Hikosaka et al., 2006; Sage & Kubien, 2007; Yamori et al., 2005) and the photosystem II (PSII), and increase electron transport rates. On the other hand, CO<sub>2</sub> release by respiration depends on cellular respiration in the mitochondria that varies depending on the metabolic requirements of the plant under the new conditions. There is evidence that photosynthesis and respiration can acclimate to some extent in response to temperature in several species (Slot & Kitajima, 2015; Slot & Winter, 2017; Way & Yamori, 2014).

A plant acclimates its photosynthesis if it adjusts CO<sub>2</sub> assimilation, improving its performance at higher temperatures (Slot & Winter, 2016; Way & Yamori, 2014). An acclimation response should allow the plant to be equal or better in its carbon gain under the new higher temperatures and maintain a positive carbon balance. Acclimated plants should be able to invest that carbon in growth, i.e., the photosynthetic rate should increase and be able to compensate for any loss of carbon by respiration. Typically, as a response to warming, the temperature optimum of photosynthesis ( $T_{opt}$ ) and the rate of photosynthesis ( $A_{max}$ ) will increase and the respiration rates decrease (Dusenge et al., 2019). But, since most of the studies on the mechanisms in the photosynthesis and respiration acclimation processes come from trees in temperate regimes (Atkin, Edwards, et al., 2000; Gauthier et al., 2014; Kroner & Way, 2016; O'Sullivan et al., 2013; Sendall et al., 2015; Way & Sage, 2008); and in the tropics, studies of photosynthesis acclimation are also concentrated on trees, mainly from the lowlands (Akaji et al., 2019; Doughty, 2011; Drake et al., 2015; Dusenge et al., 2015; Dusenge & Way, 2017; Hernández et al., 2020; Slot et al., 2014; Slot & Winter, 2017), information on the thermal acclimation of high elevation tropical ecosystems, including páramo species is desperately needed.

The tropics are usually pictured as sites with relatively constant temperatures year-round, without large annual temperature fluctuations, as in the seasonal temperate zone. Therefore, there is a prevailing notion is that tropical species live already close to their thermal optimum, and they lack the capacity to acclimate to temperature as their temperate counterparts (Sentinella et al., 2020), and that small temperature changes can strongly affect them (Doughty & Goulden, 2008; Janzen, 1967; Way & Oren, 2010). In that sense, we have labeled all the tropics as highly vulnerable to climate change. In that sense, we have labeled all the tropics as highly vulnerable to climate change. However, not all the tropics are the same, and tropical high mountain vegetation may tell another story yet to be told. In this project, we evaluate the metabolic thermal acclimation of páramo plants. We hypothesize that species from the páramos, exposed to high diurnal variability in temperatures (-2 to 26°C during a single day), with high thermal tolerance in natural conditions (Leon-Garcia & Lasso, 2019) will show high thermal acclimation as other temperate alpine species (Ghalambor et al., 2006). We evaluated the respiration and photosynthetic acclimation to warming of five species in a growth chamber experiment. We propose that photosynthesis ( $A_{max}$ ) will increase in response to warming. At the same time, respiration will decrease. Given that the lower temperatures typical of the mountain tops provide the impression of maintaining metabolic processes at sub-optimal temperatures (Hernández-Fuentes et al., 2015), warming will positively affect this processes. Alternatively, if plants are not as thermotolerant as expected, warming may damage their PSII, resulting in increased photoinhibition and reduced photosynthesis and growth.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Plant material and growth conditions

We studied five common páramo species with different growth forms found at “Parque Ecologico Matarredonda ” (4° 33' N, 74° 00' W, Eastern Colombian Andean range). According to our previous work, those species belong to different functional groups and ecological “CSR” strategies (Cruz and Lasso, 2021). They also have a distinct geographic origin (Sklenář 2010), and vary in their distribution range (**Table 1**).

**Table 1.** List of Páramo species studied together with their growth form following the classification of Hedberg and Hedberg (1979) and Ramsay (2001), their geographic origin according to Sklenář (2010), and their distribution range obtained from GBIF occurrence data. The data on plant height (H) are from Cruz and Lasso (2021). Growth forms (GF) are as follow: US correspond to upright shrub, SR to stem rosette, BR to basal rosette, EH to erect herb.

Scientific name	Family	GF	Distribution	Origin	Height (m)
<i>Diplostephium phyllicoides</i>	Asteraceae	US	Mexico, Colombia, Ecuador	Neotropical	1.06 ± 0.1
<i>Espeletia grandiflora</i>	Asteraceae	SR	Colombia	Páramo endemic	1.77 ± 0.3
<i>Puya goudotiana</i>	Bromeliaceae	BR	Colombia	Páramo endemic	1.63 ± 0.2
<i>Orthrosanthus chimboracensis</i>	Iridaceae	EH	Central and South America	Austral antartic	0.66 ± 0.1
<i>Valeriana pilosa</i>	Caprifoliaceae	EH	Costa Rica, Colombia, Venezuela, Peru	Wide temperate	0.29 ± 0.1

In February 2017, at the greenhouse of the University of Los Andes we planted 200 seedlings, forty from each species, into individual pots (pot ref. CP512, Treepot, Stuewe and sons, Tangent, Oregon, USA). Pots were 0.09 × 0.09 × 0.25 m (l × w × h), each having approximately 1.6 L of regular commercial soil (70%) and páramo soil (30%). Three months later, we randomly assigned twenty individuals per specie to one of two-temperature treatments (control and warming) in two growth chambers (Percival Scientific Low Temperature Chamber Model LT-105, Perry, Iowa, USA). The conditions in the control chamber were adjusted to match today's páramo temperature, simulating a day and night cycle (**Table 2**). The warming treatment followed the same diurnal cycle of temperature changes as the control but with temperatures 4.8°C higher (**Table 2**), which is the projected change in the annual average surface temperature by 2100 under the RCP8.5 scenario of the IPCC. All other environmental conditions were the same. The light conditions in both chambers using all the lights on were 380-μmol photons m<sup>-2</sup> s<sup>-1</sup>. The relative humidity ranged between 55% and 65% during the day, and 50% and 55% during the night. The irrigation regime was decided previously to the start of the experiment by estimating the permanent wilting point of the soil, that occurred twelve days after saturating the soil with 100 ml of water. All plants were irrigated every ten days with 100 ml of regular water.

**Table 2.** Daily temperature cycle conditions in the growth chambers for the control and the warming treatment

Time	Control (°C)	Warming (°C)
7:00 am – 10:00 am	12.0	16.8
10:00 am – 12:00 pm	20.0	24.8
12:00 pm – 1:00 pm	25.0	29.8
1:00 pm – 4:00 pm	20.0	24.8
4:00 pm – 7:00 pm	12.0	16.8
7:00 pm – 7:00 am	5.0	9.8
<b>Average</b>	<b>10</b>	<b>15</b>

### 2.2.2 Photosynthetic response curves to temperature

To estimate the carbon assimilation parameters, we constructed A-Ci curves by measuring the net photosynthesis at saturating irradiance ( $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) on twelve CO<sub>2</sub> concentrations. The first point was measured at a reference CO<sub>2</sub> concentration of 400 ppm, followed by 300, 200, 100, 50, 400, 400, 600, 800, 1200, 1600 and 2000 ppm. Data for the curves were taken from December 2018 until June 2019 at one mature leaf that had grown in the experimental condition from five plants per species on each treatment. All gas exchange measurements were taken with a LI-6400XT portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) at four different temperatures ( $T_{\text{set}} = 10^\circ\text{C}, 20^\circ\text{C}, 30^\circ\text{C}$  and  $40^\circ\text{C}$ ). To ensure that the leaf reached the desired temperature, the entire plant, together with the IRGA cuvette already placed on the leaf, was placed in the growth chamber where the temperature was adjusted and left until the temperature stabilized and the leaf reached the expected temperature. All A-Ci curves at those temperatures were obtained from the same leaf from 09:00 to 13:00 h. Afterward; we measured the leaf area and harvested the entire leaf to determine the foliar nitrogen concentration. The leaf area in the cuvette was estimated from drawing the foliar area in a paper, and then using ImageJ (Schneider et al., 2012) to correct the area in the gas exchange measurements.

### 2.2.3 Photosynthesis model parameterization

To assess the temperature response of net photosynthesis, measurements of photosynthesis at 400 ppm CO<sub>2</sub> ( $A_{400}$ ) were extracted from the A-Ci curves. We estimated  $A_{400,\text{opt}}$ , that represents the photosynthesis rate at the optimum temperature  $T_{\text{opt}}$ , and the parameter  $b$  that defines the shape of the parabola, where a greater  $b$  means a narrower parabola (Battaglia et al., 1996). The temperature response was fitted following Gunderson et al., 2009 as:

$$A_{400} = A_{400,\text{opt}} - b(T_{\text{leaf}} - T_{\text{opt}})^2$$

Where  $T_{\text{leaf}}$  is the leaf temperature measured abaxially with a Type E thermocouple in the cuvette of the LI-6400. We modeled  $T_{\text{opt}}$  and  $A_{400,\text{opt}}$  and their standard errors using non-linear least squares regression with the `nls_multstart` function from the `nls.multstart` package in R (Padfield D & Matheson GJ 2018). Maximum rates of RuBP carboxylation ( $V_{\text{cmax}}$ ) and RuBP regeneration ( $J_{\text{max}}$ ) were calculated from the A-Ci curves with the Farquhar, von Caemmerer, and Berry model (Farquhar et al. 1980; von Caemmerer & Farquhar 1981) using the ‘`fitaci`’ function from the ‘`plantecophys`’ package (Duursma 2015) in R version 3.5 (R Development Core Team, 2018). To better compare the photosynthetic acclimation capability of our species, we calculated the acclimation percentage index for  $T_{\text{opt}}$ ,  $A_{400,\text{opt}}$  and  $b$ , this index is calculated as the difference of each parameter between the warming and control treatments over the experimental warming effect, according to Zhou et al., 2018.

#### 2.2.4 Leaf dark respiration response curves to temperature

After we finished A-Ci curves, we turned off the lights in the growth chamber and waited 20 minutes before measuring dark respiration on a healthy mature leaf nearest to the one previously collected. Dark respiration ( $R_d$ ) was measured at a cuvette  $CO_2$  concentration of 400 ppm, at seven different temperatures from the highest to the lowest (40°C, 35°C, 30°C, 25°C, 20°C, 15°C and 10°C). We recorded three measurements at each temperature after values had stabilized.

#### 2.2.5 Temperature sensitivity and Acclimation of respiration

The temperature sensitivity of respiration was determined as the  $Q_{10}$ , the proportional increase in respiration with a 10°C increase in temperature following Slot & Winter (2018) as:

$$Q_{10} = e^{(\text{slope} \times 10)}$$

where slope refers to the slope of the respiration rate plotted against leaf temperature (**Figure 3**).

To better understand the type of acclimation occurring, we compared the slopes (b) and intercepts (m) of the log-transformed curves of respiration response to temperature following Slot & Kitajima (2015). Finally, due to Heskell et al., (2016) supports that a second-order log-polynomial model is the best way to represent how respiration curves response to temperature, we fitted a quadratic regression on natural log-transformed respiration rates according to Heskell et al., (2016):

$$\ln R_d = a + bT + cT^2$$

where  $R_d$  is dark respiration rate at each temperature (T), and the three coefficients are:  $a$ , the y axis intercept;  $b$ , the slope when  $T=0^\circ\text{C}$ , and  $c$ , is the curvature of the slope (Dusenge et al., 2020; Heskell et al., 2016)

#### 2.2.6 Growth analysis

One year after the experiment started, we harvested five individuals per species and treatment and oven dried them at 70°C for 72 h (Lab-line Imperial V convection oven) to calculate the initial dry mass. At the end of the experiment (1000 days later), the final dry mass was determined from five plants, and mean relative growth rate (RGR) was calculated following Slot & Winter (2018) as:

$$\text{RGR} = [\ln(\text{final dry mass}) - \ln(\text{average initial dry mass})] / \# \text{ days in the experiment } (\Delta \text{ time})$$

#### 2.2.7 Leaf traits

Leaf mass per area (LMA) was measured in five mature healthy leaves per species and treatment. Leaf area was processed using ImageJ on pictures obtained with a flatbed scanner (Hewlett Packard G3010). Leaf dry mass was obtained after drying samples in the oven at 70°C for 72 h and weighted them using a 0.01 mg precision balance (Sartorius MSE 125p). LMA was calculated as the ratio of leaf dry mass and leaf area. Foliar nitrogen concentration was determined in an elemental analyzer at the Soil and Water laboratory at the Universidad Nacional de Colombia, Bogota.

#### 2.2.8 Heat tolerance

We also evaluated the acclimation of parameters related to heat tolerance of photosystem II (PSII), the protein complex involved in the oxidation of water and the initiation of electron transport during photosynthesis. When PSII is exposed to stress-generating heat levels, its ability to process light in photochemical reactions decreases and unused energy is re-emitted in the form of fluorescence.

Therefore, different chlorophyll fluorescence parameters are used as an indicator of photoinhibition, with the maximum efficiency of PSII or  $F_v/F_m$  being reliable and commonly employed for thermal tolerance assessment. PSII heat tolerance in plants can be estimated by recording the decline in  $F_v/F_m$  in response to a temperature increase. Three parameters can be obtained from this curve describing different stages of the heat response on plants:  $T_{crit}$ ,  $T_{50}$  and  $T_{95}$ .  $T_{crit}$  is the critical temperature at which  $F_v/F_m$  starts to decline, or the inflection point between the plateau and the exponential phase of the  $F_v/F_m$  versus temperature curve.  $T_{50}$  corresponds to the temperature that causes a 50% reduction in  $F_v/F_m$ , which typically occurs when damage to PSII is irreversible, leaves become necrotic and net carbon assimilation decreases (Krause et al., 2010; Perez et al., 2016; Ruiz et al., 2008). Lastly,  $T_{95}$  indicates the temperature that causes a 95% reduction in  $F_v/F_m$  and corresponds to a yield close to zero.

To construct the curves, we cut leaf disks from the last fully expanded leaves from three well-watered individuals of each species in both the warm and control growth chambers after one year of treatment (between May and August 2018). Initial  $F_v/F_m$  values were recorded to ensure healthy leaves were collected and to later calculate  $T_{50}$ . Typical values of  $F_v/F_m$  of non-stressed leaves are around 0.83. Afterwards, leaf disks were heated in water baths set at seven different temperatures: 34°C, 38°C, 42°C, 48°C, 52°C, 56°C and 58°C following the protocol by Krause et al., (2010). Leaf disks were placed inside tea cloth bags to prevent anaerobiosis and then enclosed in zipped bags that were introduced into a second zipped bag containing a weight to ensure complete immersion into the preheated water baths. Control disks were maintained in cloth and zipped bags at room temperature ( $\approx 21$  °C). After a 15-minute immersion in the different water baths, leaf disks were placed on petri dishes with wet paper towel to prevent desiccation and stored in the dark for 24h. After the recovery period, fluorescence was recorded with a modulated fluorometer OS30p+ (Opti-Sciences, Inc. NH, USA). We fitted a logistic curve on the  $F_v/F_m$  response to temperature per individual and obtained  $T_{crit}$ ,  $T_{50}$  and  $T_{95}$ . Curves were fitted with the “fitplc” R package, with the Weibull model and a 95% confidence interval modifying the “Kmax” argument so that it corresponded to the mean initial value for each species.

### 2.2.9 Data analysis

For photosynthetic parameters obtained from the A-C<sub>i</sub> curves ( $V_{cmax}$ , and  $J_{max}$ ), we ran for each species, a Linear Mixed-effects model (ANOVA) to evaluate the effect of the treatment (control, warming), the effect of temperature ( $T_{set}=10^\circ\text{C}$ ,  $20^\circ\text{C}$ ,  $30^\circ\text{C}$ ,  $40^\circ\text{C}$ ) and the interaction of both (**Table 4**). After that, post-hoc tests were used to evaluate differences in the pairwise comparisons,  $p$ -values were adjusted using the Bonferroni multiple testing correction method (**Table S4**).

The effect of treatments on photosynthetic parameters ( $A_{400,opt}$ ,  $T_{opt}$ ,  $b$ ,  $V_{cmax20}$ ,  $J_{max20}$ ; **Table S1**), respiration traits ( $Q_{10}$ ,  $b$ ,  $m$ ,  $a$ ,  $b$ ,  $c$ ; **Table S2**), measures of growth rate, leaf traits and thermotolerance parameters (RGR, LMA,  $N_{mass}$ ,  $T_{crit}$ ,  $T_{50}$ ,  $T_{95}$ ; **Table S3**) were evaluated by t-tests separately for each species after testing the normal distributions and homoscedasticity hypotheses of the variables. In cases where these assumptions were not met, Mann-Whitney U-tests were performed. Heteroscedasticity T-tests was applied for normally distributed data with significant differences between the two variances or to unpaired samples. All data are reported as means  $\pm$  standard error of the mean (SEM) and all analyses were performed in R (Version 1.3.1093 - R Development Core Team, 2009–2016).

## 2.3 RESULTS

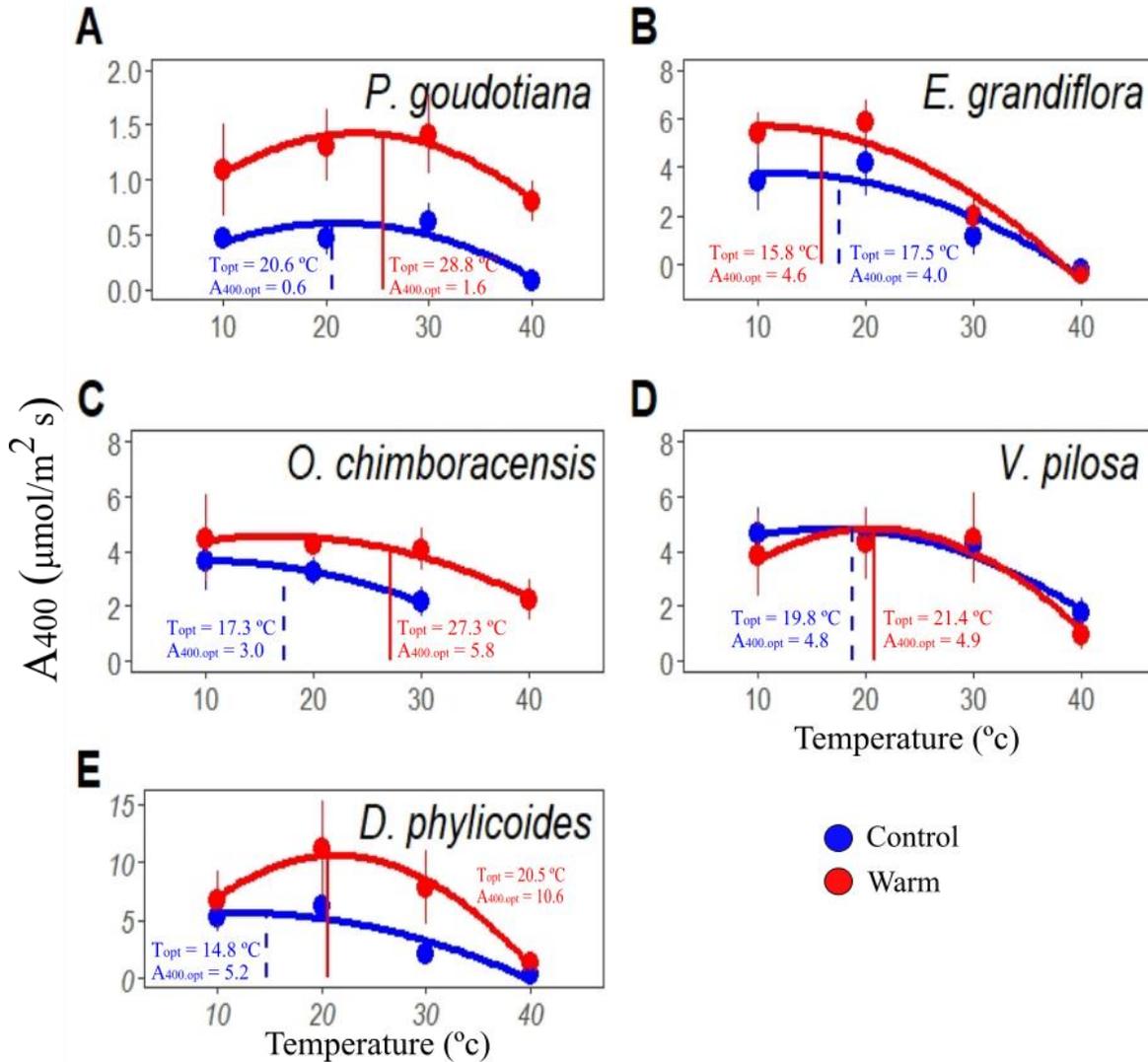
### 2.3.1 Thermal photosynthetic acclimation in páramo plant species

#### Photosynthetic parameters

The temperature response curves of photosynthesis show that all species can slightly modify their photosynthetic parameters in response to warming (**Figure 1**) but in most cases these differences are not statistically significant (**Table 3**). Only two of the species clearly increased their optimum temperature of assimilation ( $T_{opt}$ ) *Orthrosanthus chimboracensis*, a forb, and *Diplostephium phyllicoides*, a shrub. The  $T_{op}$  of *O. chimboracensis* increased from 17°C to 27°C in response to warming, i.e. an increase of 37% ( $t=-4.9$ ,  $df=4$ ,  $p=0.008$ ; **Figure 1**; **Table 3**); while the  $T_{op}$  of *D. phyllicoides*, increased from 14°C to 20°C a 28% increase ( $t=0.7$ ,  $df=5$ ,  $p=0.04$ ). The maximum photosynthesis at 400 ppm CO<sub>2</sub> ( $A_{400}$ ) increased in response to warming for all species but only for *O. chimboracensis* this was a significant increase ( $t=-3.8$ ,  $df=4$ ,  $p=0.02$ ). *Puya goudotiana* increased their maximum assimilation ( $A_{400,opt}$ ) by about 64% in response to warming, although these differences were only marginally significant ( $p = 0.09$ ) and assimilation was still quite low (1.6  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). *Valeriana pilosa*, a forb, maintained its parameters almost identical under both treatments, showing almost completely overlapping curves and very low acclimation rates.

**Table 3.** Summary of means and the standard error of each group of photosynthetic parameters in 5 different páramo species, with its respective acclimation Index. The significance of treatments results from Student's t-test (control vs. warmed) are shown in bold.

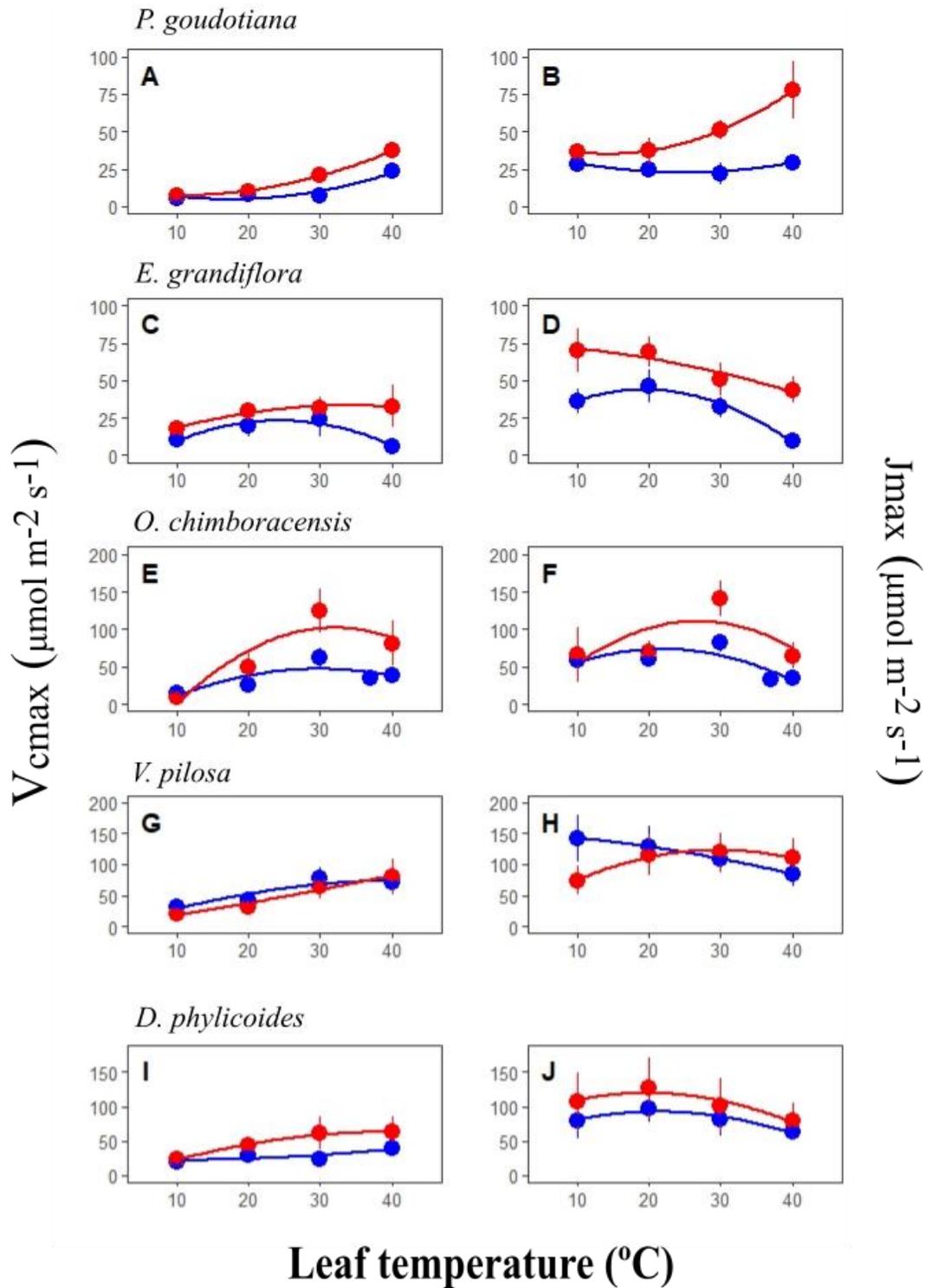
Species/parameters	Control	Warm	<i>p</i> -value	Acclimation Index
<i>Puya goudotiana</i>				
$A_{400,opt}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	0.6±0.1	1.6±0.4	0.09	64%
$T_{opt}$ (°C)	20.6±3	28.8±3.5	0.15	28%
<b>b</b>	0.001±0.0001	0.003±0.001	0.17	67%
<i>Espeletia grandiflora</i>				
$A_{400,opt}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	4.0±1.7	4.6±0.6	0.76	13%
$T_{opt}$ (°C)	17.6±0.5	15.8±0.7	0.12	-11%
<b>b</b>	0.02±0.008	0.01±0.0008	0.47	-56%
<i>Orthrosanthus chimboracensis</i>				
$A_{400,opt}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	3.0±0.6	5.8±0.4	<b>0.02</b>	48%
$T_{opt}$ (°C)	17.3±1.9	27.3±0.8	<b>0.008</b>	37%
<b>b</b>	0.007±0.002	0.01±0.0006	0.06	48%
<i>Valeriana pilosa</i>				
$A_{400,opt}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	4.8±0.4	4.9±2.4	0.61	2%
$T_{opt}$ (°C)	19.8±3.3	21.4±1.0	0.62	8%
<b>b</b>	0.01±0.0003	0.01±0.006	0.70	0%
<i>Diplostephium phyllicoides</i>				
$A_{400,opt}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	5.2±0.5	10.6±5.1	0.35	51%
$T_{opt}$ (°C)	14.8±2.0	20.5±1.1	<b>0.04</b>	28%
<b>b</b>	0.02±0.007	0.03±0.01	0.58	36%



**Figure 1.** Temperature response of net CO<sub>2</sub> assimilation at a CO<sub>2</sub> concentration of 400 ppm ( $A_{400}$ ) of five páramo species in control (blue) and warmed (red) conditions. Error bars indicate standard error for mean values at each temperature, treatment, and species. Vertical lines indicate the mean of  $T_{opt}$  for control (dashed blue lines) and warming (solid red lines) individuals.

#### Photosynthetic capacity

Rubisco maximum carboxylation rates ( $V_{cmax}$ ) and maximum electron transport rates ( $J_{max}$ ) increased with leaf temperature in *P. goudotiana*, *E. grandiflora*, and *O. chimboracensis* (**Figure 2**) as shown by the significant effect of  $T_{set}$  on  $V_{cmax}$  and  $J_{max}$  (**Table 4**). For *D. phyllicoides* the carboxylation rate increased with increasing leaf temperature, but the electron transport rate did not. *V. pilosa* was the only species where no significant changes were observed in either  $V_{cmax}$  or  $J_{max}$ , indicating that both parameters remain constant even if leaf temperature changes. The only two species showing acclimation, i.e., a significant effect of treatment on  $V_{cmax}$ , were *P. goudotiana* ( $F = 8.6$ ;  $p = 0.043$ ) and *O. chimboracensis* ( $F = 11.1$ ;  $p = 0.021$ ). While *P. goudotiana* was the only one that also modifies its  $J_{max}$  in response to the warming treatment ( $F = 19.6$ ;  $p = 0.011$ ).



**Figure 2.** Biochemical temperature response parameters ( $V_{cmax}$  in the left and  $J_{max}$  in the right) of five páramo species in control (blue) and warming (red) experiments. Dots indicate the mean value and error bars indicate standard error for mean values at each temperature, treatment, and species.

**Table 4.** Summary of the mixed-effect model for  $V_{cmax}$  and  $J_{max}$ . DF<sub>n</sub> and DF<sub>d</sub> are degrees of freedom in the numerator and denominator, respectively. Statistically significant values are shown in bold.

		<i>P. goudotiana</i>				<i>E. grandiflora</i>			
$V_{\text{cmax}}$	DFn	DFd	<i>F</i> -value	<i>p</i> -value	DFn	DFd	<i>F</i> -value	<i>p</i> -value	
Treatment	1	4	8.603	<b>0.043</b>	1	4	0.461	0.534	
$T_{\text{set}}$	3	12	64.684	<b>0.00000012</b>	2	8	5.973	<b>0.026</b>	
Interaction	3	12	3.342	0.056	2	8	0.215	0.811	
$J_{\text{max}}$									
Treatment	1	4	19.632	<b>0.011</b>	1	4	1.742	0.257	
$T_{\text{set}}$	3	12	3.514	<b>0.049</b>	2	8	6.046	<b>0.025</b>	
Interaction	3	12	3.118	0.066	2	8	0.948	0.427	

		<i>O. chimboracensis</i>				<i>V. pilosa</i>			
$V_{\text{cmax}}$	DFn	DFd	<i>F</i> -value	<i>p</i> -value	DFn	DFd	<i>F</i> -value	<i>p</i> -value	
Treatment	1	5	11.103	<b>0.021</b>	1	6	0.002	0.967	
$T_{\text{set}}$	3	15	29.768	<b>0.00000146</b>	3	18	7.201	<b>0.002</b>	
Interaction	3	15	5.833	<b>0.008</b>	3	18	1.625	0.219	
$J_{\text{max}}$									
Treatment	1	5	5.027	0.075	1	6	0.495	0.508	
$T_{\text{set}}$	3	15	11.765	<b>0.0003</b>	3	18	0.688	0.571	
Interaction	3	15	0.695	0.569	3	18	1.891	0.167	

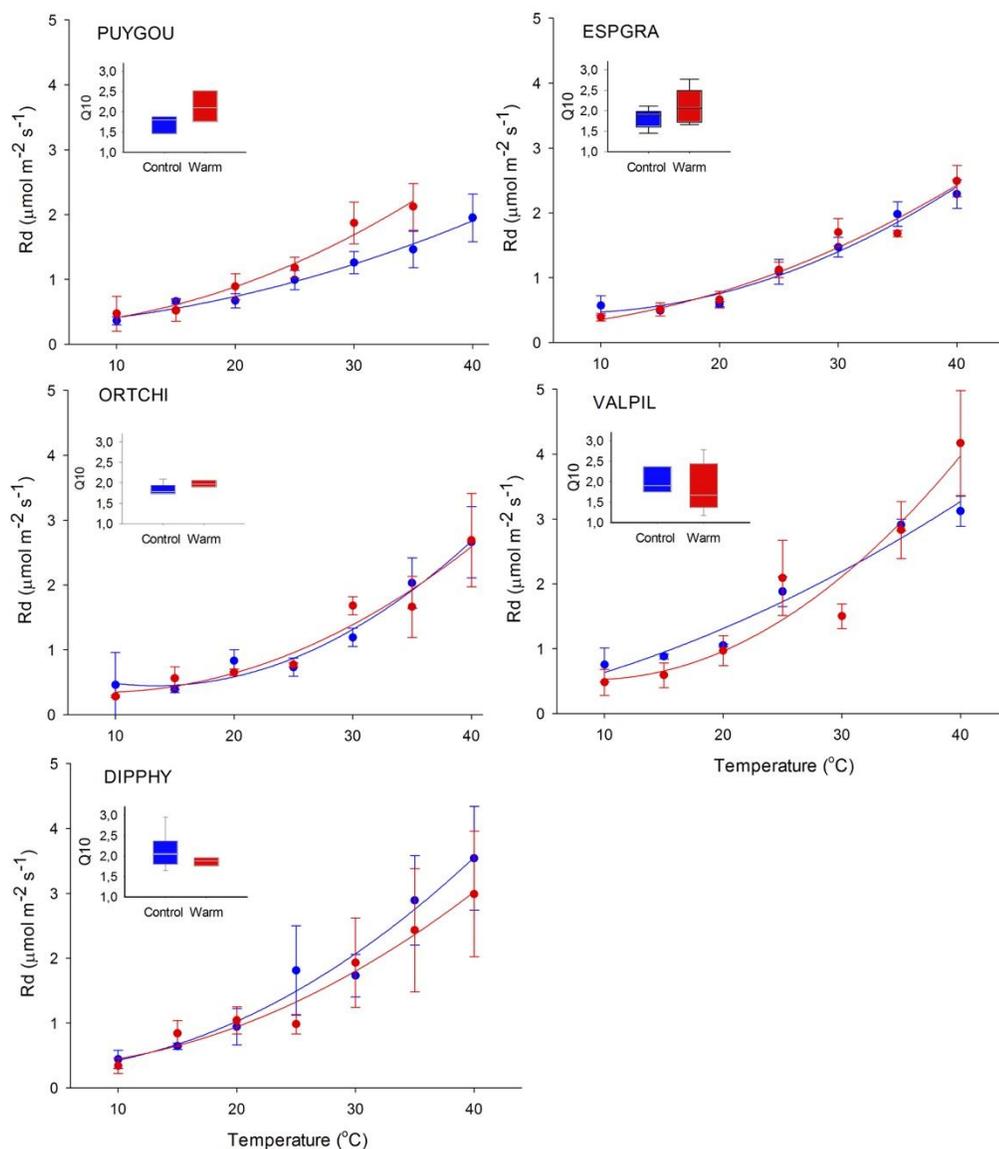
  

		<i>D. phyllicoides</i>			
$V_{\text{cmax}}$	DFn	DFd	<i>F</i> -value	<i>P</i> -value	
Treatment	1	6	0.916	0.376	
$T_{\text{set}}$	3	18	5.282	<b>0.009</b>	
Interaction	3	18	0.813	0.503	
$J_{\text{max}}$					
Treatment	1	6	0.447	0.529	
$T_{\text{set}}$	3	18	2.66	0.079	
Interaction	3	18	1.098	0.376	

### 2.3.2 Thermal acclimation of respiration in páramo plant species

#### Respiration and its temperature sensitivity – $Q_{10}$

We found no evidence of respiration acclimation for any species (**Figure 3; Table 5**). The curves from both treatments overlapped for all species. If acclimation of respiration occurs, it is expected that respiration values decrease in the warming treatment, as observed in the curves of *D. phyllicoides* (**Figure 3**). However, even for this species, this decrease is not significant. Values on the sensitivity of respiration to temperature ( $Q_{10}$ ) did not differ between treatments either (**Table 5**), supporting our previous findings of no-acclimation of respiration in all species. The slopes (b) and intercepts (m) of the log-transformed curves of respiration response to temperature were also not statistically different between treatments, as any of the three descriptive parameters of the respiration response to temperature (*a*, *b*, *c*) (**Table 5** and **Table S2**) confirming that no acclimation is taking place.



**Figure 3.** Effect of warming treatment in dark respiration ( $R_d$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $Q_{10}$  of five páramo species in control (blue) and warming (red) experiments. Error bars indicate standard error for mean values at each temperature, treatment, and species.

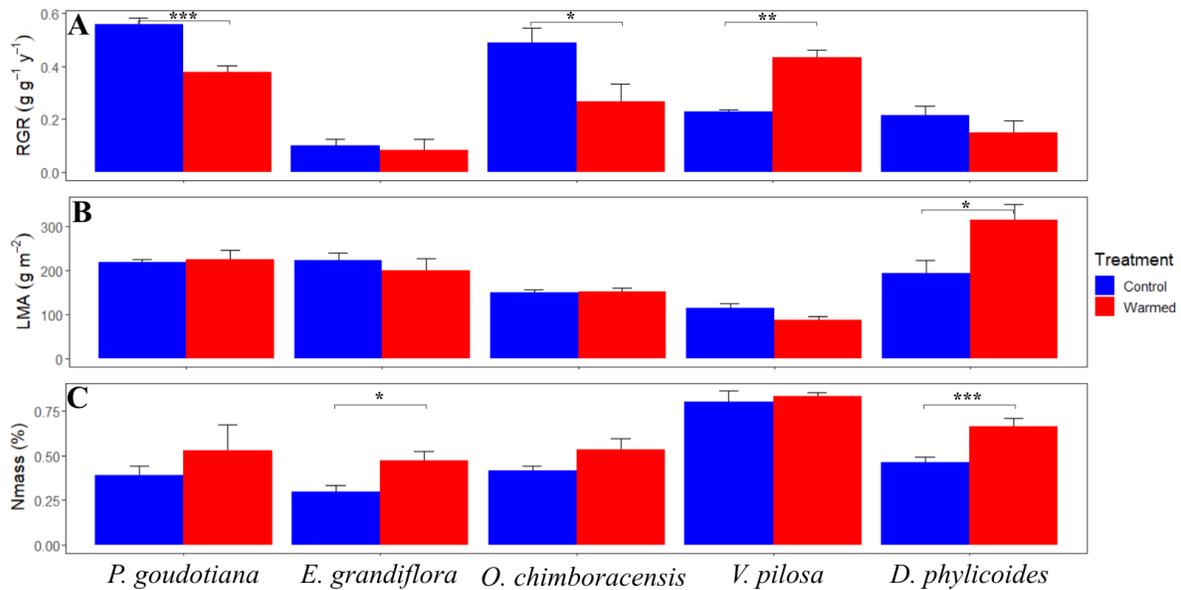
**Table 5.** Summary of means and the standard error of respiration traits measured in five páramo species. The Student's t-test comparing treatments (control vs. warm) are reported in the last column.

Species/parameters	Control	Warm	<i>p</i> -value
<i>Puya goudotiana</i>			
$Q_{10}$	$1.68 \pm 0.05$	$2.14 \pm 0.17$	0.24
$R_{10}$	$-0.36 \pm 0.06$	$-0.47 \pm 0.27$	0.72
$R_{15}$	$-0.66 \pm 0.04$	$-0.52 \pm 0.17$	0.49
$R_{20}$	$-0.67 \pm 0.11$	$-0.89 \pm 0.20$	0.49
<i>Espeletia grandiflora</i>			

Q <sub>10</sub>	1.81 ± 0.12	2.13 ± 0.21	0.22
R <sub>10</sub>	-0.57 ± 0.15	-0.39 ± 0.06	0.33
R <sub>15</sub>	-0.49 ± 0.08	-0.51 ± 0.10	0.89
R <sub>20</sub>	-0.59 ± 0.04	-0.66 ± 0.13	0.64
<i>Orthrosanthus chimboracensis</i>			
Q <sub>10</sub>	1.85 ± 0.07	1.98 ± 0.17	0.17
R <sub>10</sub>	-0.46 ± 0.05	-0.28 ± 0.02	<b>0.03</b>
R <sub>15</sub>	-0.40 ± 0.05	-0.56 ± 0.18	0.45
R <sub>20</sub>	-0.83 ± 0.17	-0.65 ± 0.05	0.43
<i>Valeriana pilosa</i>			
Q <sub>10</sub>	2.06 ± 0.25	1.88 ± 0.30	0.66
R <sub>10</sub>	-0.75 ± 0.26	-0.48 ± 0.20	0.48
R <sub>15</sub>	-0.88 ± 0.04	-0.59 ± 0.19	0.20
R <sub>20</sub>	-1.05 ± 0.02	-0.97 ± 0.23	0.84
<i>Diplostephium phyllicoides</i>			
Q <sub>10</sub>	2.13 ± 0.22	1.86 ± 0.08	0.34
R <sub>10</sub>	-0.44 ± 0.14	-1.16 ± 0.82	0.47
R <sub>15</sub>	-0.64 ± 0.05	-2.01 ± 1.18	0.37
R <sub>20</sub>	-0.94 ± 0.28	-1.59 ± 0.56	0.38

### 2.3.3 Relative growth rate

There is a trend whereby plants of most species growing under higher temperatures grow less, but this is only statistically significant for the rosette *P. goudotiana* ( $t=5.12$ ,  $df=7$ ,  $p=0.001$ ) and the forb *O. chimboracensis* ( $t=2.54$ ,  $df=5$ ,  $p=0.05$ ). On the other hand, one of the forb species, *V. pilosa*, substantially increased its growth from 0.23 to 0.43  $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$  ( $t=-6.1$ ,  $df=7$ ,  $p=0.0005$ ) under the warming treatment (**Figure 4; Table S3**).



**Figure 4.** (A) Relative growth rate (RGR), (B) Leaf mass per area (LMA) and (C) leaf nitrogen content (Nmass) of five species grown under two growth temperatures, control in blue and warmed in red.

Significant differences between treatments comparison by Student's t-test analysis are reported as: \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ . Bars are means and error bars represent standard error.

#### 2.3.4 Leaf traits

Leaf mass per area (LMA) remained unchanged in response to warming for most species (**Table S3**), except for *D. phyllicoides*, for which warmed plants produced tougher and thicker leaves with higher LMA ( $t=-2.59$ ,  $df=8$ ,  $p=0.03$ ; **Figure 5B**). Leaf nitrogen content on a mass basis ( $N_{mass}$ ) increased in response to warming in all species but was only significantly for *D. phyllicoides* ( $t=-5.67$ ,  $df=7$ ,  $p=0.008$ ) and *E. grandiflora* ( $t=-2.63$ ,  $df=8$ ,  $p=0.03$ ; **Figure 5C, Table S3**).

#### 2.3.5 Heat tolerance

All species had high heat tolerance values (**Table 5**). Leaves of these species must reach at least 40°C for a decrease in  $F_v/F_m$  to begin to occur ( $T_{crit}$ ) and must be heated to at least 45°C for  $F_v/F_m$  to be reduced by half ( $T_{50}$ ) and about 50°C to be reduced by 95% ( $T_{95}$ ). However, we see no acclimation of this value in most species except for *P. goudotiana*, for which there was an increase in  $T_{crit}$  ( $t=-6.3$ ,  $df=4$ ,  $p=0.003$ ) and  $T_{50}$  ( $t=-4.4$ ;  $df=4$ ;  $p=0.01$ ) in response to warming (**Table 5**).

**Table 5.** Summary of means and standard error of heat tolerance traits measured in this study in five different páramo species. Significance of treatments results from Student's t-test (control vs. heated) for normally distributed variables. Statistically significant values are shown in bold.

Species/parameters	Control	Warmed	Statistical summary
<i>Puya goudotiana</i>			
$T_{crit}$	40.4±0.4	43.1±0.2	$t$ -value=-6.3; $df=4$ ; $p$ -value= <b>0.003</b>
$T_{50}$	48.2±0.4	50.4±0.3	$t$ -value=-4.4; $df=4$ ; $p$ -value= <b>0.01</b>
$T_{95}$	56.2±1.4	57.6±0.7	$t$ -value=-0.9; $df=4$ ; $p$ -value= 0.42
<i>Espeletia grandiflora</i>			
$T_{crit}$	45.4±0.0	44.7±1.6	$t$ -value=0.3; $df=1$ ; $p$ -value= 0.83
$T_{50}$	53.4±0.0	49.8±0.1	$t$ -value=42.2; $df=1$ ; $p$ -value= <b>0.01</b>
$T_{95}$	61.4±0.0	54.7±1.8	$t$ -value=2.2; $df=1$ ; $p$ -value= 0.28
<i>Orthrosanthus chimboracensis</i>			
$T_{crit}$	40.0±2.6	42.8±1.5	$t$ -value=-0.9; $df=4$ ; $p$ -value= 0.41
$T_{50}$	45.4±0.8	46.5±0.9	$t$ -value=-0.9; $df=4$ ; $p$ -value= 0.40
$T_{95}$	50.7±1.5	50.0±0.8	$t$ -value=0.5; $df=4$ ; $p$ -value= 0.67
<i>Valeriana pilosa</i>			
$T_{crit}$	42.5±1.1	41.7±0.7	$t$ -value=0.7; $df=3$ ; $p$ -value= 0.53
$T_{50}$	45.1±0.6	46.6±0.5	$t$ -value=-1.9; $df=3$ ; $p$ -value= 0.15
$T_{95}$	47.4±0.0	51.3±1.7	$t$ -value=-1.7; $df=3$ ; $p$ -value= 0.18
<i>Diplostephium phyllicoides</i>			
$T_{crit}$	44.7±1.6	45.5±0.7	$t$ -value=-0.5; $df=4$ ; $p$ -value= 0.65
$T_{50}$	48.4±0.7	49.4±0.5	$t$ -value=-1.3; $df=4$ ; $p$ -value= 0.27
$T_{95}$	51.8±0.2	53.1±0.5	$t$ -value=-2.5; $df=4$ ; $p$ -value= 0.07

## 2.4 DISCUSSION

This study is one of the first to evaluate the capacity of páramo plants to acclimate to the higher temperatures expected by the end of the century and one of the few in which A-C<sub>i</sub> curves have been constructed to evaluate in detail the carbon assimilation capacity of páramo species and the effects of temperature on them. The results for this small group of five species suggest that the acclimation capacity of páramo species is low if we consider that a plant acclimates its photosynthesis if it adjusts CO<sub>2</sub> assimilation, improving its performance at higher temperatures (Slot & Winter, 2016; Way & Yamori, 2014). An acclimation response should allow the plant to be equal or better in its carbon gain under the new higher temperatures and maintain a positive carbon balance. Although we don't see that more elevated temperatures negatively affect CO<sub>2</sub> assimilation, we also didn't find an increase in temperature significantly enhances CO<sub>2</sub> assimilation and plant growth in most species to compensate for the CO<sub>2</sub> released by other means (Smith et al., 2020). Moreover, none of the species seem to be able to downregulate their respiration in response to warming. While this is a small sample of páramo species, we have species from various growth forms and functional groups (Cruz and Lasso, 2021). In all, there appears to be a similar homeostatic response.

Rates of CO<sub>2</sub> assimilation obtained in this study were particularly low as has been previously reported for the páramo (Baruch, 1979; Rada, 2016), and were not reduced in response to warming, on the contrary, photosynthetic rates slightly increased, although not significantly. Only the forb *O. chimboracensis* had the ability to acclimate photosynthesis to warming. This native forb adjusted its photosynthetic capacities ( $V_{cmax}$  and  $J_{max}$ ) specially in temperatures above 20°C and decreased its rate of respiration at 10°C. Although a lack of thermal acclimation of photosynthesis seems prevalent in páramo plants, other kind of adjustments could be observed, *D. phylloides* showed the ability to shift upward the thermal optimum ( $T_{opt}$ ) in warmer growth conditions apparently due to increases in the nitrogen content of leaves or by changes in its foliar structure. In *P. goudotiana*, warming treatment improved the maximum rates of Rubisco carboxylation ( $V_{cmax}$ ) and the potential rate of electron transport ( $J_{max}$ ), this translated into just a slight improvement in its CO<sub>2</sub> assimilation rate. In *E. grandiflora*, high leaf nitrogen content in warmed conditions seems to play an insignificant role in photosynthetic or respiratory acclimation. *V. pilosa* appeared relatively insensitive to growth temperature since the treatment had no effect on any of the parameters measured, apart from RGR. As opposed to what we had hypothesized, we did not find high thermal acclimation in páramo plant species despite the wide temperature ranges to which they are exposed daily (Lasso et al., 2021; Llambí & Rada, 2019; Rada et al., 2019).

None of the species down regulated their respiration rates or acclimated their sensitivity to temperature ( $Q_{10}$ ) in response to the warming, as hypothesized. This small representation of páramo species suggest that changes in growth temperature did not induce coupled changes in photosynthesis and respiration, thus our data do not support the optimal photosynthetic theory (Dusenge et al., 2019; Wang et al., 2020). In the absence of acclimation of respiration, warming in páramo plants could increase CO<sub>2</sub> release which will impact negatively global temperatures (Atkin & Tjoelker, 2003).

On the other hand, the results found in this study are consistent with rosettes being the páramo group considered to have the highest thermal tolerance (Leon-Garcia & Lasso, 2019), as *P. goudotiana* and *E. grandiflora* showed the highest values of heat tolerance, and both modified at least one of those parameters in the warming treatment (e.g., temperature at which irreversible thermal damage of 50% of the photosynthetic apparatus is observed ( $T_{50}$ ) was greater at a higher temperature), which makes its

photosynthetic apparatus still safe at warmer growth temperatures. We found high values for heat tolerance that will translate to broad thermal safety margins, but PSII heat tolerance have recently been shown not to improve carbon assimilation (specifically  $T_{opt}$  and  $P_{opt}$ ; Perez et al., 2020), and thermal tolerance under drought stress was found to result in narrower thermal safety margins (Cook et al., 2021). Therefore, these findings, accompanied with the low acclimation capacity of heat tolerance found in our study, suggests more frequent combined stress of heat and drought could still leave paramo plants at risk of thermal damage.

Higher temperatures are expected to be detrimental to plant species because they tend to decrease their photosynthetic rates by reducing stomatal conductance, or the carboxylation capacity of Rubisco, or because elevated temperatures could increase the permeability of the thylakoid membrane, which decreases electron transport rates (Carter et al., 2021; Kumarathunge et al., 2020). At the same time, higher temperatures will increase respiration rates by underlying mechanisms still unknown (Dusenge et al., 2019; Mujawamariya et al., 2021; Wang et al., 2020). Thermal acclimation is the mechanism by which these harmful effects on the plant can be reversed, affecting the vegetation feedbacks to the Earth's carbon cycle (Gunderson et al., 2009; Sklenář et al., 2016; Way & Sage, 2008; Way & Yamori, 2014). In this study we found a greater degree of acclimation of photosynthesis in fast-growing species (*O. chimboracensis* and *D. phyllicoides*, (Cruz & Lasso, 2021)) compared to those considered slow-growing (*P. goudotiana* and *E. grandiflora*, (Cruz & Lasso, 2021)), as has been reported in other studies (Atkin et al., 2006; Loveys et al., 2003). However, none of the evaluated species the optimum photosynthetic rate was reduced by the effect of warming, these suggests increases in temperature in the páramo will not be detrimental to the plants, because the fast-growing species will have some capacity for acclimation, while the slow-growing ones will maintain greater thermal safety margins of their photosynthetic apparatus. Furthermore, plants grown under control and warming treatments had the same rates of respiration, thus warming has not a detrimental effect in respiration rates, because any of the species had the expected increase in leaf respiration (Reich et al., 2016). It has been reported that daily high thermal fluctuations experienced by plants is one of the key factors in plant development since the most important metabolic processes are biochemical reactions in which temperature-dependent enzymes are involved (Berry & Bjorkman, 1980; Niinemets, 2018), and maybe that is why it is expected some homeostatic behavior (Lasso et al., 2021), because páramo plants are not living in narrow temperature ranges, as the forest trees in tropical lowlands (Cheesman & Winter, 2013; Doughty, 2011; Slot et al., 2014; Ziegler, 2020).

For the páramo, the expected rising temperatures and drying trends for the region (Buytaert et al., 2011; Urrutia & Vuille, 2009) could cause all the carbon stored in the soils to be released, as is happening with tundra soils, but on a smaller scale with the possibility of impacting the worldwide carbon cycle (Chimner & Karberg, 2008). The lack of acclimation observed in these controlled conditions suggests that the páramo vegetation will not increase its photosynthetic capacity and thus  $CO_2$  assimilation in a substantial manner to offset the increase in  $CO_2$  from soil respiration because of warming (Curiel-Yuste et al., 2017) and the páramo could cease to be a carbon sink in the future. While this Colombian Páramos has shown a homeostatic response to warmings after three years of *in situ* manipulation with no significant changes neither in soil and vegetation respiration nor photosynthesis (Lasso et al., 2021), data from an Eddy covariance tower indicates that one Ecuadorian páramo in no longer behaving as a sink but as a source, releasing more carbon than it takes up per day (Carrillo-Rojas et al., 2019).

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## REFERENCES

- Akaji, Y., Inoue, T., Tomimatsu, H., & Kawanishi, A. (2019). Photosynthesis, respiration, and growth patterns of *Rhizophora stylosa* seedlings in relation to growth temperature. *Trees - Structure and Function*, *0*(0), 0–0. <https://doi.org/10.1007/s00468-019-01840-7>
- Atkin, O. K., Edwards, E. J., & Loveys, B. R. (2000). Response of root respiration to changes in temperature and its relevance to global warming: REVIEW Temperature and root respiration. *New Phytologist*, *147*(1), 141–154. <https://doi.org/10.1046/j.1469-8137.2000.00683.x>
- Atkin, O. K., & Tjoelker, M. G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, *8*(7), 343–351. [https://doi.org/10.1016/S1360-1385\(03\)00136-5](https://doi.org/10.1016/S1360-1385(03)00136-5)
- Battaglia, M., Beadle, C., & Loughhead, S. (1996). Photosynthetic temperature responses of *Eucalyptus globulus* and *Eucalyptus nitens*. *Tree Physiology*, *16*(1–2), 81–89. <https://doi.org/10.1093/treephys/16.1-2.81>
- Bernacchi, C. J., Pimentel, C., & Long, S. P. (2003). *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis: Modelling RuBP-limited photosynthesis. *Plant, Cell & Environment*, *26*(9), 1419–1430. <https://doi.org/10.1046/j.0016-8025.2003.01050.x>
- Berry, J., & Bjorkman, O. (1980). Photosynthetic Response and Adaptation to Temperature in Higher Plants. *Annual Review of Plant Physiology*, *31*(1), 491–543. <https://doi.org/10.1146/annurev.pp.31.060180.002423>
- Bond-Lamberty, B., & Thomson, A. (2010). Temperature-associated increases in the global soil respiration record. *Nature*, *464*(7288), 579–582. <https://doi.org/10.1038/nature08930>
- Buytaert, W., Cuesta, F., & Tobon, C. (2011). Potential Impacts of Climate Change on the Environmental Services of Humid Tropical Alpine Regions. *Global Ecology and Biogeography*, *20*, 19–33. <https://doi.org/10.1111/j.1466-8238.2010.00585.x>
- Buytaert, W., Iñiguez, V., & Bièvre, B. D. (2007). The effects of afforestation and cultivation on water yield in the Andean páramo. *Forest Ecology and Management*, *251*(1–2), 22–30. <https://doi.org/10.1016/j.foreco.2007.06.035>
- Castaño-Uribe, C. (2002). Colombia alto andina y la significancia ambiental del bioma páramo en el contexto de los andes tropicales: Una aproximación a los efectos futuros por el cambio climático global (Global Climatic Tensor). In C. Castaño-Uribe (Ed.), *Aproximación al efecto del Global Climatic Tensor en el Bioma Páramo* (pp. 387–387). IDEAM.
- Chapin III, S. J., McFarland, J., McGuire, David, A., Euskirchen, E. S., Ruess, R. W., & Kielland, K. (2009). The changing global carbon cycle: Linking plant-soil carbon dynamics to global consequences. *Journal of Ecology*, *97*(5), 840–850. <https://doi.org/10.1111/j.1365-2745.2009.01529.x>

- Chimner, R. A., & Karberg, J. M. (2008). *Long-term carbon accumulation in two tropical mountain peatlands, Andes Mountains, Ecuador*. 10.
- Cruz, M., & Lasso, E. (2021). Insights into the functional ecology of páramo plants in Colombia. *Biotropica*, 53(5), 1415–1431. <https://doi.org/10.1111/btp.12992>
- Curiel-Yuste, J., Hereş, A. M., Ojeda, G., Paz, A., Pizano, C., García-Angulo, D., & Lasso, E. (2017). Soil heterotrophic CO<sub>2</sub> emissions from tropical high-elevation ecosystems (Páramos) and their sensitivity to temperature and moisture fluctuations. *Soil Biology and Biochemistry*. <https://doi.org/10.1016/j.soilbio.2017.02.016>
- Doughty, C. E. (2011). An In Situ Leaf and Branch Warming Experiment in the Amazon: Warming Experiment in the Amazon. *Biotropica*, 43(6), 658–665. <https://doi.org/10.1111/j.1744-7429.2010.00746.x>
- Doughty, C. E., & Goulden, M. L. (2008). Are tropical forests near a high temperature threshold? *Journal of Geophysical Research: Biogeosciences*, 113(G1), n/a-n/a. <https://doi.org/10.1029/2007JG000632>
- Drake, J. E., Aspinwall, M. J., Pfautsch, S., Rymer, P. D., Reich, P. B., Smith, R. A., Crous, K. Y., Tissue, D. T., Ghannoum, O., & Tjoelker, M. G. (2015). The capacity to cope with climate warming declines from temperate to tropical latitudes in two widely distributed *Eucalyptus* species. *Global Change Biology*, 21(1), 459–472. <https://doi.org/10.1111/gcb.12729>
- Duchicela, S. A., Cuesta, F., Tovar, C., Muriel, P., Jaramillo, R., Salazar, E., & Pinto, E. (2021). Microclimatic Warming Leads to a Decrease in Species and Growth Form Diversity: Insights From a Tropical Alpine Grassland. *Frontiers in Ecology and Evolution*, 9, 673655. <https://doi.org/10.3389/fevo.2021.673655>
- Dusenge, M. E., Duarte, A. G., & Way, D. A. (2019). Plant carbon metabolism and climate change: Elevated CO<sub>2</sub> and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist*, 221(1), 32–49. <https://doi.org/10.1111/nph.15283>
- Dusenge, M. E., Madhavji, S., & Way, D. A. (2020). Contrasting acclimation responses to elevated CO<sub>2</sub> and warming between an evergreen and a deciduous boreal conifer. *Global Change Biology*, 26(6), 3639–3657. <https://doi.org/10.1111/gcb.15084>
- Dusenge, M. E., Wallin, G., Gårdesten, J., Niyonzima, F., Adolfsson, L., Nsabimana, D., & Uddling, J. (2015). Photosynthetic capacity of tropical montane tree species in relation to leaf nutrients, successional strategy and growth temperature. *Oecologia*, 177(4), 1183–1194. <https://doi.org/10.1007/s00442-015-3260-3>
- Dusenge, M. E., & Way, D. A. (2017). Warming puts the squeeze on photosynthesis – lessons from tropical trees. *Journal of Experimental Botany*, 68(9), 2073–2077. <https://doi.org/10.1093/jxb/erx114>
- Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta*, 149(1), 78–90. <https://doi.org/10.1007/BF00386231>
- Gauthier, P. P. G., Crous, K. Y., Ayub, G., Duan, H., Weerasinghe, L. K., Ellsworth, D. S., Tjoelker, M. G., Evans, J. R., Tissue, D. T., & Atkin, O. K. (2014). Drought increases heat tolerance of leaf respiration in *Eucalyptus globulus* saplings grown under both ambient and elevated atmospheric [CO<sub>2</sub>] and temperature. *Journal of Experimental Botany*, 65(22), 6471–6485. <https://doi.org/10.1093/jxb/eru367>
- Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J., & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, 46(1), 5–17. <https://doi.org/10.1093/icb/icj003>

- Gunderson, C. A., O'Hara, K. H., & Champion, C. M. (2009). Thermal plasticity of photosynthesis: The role of acclimation in forest responses to a warming climate. *Global Change Biology*, 15.
- Hernández, G. G., Winter, K., & Slot, M. (2020). Similar temperature dependence of photosynthetic parameters in sun and shade leaves of three tropical tree species. *Tree Physiology*, 40(5), 637–651. <https://doi.org/10.1093/treephys/tpaa015>
- Hernández-Fuentes, C., Bravo, L. A., & Cavieres, L. A. (2015). Photosynthetic responses and photoprotection strategies of *Phacelia secunda* plants exposed to experimental warming at different elevations in the central Chilean Andes. *Alpine Botany*, 125(2), 87–99. <https://doi.org/10.1007/s00035-015-0151-5>
- Heskel, M. A., O'Sullivan, O. S., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., Egerton, J. J. G., Creek, D., Bloomfield, K. J., Xiang, J., Sinca, F., Stangl, Z. R., Martinez-de la Torre, A., Griffin, K. L., Huntingford, C., Hurry, V., Meir, P., Turnbull, M. H., & Atkin, O. K. (2016). Convergence in the temperature response of leaf respiration across biomes and plant functional types. *Proceedings of the National Academy of Sciences*, 113(14), 3832–3837. <https://doi.org/10.1073/pnas.1520282113>
- Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., & Onoda, Y. (2006). Temperature acclimation of photosynthesis: Mechanisms involved in the changes in temperature dependence of photosynthetic rate. *Journal of Experimental Botany*, 57(2), 291–302. <https://doi.org/10.1093/jxb/erj049>
- Janzen, D. H. (1967). Why Mountain Passes are Higher in the Tropics. *The American Naturalist*, 101(919), 233–249. <https://doi.org/10.1086/282487>
- Jump, A. S., & Peñuelas, J. (2005). Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters*, 8(9), 1010–1020. <https://doi.org/10.1111/j.1461-0248.2005.00796.x>
- Keating, P. L. (1999). Changes in Páramo Vegetation Along an Elevation Gradient in Southern Ecuador. *The Journal of the Torrey Botanical Society*, 126(2), 159–175. JSTOR. <https://doi.org/10.2307/2997292>
- Kroner, Y., & Way, D. A. (2016). Carbon fluxes acclimate more strongly to elevated growth temperatures than to elevated CO<sub>2</sub> concentrations in a northern conifer. *Global Change Biology*, 22(8), 2913–2928. <https://doi.org/10.1111/gcb.13215>
- Kumarathunge, D. P., Medlyn, B. E., Drake, J. E., Tjoelker, M. G., Aspinwall, M. J., Battaglia, M., Cano, F. J., Carter, K. R., Cavaleri, M. A., Cernusak, L. A., Chambers, J. Q., Crous, K. Y., De Kauwe, M. G., Dillaway, D. N., Dreyer, E., Ellsworth, D. S., Ghannoum, O., Han, Q., Hikosaka, K., ... Way, D. A. (2019). Acclimation and adaptation components of the temperature dependence of plant photosynthesis at the global scale. *New Phytologist*, 222(2), 768–784. <https://doi.org/10.1111/nph.15668>
- Lasso, E., Matheus-Arbeláez, P., Gallery, R. E., Garzón-López, C., Cruz, M., Leon-Garcia, I. V., Aragón, L., Ayarza-Páez, A., & Curiel Yuste, J. (2021). Homeostatic Response to Three Years of Experimental Warming Suggests High Intrinsic Natural Resistance in the Páramos to Warming in the Short Term. *Frontiers in Ecology and Evolution*, 9, 55. <https://doi.org/10.3389/fevo.2021.615006>
- Leon-Garcia, I. V., & Lasso, E. (2019). High heat tolerance in plants from the Andean highlands: Implications for páramos in a warmer world. *PLoS ONE*, 14(11), 1–14. <https://doi.org/10.1371/journal.pone.0224218>
- Loveys, B. R., Atkinson, L. J., Sherlock, D. J., Roberts, R. L., Fitter, A. H., & Atkin, O. K. (2003). Thermal acclimation of leaf and root respiration: An investigation comparing inherently fast-

- and slow-growing plant species. *Global Change Biology*, 9(6), 895–910.  
<https://doi.org/10.1046/j.1365-2486.2003.00611.x>
- Luteyn, J. (1999). Páramos: A checklist of plant diversity, geographical distribution, and botanical literature. Costa Rica and Panama. *Memoirs of the New York Botanical Garden*, 84, 138–141.
- Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, 4(192).  
<https://doi.org/10.3389/fgene.2013.00192>
- Moore, C. E., Meacham-Hensold, K., Lemonnier, P., Slattery, R. A., Benjamin, C., Bernacchi, C. J., Lawson, T., & Cavanagh, A. P. (2021). The effect of increasing temperature on crop photosynthesis: From enzymes to ecosystems. *Journal of Experimental Botany*, 72(8), 2822–2844. <https://doi.org/10.1093/jxb/erab090>
- O'Sullivan, O. S., Weerasinghe, K. W. L. K., Evans, J. R., Egerton, J. J. G., Tjoelker, M. G., & Atkin, O. K. (2013). High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal high-temperature limits to respiratory function. *Plant, Cell and Environment*, 36(7), 1268–1284. <https://doi.org/10.1111/pce.12057>
- Peng, F., Jung, C. G., Jiang, L., Xue, X., & Luo, Y. (2019). Thermal acclimation of leaf respiration varies between legume and non-legume herbaceous. *Journal of Plant Ecology*, 12(3), 498–506. <https://doi.org/10.1093/jpe/rty042>
- Sage, R. F., & Kubien, D. S. (2007). The temperature response of C<sub>3</sub> and C<sub>4</sub> photosynthesis. *Plant, Cell & Environment*, 30(9), 1086–1106. <https://doi.org/10.1111/j.1365-3040.2007.01682.x>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Sendall, K. M., Reich, P. B., Zhao, C., Jihua, H., Wei, X., Stefanski, A., Rice, K., Rich, R. L., & Montgomery, R. A. (2015). Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming. *Global Change Biology*, 21(3), 1342–1357. <https://doi.org/10.1111/gcb.12781>
- Sentinella, A. T., Warton, D. I., Sherwin, W. B., Offord, C. A., & Moles, A. T. (2020). Tropical plants do not have narrower temperature tolerances but are more at risk from warming because they are close to their upper thermal limits. *Global Ecology and Biogeography*, 29(8), 1387–1398. <https://doi.org/10.1111/geb.13117>
- Slot, M., & Kitajima, K. (2015). General patterns of acclimation of leaf respiration to elevated temperatures across biomes and plant types. *Oecologia*, 177(3), 885–900. <https://doi.org/10.1007/s00442-014-3159-4>
- Slot, M., Rey-Sánchez, C., Gerber, S., Lichstein, J. W., Winter, K., & Kitajima, K. (2014). Thermal acclimation of leaf respiration of tropical trees and lianas: Response to experimental canopy warming, and consequences for tropical forest carbon balance. *Global Change Biology*, 20(9). <https://doi.org/10.1111/gcb.12563>
- Slot, M., & Winter, K. (2016). The Effects of Rising Temperature on the Ecophysiology of Tropical Forest Trees. In G. Goldstein & L. S. Santiago (Eds.), *Tropical Tree Physiology* (Vol. 6, pp. 385–412). Springer International Publishing. [https://doi.org/10.1007/978-3-319-27422-5\\_18](https://doi.org/10.1007/978-3-319-27422-5_18)
- Slot, M., & Winter, K. (2017). In situ temperature relationships of biochemical and stomatal controls of photosynthesis in four lowland tropical tree species. *Plant Cell and Environment*, 40(12). <https://doi.org/10.1111/pce.13071>
- Smith, N. G., & Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale models: Incorporating acclimation to temperature and CO<sub>2</sub>. *Global Change Biology*, 19(1), 45–63. <https://doi.org/10.1111/j.1365-2486.2012.02797.x>

- Smith, N. G., McNellis, R., & Dukes, J. S. (2020). No acclimation: Instantaneous responses to temperature maintain homeostatic photosynthetic rates under experimental warming across a precipitation gradient in *Ulmus americana*. *AoB PLANTS*, *12*(4), plaa027. <https://doi.org/10.1093/aobpla/plaa027>
- Urrutia, R., & Vuille, M. (2009). Climate Change Projections for the Tropical Andes Using a Regional Climate Change Model: Temperature and Precipitation Simulations for the 21st Century. *Journal of Geophysical Research*, *114*. <https://doi.org/10.1029/2008JD011021>
- Von Caemmerer, S. (2000). *Biochemical models of leaf photosynthesis*. CSIRO Publishing.
- Wang, H., Atkin, O. K., Keenan, T. F., Smith, N. G., Wright, I. J., Bloomfield, K. J., Kattge, J., Reich, P. B., & Prentice, I. C. (2020). Acclimation of leaf respiration consistent with optimal photosynthetic capacity. *Global Change Biology*, *26*(4), 2573–2583. <https://doi.org/10.1111/gcb.14980>
- Way, D. A., & Oren, R. (2010). Differential responses to changes in growth temperature between trees from different functional groups and biomes: A review and synthesis of data. *Tree Physiology*, *30*(6), 669–688. <https://doi.org/10.1093/treephys/tpq015>
- Way, D. A., & Sage, R. F. (2008). Thermal acclimation of photosynthesis in black spruce [*Picea mariana* (Mill.) B.S.P.]. *Plant, Cell and Environment*, *31*(9), 1250–1262. <https://doi.org/10.1111/j.1365-3040.2008.01842.x>
- Way, D. A., & Yamori, W. (2014). Thermal acclimation of photosynthesis: On the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research*, *119*(1–2). <https://doi.org/10.1007/s11120-013-9873-7>
- Wythers, K. R., Reich, P. B., Tjoelker, M. G., & Bolstad, P. B. (2005). Foliar respiration acclimation to temperature and temperature variable Q<sub>10</sub> alter ecosystem carbon balance. *Global Change Biology*, *11*(3), 435–449. <https://doi.org/10.1111/j.1365-2486.2005.00922.x>
- Yamori, W., Noguchi, K., & Terashima, I. (2005). Temperature acclimation of photosynthesis in spinach leaves: Analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell and Environment*, *28*(4), 536–547. <https://doi.org/10.1111/j.1365-3040.2004.01299.x>

## SUPPORTING INFORMATION

**Table S1.** Summary statistics results for photosynthetic parameters: Optimal temperature ( $T_{opt}$ ); optimal net assimilation rate ( $A_{400,opt}$ ); unitless shape parameter describing spread of the parabola (b); Maximum potential rate of electron transport ( $J_{max}$ ); Maximum Rubisco activity rate ( $V_{cmax}$ ) in 5 different páramo species. T-test (no marks), Mann-Whitney U-Test ( $\Delta$ ), Heteroscedasticity t-test ( $\Delta\Delta$ ). Statistically significant values are shown in bold.

Species	Control			Warm			Acclimation Index	Shapiro-test		F-test for homocedasticity		T-test		
	Mean	Se	n	Mean	Se	n		w	p-value	F	p-value	t	df	p-value
<i>Puya goudotiana</i>														
$P_{400,opt}$	0.56	0.13	3	1.57	0.44	3	64%	0.89	0.29	0.08	0.16	-2.18	4	0.09
$T_{opt}$	20.63	3.00	3	28.83	3.54	3	28%	0.91	0.44	0.72	0.84	-1.77	4	0.15
b	0.001	0.0001	3	0.003	0.001	3	67%	0.73	<b>0.01</b>	0.94	0.39	3.00	-	0.7 $\Delta$
$J_{max20}$	23.15	6.29	4	22.03	5.64	4		0.96	0.82	1.25	0.86	0.13	6	0.89
$V_{cmax20}$	25.00	0.00	4	25.00	0.00	4		-	-	-	-	-	-	-
<i>Espeletia grandiflora</i>														
$A_{400,opt}$	3.98	1.71	3	4.58	0.63	3	13%	0.99	0.99	6.70	0.26	-0.33	4	0.76
$T_{opt}$	17.55	0.52	3	15.84	0.70	3	-11%	0.97	0.87	0.83	0.90	1.96	4	0.12
b	0.02	0.01	3	0.01	0.001	3	-56%	0.80	0.08	67.07	<b>0.03</b>	0.79	4	0.47 $\Delta\Delta$
$J_{max20}$	42.96	11.61	4	74.25	10.16	4		0.99	0.99	1.04	0.93	-2.03	7	0.08
$V_{cmax20}$	23.68	1.32	4	25.10	2.59	4		0.93	0.45	0.21	0.23	-0.45	7	0.67
<i>Orthrosanthus chimboracensis</i>														
$A_{400,opt}$	2.99	0.63	3	5.79	0.37	3	48%	0.93	0.59	0.52	0.62	-3.83	4	<b>0.019</b>
$T_{opt}$	17.26	1.91	3	27.35	0.80	3	37%	0.92	0.48	1.18	0.92	-4.87	4	<b>0.008</b>
b	0.01	0.002	3	0.01	0.001	3	48%	0.98	0.96	1.06	0.97	-2.66	4	0.056
$J_{max20}$	61.94	5.21	4	59.52	9.48	4		0.89	0.25	0.30	0.35	0.22	6	0.83
$V_{cmax20}$	28.41	7.00	4	21.54	4.60	4		0.95	0.74	2.32	0.51	0.82	6	0.44
<i>Valeriana pilosa</i>														
$A_{400,opt}$	4.77	0.42	3	4.87	2.38	3	2%	0.98	0.93	0.03	0.06	-0.60	2.12	0.61
$T_{opt}$	19.75	3.25	3	21.43	0.98	3	8%	0.92	0.49	10.96	0.17	0.53	4	0.62
b	0.01	0.0003	3	0.01	0.01	3	0%	0.75	0.30	2.36	0.20	3.00	-	0.7
$J_{max20}$	129.26	32.57	5	132.37	33.47	5		0.91	0.32	0.95	0.96	0.07	8	0.95
$V_{cmax20}$	37.18	8.85	5	38.78	4.85	5		0.97	0.92	3.33	0.27	-0.16	8	0.88
<i>Diplostephium phyllicoides</i>														
$A_{400,opt}$	5.16	0.53	3.00	10.58	5.15	5	51%	0.82	<b>0.02</b>	23.49	<b>0.01</b>	9.00	-	0.90 $\Delta$
$T_{opt}$	14.79	1.96	3.00	20.48	1.14	4	28%	0.81	<b>0.03</b>	4.06	0.10	1.00	-	<b>0.032<math>\Delta</math></b>
b	0.02	0.01	3.00	0.03	0.01	3	36%	0.81	<b>0.02</b>	23.40	<b>0.01</b>	5.00	-	0.29 $\Delta$
$J_{max20}$	98.92	16.40	5.00	124.61	41.08	5		0.88	0.12	0.16	0.11	-0.58	8	0.58
$V_{cmax20}$	28.65	3.33	5.00	38.43	10.18	5		0.81	0.02	1.54	0.25	11.00	-	0.84

**Table S2.** Summary statistics results of the descriptive parameters for the response of respiration to temperature in 5 different páramo species. T-test (no marks), Mann-Whitney U-Test (A) or Heteroscedasticity T-test (AA) for respiration parameters: the temperature sensitivity term ( $Q_{10}$ ), slopes (b) and intercepts (m) of the log-transformed curves of respiration response to temperature, and the three coefficients of quadratic regressions on natural log-transformed respiration rates ( $a$ ,  $b$ ,  $c$ ) Statistically significant values are shown in bold.

Species	Control			Warm			Shapiro-test		F-test for homocedasticity		T-test			
	Parameters	Mean	Se	n	Mean	Se	n	w	p- value	F	p- value	t	df	p- value
<i>Puya goudotiana</i>														
$Q_{10}$	1.70	0.18	3	2.09	0.24	4	0.97	0.90	0.43	0.62	1.16	5	0.30	
b	3.78	0.69	3	5.77	0.92	3	0.96	0.83	0.57	0.73	-1.74	4	0.16	
m	1.17	0.25	3	1.79	0.27	3	0.97	0.89	0.87	0.93	-1.68	4	0.17	
a	-0.002	0.0006	3	-0.003	0.001	3	0.95	0.74	0.12	0.47	0.28	4	0.80	
b	0.12	0.04	3	0.22	0.08	3	0.84	0.17	0.17	0.56	-0.95	4	0.41	
c	-2.20	0.42	3	-3.78	1.07	3	0.82	0.13	0.10	0.44	1.11	4	0.35	
<i>Espeletia grandiflora</i>														
$Q_{10}$	1.82	0.12	5	2.16	0.20	5	0.95	0.70	0.35	0.34	-1.44	8	0.19	
b	4.38	0.49	4	4.36	0.41	4	0.86	0.11	1.37	0.80	0.03	6	0.97	
m	1.38	0.13	4	1.42	0.14	4	0.88	0.20	0.90	0.93	-0.23	6	0.83	
a	-	0.0004	0.0003	3	-0.001	0.0003	3	0.95	0.72	0.99	1.00	4	0.16	
b	0.09	0.01	3	0.12	0.02	3	0.94	0.67	0.06	0.75	-1.50	4	0.21	
c	-2.06	0.34	3	-2.24	0.13	3	0.93	0.55	7.01	0.25	0.51	4	0.64	
<i>Orthrosanthus chimboracensis</i>														
$Q_{10}$	1.94	0.07	4	1.93	0.08	3	0.91	0.41	1.05	0.95	0.07	5	0.95	
b	4.52	0.68	3	4.76	0.14	3	0.78	<b>0.04</b>	0.59	0.49	6.00	-	0.70 <sub>A</sub>	
m	1.38	0.17	3	1.48	0.09	3	0.92	0.48	3.21	0.48	-0.51	4	0.63	
a	-	0.0006	0.0001	3	-0.0021	0.0007	3	0.83	0.11	0.04	0.08	4	0.12	
b	0.09	0.002	3	0.17	0.03	3	0.83	0.11	0.01	<b>0.01</b>	-2.68	2.02	0.115 <sub>AA</sub>	
c	-2.07	0.13	3	-2.79	0.33	3	0.97	0.92	0.76	0.86	1.65	4	0.18	
<i>Valeriana pilosa</i>														
$Q_{10}$	2.16	0.24	4	1.71	0.12	4	0.89	0.24	3.96	0.29	1.71	6	0.14	
b	5.19	0.76	3	3.77	0.84	3	0.97	0.89	0.81	0.89	1.26	4	0.28	
m	1.77	0.23	3	1.26	0.23	3	0.99	0.99	1.01	0.99	1.60	4	0.19	
a	-0.003	0.0009	3	-0.002	0.002	3	0.89	0.31	0.37	0.54	-0.37	4	0.73	
b	0.17	0.04	3	0.18	0.09	3	0.82	0.10	0.22	0.36	-0.09	4	0.93	
c	-2.57	0.58	3	-2.47	0.90	3	0.89	0.34	0.28	0.44	-0.09	4	0.94	

<i>Diplostephium phyllicoides</i>													
Q <sub>10</sub>	2.07	0.07	5	1.91	0.06	3	0.96	0.80	2.42	0.63	1.64	6	0.15
b	4.10	1.10	3	3.17	0.86	3	0.97	0.87	1.64	0.76	0.67	4	0.54
m	1.49	0.26	3	1.25	0.12	3	0.83	0.10	4.39	0.37	0.87	4	0.43
a	-0.002	0.0008	3	-0.0007	0.0003	3	0.82	0.11	13.68	0.38	-1.26	4	0.30
b	0.16	0.04	3	0.09	0.009	3	0.81	0.11	37.31	0.23	1.29	4	0.29
c	-2.11	0.74	3	-1.62	0.28	3	0.84	0.16	9.99	0.44	-0.50	4	0.65

**Table S3.** Summary statistics results. Heteroscedasticity T-test for other leaf traits measured: Relative Growth Rate (RGR); Leaf mass per area (LMA), Leaf nitrogen content (N<sub>mass</sub>), the critical temperature at which F<sub>v</sub>/F<sub>m</sub> starts to decline (T<sub>crit</sub>), the temperature at which 50% and 95% reduction of initial F<sub>v</sub>/F<sub>m</sub> value occurred (T<sub>50</sub> and T<sub>95</sub>, respectively) in 5 different páramo species. Statistically significant values are shown in bold.

Species	Control			Warm			Shapiro-test		T- test			
	Parameters	Mean	Se	n	Mean	Se	n	w	p- value	t	df	p- value
<i>Puya goudotiana</i>												
RGR	0.56	0.02	4	0.38	0.02	5	0.92	0.39	5.12	7	<b>0.001</b>	
LMA	218.2	5.80	4	224.72	21.80	3	0.97	0.93	-0.33	5	0.75	
N <sub>mass</sub>	0.34	0.02	4	0.39	0.05	4	0.98	0.96	-0.88	6	0.41	
T <sub>crit</sub>	40.4	0.40	3	43.10	0.15	3	0.83	0.11	-6.31	4	<b>0.003</b>	
T <sub>50</sub>	48.2	0.40	3	50.40	0.30	3	0.92	0.48	-4.41	4	<b>0.01</b>	
T <sub>95</sub>	56.2	1.40	3	57.60	0.70	3	0.81	0.07	-0.89	4	0.42	
<i>Espeletia grandiflora</i>												
RGR	0.1	0.02	5	0.09	0.04	4	0.92	0.41	0.39	7	0.05	
LMA	222.0	17.30	4	199.6	27.40	5	0.96	0.82	0.65	7	0.54	
N <sub>mass</sub>	0.30	0.03	5	0.47	0.06	5	0.94	0.54	-2.63	8	<b>0.03</b>	
T <sub>crit</sub>	45.40	-	1	44.70	1.60	2	0.93	0.48	0.28	1	0.83	
T <sub>50</sub>	53.40	-	1	49.80	0.05	2	0.77	<b>0.05</b>	42.15	1	<b>0.02</b>	
T <sub>95</sub>	61.40	-	1	54.70	1.80	2	1.00	0.83	2.15	1	0.28	
<i>Orthrosanthus chimboracensis</i>												
RGR	0.49	0.06	4	0.27	0.07	3	0.95	0.73	2.54	5	<b>0.05</b>	
LMA	149.8	6.30	4	152.9	7.50	5	0.92	0.40	-0.30	7	0.78	
N <sub>mass</sub>	0.44	0.02	4	0.53	0.06	5	0.83	<b>0.04</b>	-1.37	7	0.21	
T <sub>crit</sub>	40.03	2.60	3	42.80	1.50	3	0.87	0.21	-0.91	4	0.41	
T <sub>50</sub>	54.40	0.80	3	46.50	0.90	3	0.97	0.90	-0.94	4	0.40	
T <sub>95</sub>	50.70	1.50	3	50.00	0.80	3	0.80	0.06	0.46	4	0.67	

<i>Valeriana pilosa</i>											
RGR	0.23	0.007	4	0.43	0.03	5	0.85	0.07	-6.10	7	<b>0.0005</b>
LMA	114.7	11.00	5	87.30	8.60	5	0.96	0.84	1.98	8	0.08
N <sub>mass</sub>	0.80	0.06	5	0.83	0.02	4	0.89	0.21	-0.44	7	0.67
T <sub>crit</sub>	42.50	1.10	2	41.70	0.70	3	0.83	0.13	0.70	3	0.53
T <sub>50</sub>	45.10	0.60	2	46.60	0.50	3	0.88	0.32	-1.89	3	0.15
T <sub>95</sub>	47.40	0.10	2	51.30	1.70	3	0.72	<b>0.01</b>	-1.73	3	0.18
<i>Diplostephium phyllicoides</i>											
RGR	0.25	0.02	4	0.15	0.05	5	0.90	0.25	1.72	7	<b>0.13</b>
LMA	193.7	29.30	5	313.3	35.70	5	0.93	0.50	-2.59	8	<b>0.03</b>
N <sub>mass</sub>	0.46	0.03	5	0.71	0.03	4	0.96	0.76	-5.67	7	<b>0.001</b>
T <sub>crit</sub>	44.70	1.50	3	45.50	0.70	3	0.91	0.43	-0.49	4	0.65
T <sub>50</sub>	48.40	0.70	3	49.40	0.50	3	0.99	0.98	-1.28	4	0.27
T <sub>95</sub>	51.80	0.20	3	53.10	0.50	3	0.88	0.29	-2.46	4	0.07

**Table S4.** Post-hoc tests after the mixed-effect model for  $V_{\text{cmax}}$  and  $J_{\text{max}}$ . Simply pairwise comparison between treatments at different  $T_{\text{set}}$  levels (A) and simply pairwise comparisons between different  $T_{\text{set}}$  levels independently of treatment (B). Statistically significant values are shown in bold.

(A) Effect of Treatment on $V_{\text{cmax}}$ at each $T_{\text{set}}$							
<i>O. chimboracensis</i>							
$V_{\text{cmax}}$	DFn	DFd	$F$	$p$	$p. \text{adj}$		
T10	1	5	3.48	0.121	0.484		
T20	1	5	0.846	0.4	1		
T30	1	5	11.1	<b>0.021</b>	0.084		
T40	1	5	3.25	0.131	0.524		
(B) Effect of $T_{\text{set}}$ on $V_{\text{cmax}}$ and $J_{\text{max}}$							
Specie	Parameter	Pairwise comparison	n	statistic	df	$p$	$p. \text{adj}$
<i>P. goudotiana</i>	$V_{\text{cmax}}$	T10-T40	6	-9.09	5	0.0003	<b>0.002</b>
<i>P. goudotiana</i>	$V_{\text{cmax}}$	T20-T40	6	-9.44	5	0.0002	<b>0.001</b>
<i>P. goudotiana</i>	$V_{\text{cmax}}$	T30-T40	6	-9.15	5	0.0003	<b>0.002</b>
<i>D. phyllicoides</i>	$V_{\text{cmax}}$	T10-T20	8	-4.31	7	0.004	<b>0.021</b>
<i>O. chimboracensis</i>	$J_{\text{max}}$	T20-T30	7	-5.13	6	0.002	<b>0.013</b>
<i>O. chimboracensis</i>	$J_{\text{max}}$	T30-T40	7	8.87	6	0.0001	<b>0.00068</b>

### 3. CHAPTER III

#### Thermal requirements for Paramo's seed germination

(Submitted to Seed Science Research)

##### Abstract

The páramos, tropical alpine ecosystems of the northern Andes, are considered biodiversity hotspots, hosting more than 60% of endemic species. Climate models predict that global temperatures will increase by 2-5°C by the end of this century. Yet, little is known about the consequences of rising temperatures on the seed germination of páramo species, a critical stage in the life cycle of plants that could determine the survival and distribution of species in the future. We examined seed germination responses of 17 páramo species under three temperature treatments: the optimum germination temperature for many alpine species (20/10°C) and other two incubation temperatures with 5°C and 10°C above those temperatures to simulate mild (25/15°C) and extreme future events (30/20°C). We found that seed germination thermal responses were specie specific. For some species, germination may improve in a nearby future warmer scenario, while for others may be reduced. For páramo species, as for other alpine species, relatively high temperatures (20-25°C) are required for optimal germination, suggesting that species are not particularly vulnerable to warming at the germination stage. When species were pooled by their distribution range, we found that contrary to our initial expectation, seeds of species with widespread distribution have lower percentage germination and slower germination rates than seeds of species with restricted distribution and are more affected by the highest temperatures.

##### 3.1 INTRODUCTION

Tropical alpine ecosystems are one of the most vulnerable terrestrial ecosystems to global environmental change and one of the less studied (Buytaert et al., 2011). The largest extension of this type of ecosystem is found in the northern Andes, where they are known as páramos ( e.g., Hofstede et al., 2003). Páramos host a unique type of flora and are considered biodiversity hotspots (Madriñán et al., 2013; Myers et al., 2000), where more than 60% of the plant species are endemic (Luteyn, 1999; Rangel, 2000; Schmidt-Mumm & Vargas Ríos, 2012) and projected to go extinct with global warming (Malcolm et al. 2006). Páramos are also known for their excellent water regulation capacity and are crucial for local water supply for cities, agriculture, and hydropower (Chevallier et al., 2010, Buytaert et al., 2011); and are one of the most important carbon sinks in the tropics (Hofstede, 1999). Despite providing these essential environmental services at both, local and global scales and being recognized as highly vulnerable to environmental changes, páramos have been little studied concerning their flora susceptibility to warming.

Climatic models predict that temperatures will rise more in the high mountains than at lower elevations (Bradley et al., 2004, 2006), and projections for the northern Andean region converge on a mean increase of  $3 \pm 1.5^\circ\text{C}$  in the next century (Buytaert et al., 2011). There is a consensus that an increase of more than 2°C would be catastrophic for the páramos ecosystem (Greenpeace, 2009). Páramo species will have to move upward to an increasingly shrinking area (Kelly & Goulden, 2008), adapt, or go locally extinct (Holt, 1990, Buytaert et al., 2011). For instance, more than 2000 endemic plant species are predicted to go extinct during the next 100 years in the Andes (Malcolm et al., 2006). The potential threats of global climate changes on páramos ecosystem processes and the services they provide are multiple and beg for experimental studies to start understanding how climate changes will affect these ecosystems.

Seed germination is a crucial developmental process in the life cycle of plants and controls the establishment success and future survival and distribution of a species. There is a consensus in the literature that temperature is the primary environmental variable affecting the germination rate of

temperate alpine seeds and that relatively high temperatures are required for optimal germination after a cold stratification period (Billings & Mooney, 1968; Cavieres & Arroyo, 2000; Schütz et al., 2002). Soil warming above ambient temperature by solar radiation seems to be an important pre-requisite of alpine and sub-arctic plant recruitment (Körner, 2003; Milbau et al., 2009) and may ensure that seedling initiates their life in the most favorable part of the year (Billings & Mooney, 1968) and in safe sites with warmer temperatures. In the tropical mountains, however, where temperatures are pretty constant through the year but quite variable on a daily basis, where seeds can be exposed to temperatures as low as  $-2^{\circ}\text{C}$  and as high as  $26^{\circ}\text{C}$  in a single day, thermal requirements for seed germination are less clear. Some species seem to be able to germinate in relatively warm conditions but only after a cold pretreatment, as was reported for *Espeletia schultzii*, whose seeds only germinate after being cold pretreated and showed an optimum temperature of  $17^{\circ}\text{C}$  (Pannier, 1969). Vargas et al. (2014) studied germination requirements of 13 páramo species in Colombia and found that around 70% had an optimum temperature for germination of  $20/10^{\circ}\text{C}$  (day/night), and 30% of them also germinated in high numbers at higher temperatures ( $30/20^{\circ}\text{C}$ ). Besides those essential works, detailed knowledge of thermal germination requirements of most páramo species is generally lacking.

To improve our knowledge of the thermal germination requirement of páramo plants and to start understanding how climate change may eventually affect germination and subsequent establishment success in the páramo ecosystem, we established a lab-controlled experiment with three temperature incubation treatments to follow germination to determine the effect of temperature in different seed germination parameters for each species. One of the incubation temperatures selected corresponds to today's high night and day temperatures and is considered the optimum germination temperature for many alpine species ( $20/10^{\circ}\text{C}$ ; Baskin & Baskin, 1998), and the other two incubation temperatures were  $5^{\circ}\text{C}$  and  $10^{\circ}\text{C}$  above those temperatures, to simulate mild ( $25/15^{\circ}\text{C}$ ) and extreme future events ( $30/20^{\circ}\text{C}$ ). Additionally, we selected species with different geographic distributions to evaluate if the species distribution could be used as a proxy to identify species potentially susceptible to warming. The hypothesis is that species with restricted distribution, limited to the páramos at high elevation sites, will be more thermally specialized and have a higher temperature impact in germination than species with widespread distribution (Janzen, 1967). In this study, we aim to answer the following questions: 1) What is the effect of temperature on the final percentage of germination (FPG), the germination rate (GR), and the mean time of germination (MTG) in páramo species? 2) Are those parameters different among species with different distribution ranges so that we could use the current distribution range to proxy species vulnerability at the seed germination stage?

## 3.2 MATERIALS AND METHODS

### 3.2.1 Study site and plant material

All seeds for the experiment were collected in the páramo at “Parque Ecológico Matarredonda” located at 3290-3400 m.a.s.l. ( $4^{\circ} 33' \text{ N}$ ,  $74^{\circ} 00' \text{ W}$ ), in the northeast range of Los Andes, 40 minutes from Bogota city. The mean annual temperature at the páramo is  $8.7^{\circ}\text{C}$ , and the annual precipitation is 1178 mm (Curiel-Yuste et al., 2017). We selected seventeen species typically found in the páramos with different altitudinal distribution ranges (**Table 1**).

**Table 1.** Species used in the germination trials are classified by family, growth form, and distribution range. Data on occurrences of specimens for each species were obtained from <http://www.gbif.org/> and <http://www.tropicos.org/>.

Species	Family	Growth form	Elevation range	Geographical range	Distribution range
<i>Puya santosii</i>	Bromeliaceae	Rosette	3000-3700	Co	Restricted
<i>Puya goudotiana</i>	Bromeliaceae	Rosette	2760-3550	Co	Restricted
<i>Espeletia grandiflora</i>	Asteraceae	Rosette	2600-4100	Co	Restricted
<i>Acaena cylindristachya</i>	Rosaceae	Rosette	2500-4300	Cr, Co, V, Pe & Bo	Restricted
<i>Diplostephium phyllicoides</i>	Asteraceae	Shrub	2120-3800	Co	Restricted
<i>Gaultheria hapalotricha</i>	Ericaceae	Shrub	2700-3900	Co, V, Pe	Restricted
<i>Miconia summa</i>	Melastomataceae	Shrub	1900-3890	Co	Restricted
<i>Bucquetia glutinosa</i>	Melastomataceae	Shrub	2100-4104	Co	Restricted
<i>Senecio formosoides</i>	Asteraceae	Herb	3000-4425	Co, V & E	Restricted
<i>Eryngium humboldtii</i>	Apiaceae	Rosette	1990-4100	Co, Br, Bo, Ur, Ch, Ar, Ar, Bo, Ch,	Widespread
<i>Gaultheria myrsinoides</i>	Ericaceae	Shrubs	600-4700	Co, Cr, E, G, H, M, Pa, Pe, V	Widespread
<i>Vaccinium floribundum</i>	Ericaceae	Shrub	1600-4600	Cr, Co, V, E, Pe, Bo, Ar, Au, Ar, Bo, Ca, Ch, Co,	Widespread
<i>Nertera granadensis</i>	Rubiaceae	Herb	30-4500	Cr, E, H, I, M, N, Ng, Nz, Pa, Par, Pe, V, T,	Widespread
<i>Lachemilla orbiculata</i>	Rosaceae	Herbs	1500-4000	M, Cr, Co, E, Pe, Ar, Bo, Br,	Widespread
<i>Galium hypocarpium</i>	Rubiaceae	Herb	1500-4100	Ch, Co, Cr, E, H, M, N, Pa, Par, Pe, V, Bo, Cr, Co, E,	Widespread
<i>Orthrosanthus chimboracensis</i>	Iridaceae	Herb	1100-4000	H, G, M, Pa, Pe, U, V	Widespread
<i>Valeriana pilosa</i>	Caprifoliaceae	Herb	2200-4300	Co, E, Pe	Widespread

Under geographical range we mention the countries where the species has been reported being Au = Australia, Ar = Argentina, Bo = Bolivia, Br = Brazil, Ca = Caribbean Islands, Ch = Chile, Co = Colombia, Cr = Costa Rica, E = Ecuador, H = Honduras, I = Indonesia, G = Guatemala, M = Mexico, Nz = New Zealand, N = Nicaragua, Ng = New Guinea, Pa = Panama, Par = Paraguay, Pe = Peru, T = Thailand, U = United States, Ur = Uruguay, V = Venezuela,

Seeds were collected between November 2013 and October 2021, depending on each species' seed production timing. However, germination trials were always carried out on freshly collected seeds in a series of germination trials using the same environmental conditions. All seeds were extracted from their fruit immediately after collection and surface sterilized with a 10% bleach solution and stored dry until all seeds were ready for the experiment.

### 3.2.2 Seed germination trials

On a preliminary test, we evaluated whether the species required a cold stratification treatment by subjecting seeds to a 4°C and -20°C conditions for 30 days and then sowing them under a 20/10°C conditions. Those pre-treatments never improved germination and, in most species, reduced or even halted germination; therefore, those data are not presented.

Three incubation temperatures were tested. One corresponds to the highest most common temperatures seeds are exposed to today and is considered optimum germination temperature for alpine species (20/10°C; Baskin & Baskin, 1998), and the other two were  $\pm 5^\circ\text{C}$  and  $\pm 10^\circ\text{C}$  above those temperatures, simulating the maximum temperature at the end of this century in the worse emission scenarios and current heatwaves. Five replicates of 15 seeds per species for each temperature treatment were sown in wet cotton on Petri dishes. Dishes were then wrapped with parafilm to reduce water loss and were arranged haphazardly on one of the three temperature treatments. Seeds were exposed to a 12/12-day/night cycle and received 300  $\mu\text{moles}$  of photons  $\text{m}^{-2}\text{s}^{-1}$  of light intensity during the day. Experiments were conducted on growth chambers PGCL-14 (Percival-scientific, Iowa). The humidity inside the chambers was kept at 65% HR. Seeds were checked every two days for the first four weeks and then weekly for three months and were registered as germinated when we observed that the radicle extruded from the seed coat.

The final percentage of germination (FPG) was calculated as the ratio of seeds with radicles observed at the end of the experiment to all seeds in each petri dish. Germination rate was calculated from the number of seeds germinated per day following Maguire (1962), according to the formula:

$$\text{GR (germinated seeds/days)} = \sum \frac{n_i}{t_i}$$

Where  $n_i$  is the number of germinated seeds in day  $t_i$  and  $t_i$  is the number of days after sowing.

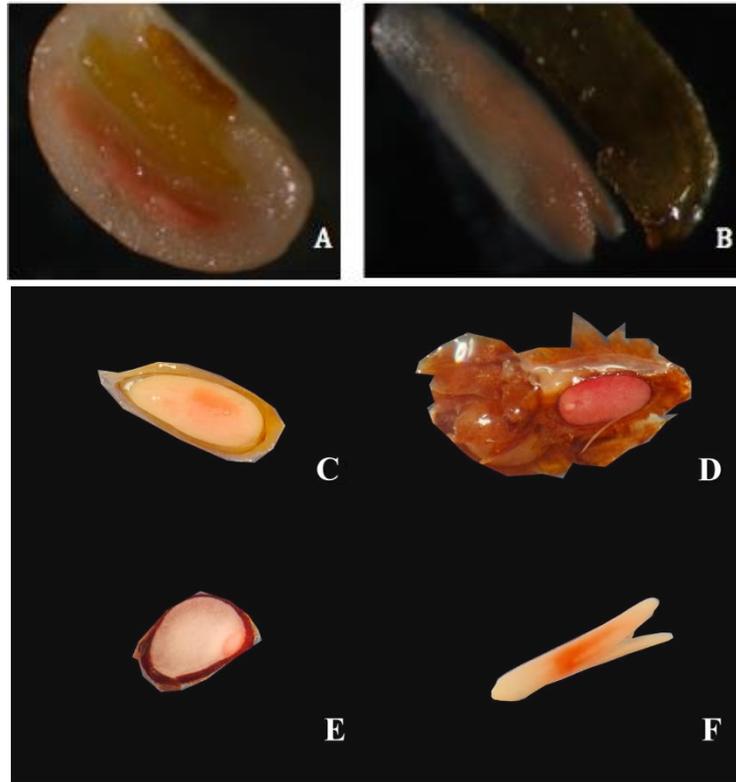
The mean germination time (MGT) is the mean number of days' seeds need to germinate, it was calculated according to González-Zertuche and Orozco-Segovia (1996):

$$\text{MGT (days)} = \frac{\sum(n_i t_i)}{\sum n_i}$$

Again,  $n_i$  is the number of germinated seeds on day  $i$ , and  $t_i$  is the number of days after sowing.

### 3.2.3 Seed viability

To determine the percentage of alive and viable seeds, we used the tetrazolium seed viability test (Craviotto et al., 2011). Seeds were soaked in water overnight and then dissected with a scalpel to expose the embryo to the solution of tetrazolium 1%. Fifteen seeds per species were examined under the microscope and were classified as viable if the embryo and endosperm stained red or pink and as not viable if not (**Figure 1**).



**Figure 1.** Viability Tetrazolium test for *G. hypocarpium* (A), *V. pilosa* (B), *D. phyllicoides* (C), *E. humboldtii* (D), *L. orbiculata* (E), *S. formosoides* (F). Viable seeds stained in light pink.

### 3.2.4 Statistical analysis

To evaluate the effect of temperature on each germination parameter, we performed Kruskal-Wallis tests for each species after testing the homoscedasticity of groups by Levene's tests. We run Dunn tests (Bonferroni adjusted) as post-hoc tests for pairwise comparisons. For this analysis, we examined the results of 225 seeds per species from day 1 to day 90 as follows: 75 seeds on 20/10°C, 75 on 25/15°C, and 75 on 30/20°C. To evaluate if the calculated germination parameter varied between distribution groups (restricted vs. widespread), we pooled all data and performed the Scheirer-Ray-Hare test; after that, we ran Dunn tests (Bonferroni adjusted) post-hoc tests for pairwise comparisons between medians. All data are reported as means  $\pm$  standard error of the mean (Se), and all analyses were performed in R (Version 1.3.1093 - R Development Core Team, 2009–2016).

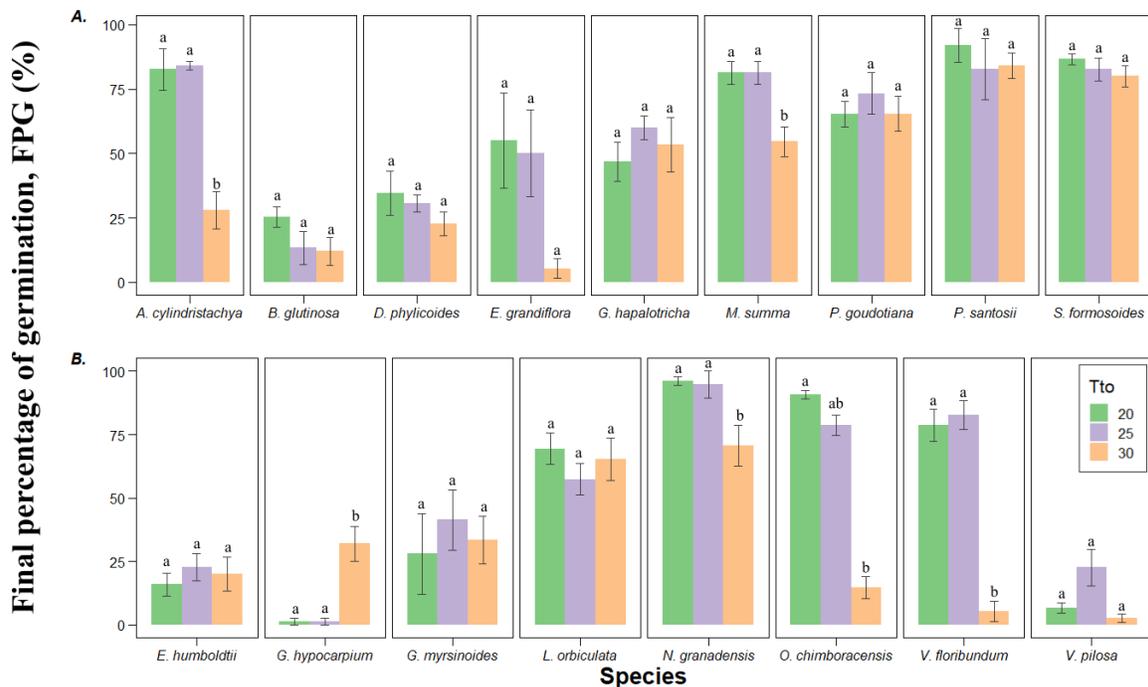
## 3.3 RESULTS

### 3.3.1. Effect of temperature in the final percentage of germination (FPG)

None of the seventeen species evaluated were significantly affected in their final germination (FPG) by the mild warming temperature (25/15°C). Their values of FPG were not significantly different between the temperature treatments that represent today's temperature and the future mild warming (**Figure 2 and S1**). However, five of the 17 species evaluated (29%) had their FPG reduced significantly at the highest temperature treatment (30/20°C). Three of them were species with widespread distribution (*N. granadensis*, *O. chimboracensis*, and *V. floribundum*), and two were species with restricted distribution (*A. cylindristhachya* and *M. summa*; **Figure 2**). One species significantly increased its FPG in response to the highest temperature (*G. hypocarpium*), with FPG values going from 1% at the two lowest

temperature treatments to 32% at 30/20°C ( $w=11.1$ ,  $df=2$ ,  $p\text{-adj}= 0.04$ ; **Figure 2 and Table S1**). The species most affected by the warmest treatment were *V. floribundum* (an *Ericaceae* shrub), whose germination dropped from 83% at 15/25°C to 5% in the 30/20°C treatments ( $w=9.7$ ,  $df=2$ ,  $p\text{-adj}= 0.008$ ) and *O. chimboracensis* (a forb in the *Iridaceae*) which went from 91% of germinated seeds in the cold treatment to 15% in the warmest treatment ( $w=11.9$ ,  $df=2$ ,  $p\text{-adj}= 0.03$ ); both species with widespread distribution (**Figure 2**).

Four species (24% of studied species) had low germination success in all treatments, with less than 25% of their seeds germinating during the whole germination period. *B. glutinosa* (FPG<sub>20/10</sub>=25.3%, FPG<sub>25/15</sub>=13.3%, FPG<sub>30/20</sub>=12%), *E. humboldtii* (FPG<sub>20/10</sub>=16%, FPG<sub>25/15</sub>=22.7%, FPG<sub>30/20</sub>=20%) *V. pilosa* (FPG<sub>20/10</sub>=6.7%, FPG<sub>25/15</sub>=22.7%, FPG<sub>30/20</sub>=2.7%) and *G. hypocarpium* (FPG<sub>20/10</sub>=1.3%, FPG<sub>25/15</sub>=1.3%, FPG<sub>30/20</sub>=32%). This last species increased its germination under the hottest treatment.



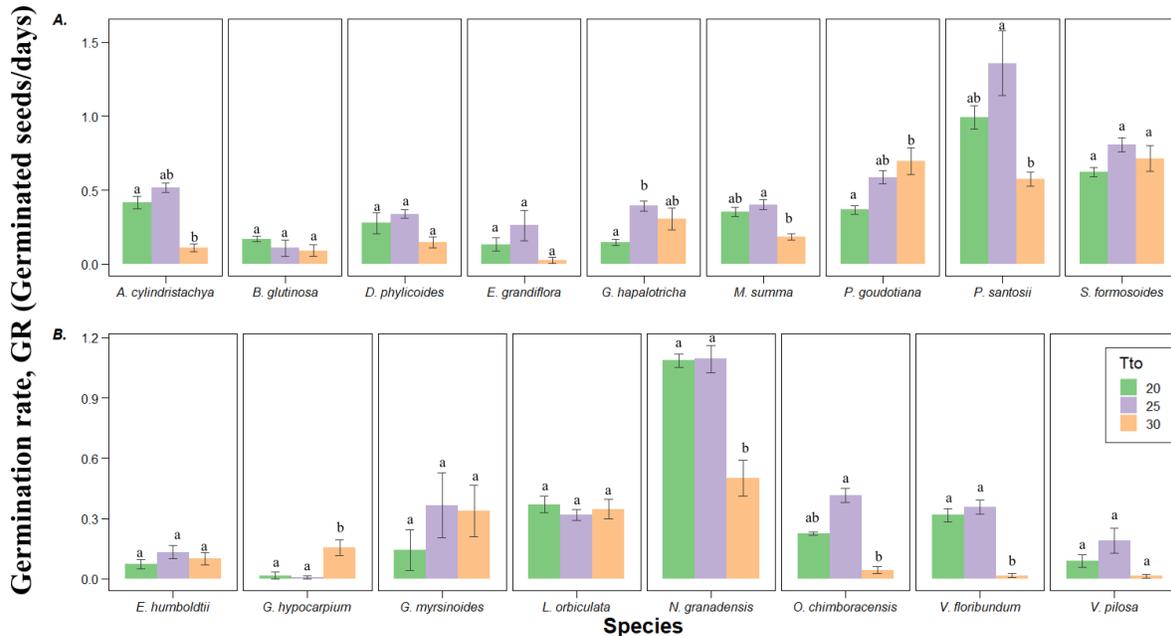
**Figure 2.** Effect of temperature on the final percentage of germination for 17 tropical alpine páramo species. In the upper panel are species with restricted distribution (A), and in the lower panel are species with a widespread distribution range (B). Different color indicates temperature treatments (Tto), green for optimal temperature, purple for mild warming, and orange for temperatures that mimic extreme events. Bars are means  $\pm$  standard error of FPG for each treatment and specie. Letters indicate statistical differences determined by a post-hoc Dunn test and K-W test.

### 3.3.2. Effect of temperature on germination rate (GR)

For the same five species where we saw a negative effect of higher temperatures on the final germination (FPG), we also see a reduction in their germination rate (**Figure 3**). These five species are *A. cylindristachya*, *M. summa*, *N. granadensis*, *O. chimboracensis*, and *V. floribundum*, which show their lowest germination rate when their seeds are exposed to the most extreme warm treatment. On the other hand, the species *G. hypocarpium* shows a positive effect of the higher temperature again, with an increase in the germination rate under the warmest treatment. For almost half of the species in this study,

the mild temperature treatment slightly increased their GR, but the effect was statistically significant only for *G. hapalotricha* (**Figure 3**).

Although we did not see an effect of temperature on the total germination of the two endemic *Puya* species, which generally have high germination values (65-92%), we see that temperature inconsistently affects their germination rate. For *P. santosii*, the germination rate is reduced in the warmest treatment, and for *P. goudotiana*, the opposite happens (**Figure 3**).

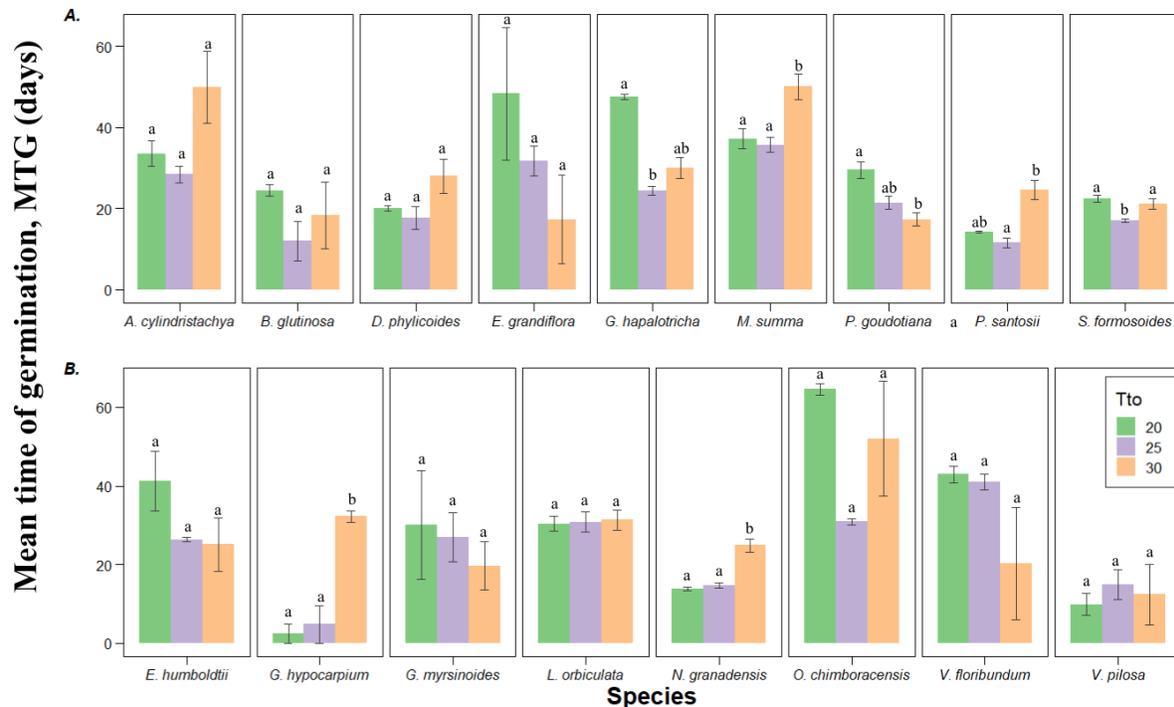


**Figure 3.** Effect of temperature on the germination rate for 17 tropical alpine páramo species. In the upper panel are species with restricted distribution (A), and in the lower panel are species with a widespread distribution range (B). Different color indicates temperature treatments (Tto), green for optimal temperature, purple for mild warming, and orange for temperatures that mimic extreme events. Bars are means  $\pm$  standard error of GR for each treatment and specie. Letters indicate statistical differences determined by a post-hoc Dunn test and K-W test.

### 3.3.3. Effect of temperature on germination times (MTG)

The mean time of germination is a measure of how fast seeds are germinating. Four species, *M. summa*, *N. granadensis*, *P. santosii*, and *G. hypocarpium*, significantly increased the average germination time when sown on the extreme warming treatment (**Figure 4, Table S1**). In *N. granadensis* and *P. santosii* extreme warming treatment almost doubled their germination time, while in *M. summa*, germination time increased by 30%, and in *G. hypocarpium* about eightfold (**Figure 4; Table S1**).

Three species germinated faster in warmer conditions. One of the *Puya* species, *P. goudotiana*, germinated faster in the extreme warming treatment (**Figure 4**), while *G. hapalotricha* and *S. formosoides* had the fastest germination in the mild warming treatment 25/15°C ( $w=10.2$ ,  $df=2$ ,  $p=0.006$  and  $w=9.64$ ,  $df=2$ ,  $p=0.008$ , respectively).



**Figure 4.** Effect of temperature on the mean time of germination for 17 tropical alpine páramo species. In the upper panel are species with restricted distribution (A), and in the lower panel are species with widespread distribution range (B). Different color indicates temperature treatments (Tto), green for optimal temperature, purple for mild warming and orange for temperatures that mimic extreme events. Letters indicate statistical differences as determined by a post-hoc Dunn test and K-W test.

### 3.3.4 Seed viability

The three species with the lowest final germination (FPG) also had the lowest percentage of viable seeds; *B. glutinosa*, *V. Pilosa*, and *G. hypocarpium* have < 33.3% viable seeds. *A. cylindristachya* also had a low percentage of viable seeds (33.3%) but high germination in the germination trials. Five species showed intermediate values of viable seeds that roughly correspond with their intermediate final germination values, *G. myrsinoides* have 50% viable seeds, *P. goudotiana* 57%, *S. formosoides* 53.3%, *V. floribundum* 53.3%, *L. orbiculata* 46.7% and *E. grandiflora* 65% and *G. hapalotricha* with 70% of viable seeds. In *E. grandiflora*, we found seeds parasitized by fly larvae. Four species that showed high germination under páramo conditions in the germination trials also had a higher percentage of viable seeds. They were *N. granadensis* 80%, *P. santosii* 85%, and *M. summa* and *O. chimboracensis*, both with 90%. Two species showed high seed viability but low germination in the trials; they are *D. phyllicoides* with 93.3% and *E. humboldtii* with 86.5% seed viability.

### 3.3.5 Distribution range as a proxy of species vulnerability at the seed germination stage

When data from all species were pooled together in one of the two geographical distribution groups (restricted vs. widespread), we found that seeds from species in the restricted distribution group showed higher values of final percentage of germinating ( $H= 10.49$ ,  $df=1$ ,  $p = 0.001$ ) and higher germination rate ( $H= 17.56$ ,  $df=1$ ,  $p<0.001$ ) than the widespread species (**Figure 5, Table 2**). However, they did not differ in their mean time for germination ( $H= 0.001$ ,  $df=1$ ,  $p = 0.97$ ).

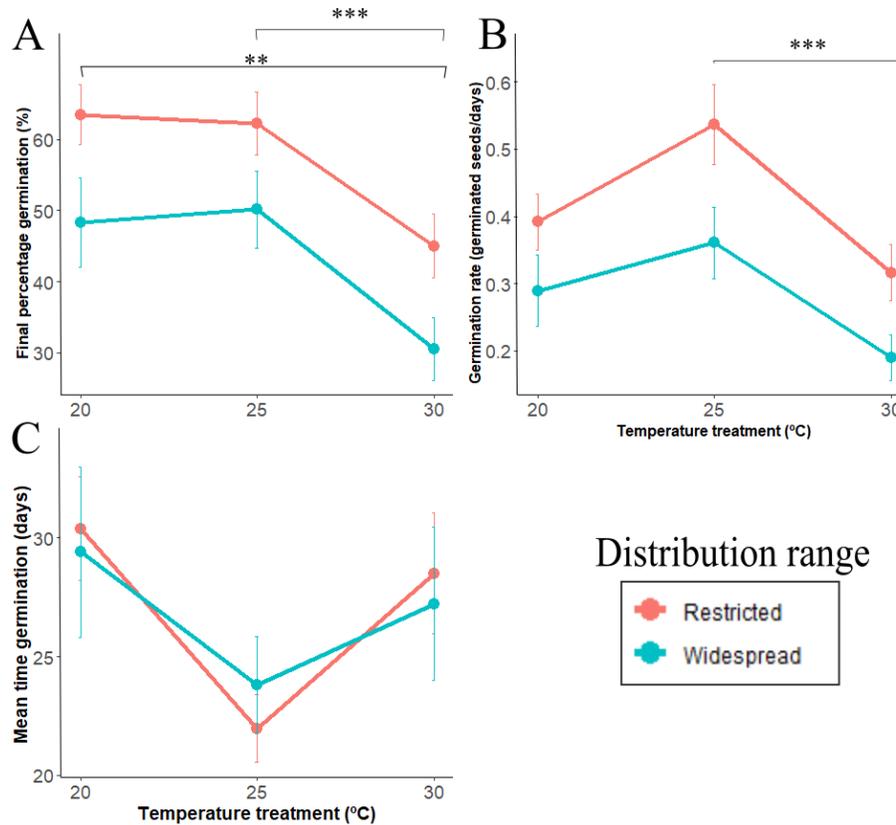
The temperature treatment had a significant effect on all the germination variables (FPG:  $H= 17.10$ ,  $df=2$ ,  $p<0.001$ ; GR:  $H=16.73$ ,  $df=2$ ,  $p= 0<0.001$ ; MTG:  $H= 6.25$ ,  $df=2$ ,  $p=0.04$ ) but the interaction between the

distribution effect and temperature effect was not significant in any case. Seeds sown at the páramo optimal temperature (20/10°C) had similar germination to those exposed to the mild warm temperature (25/15°C). Still, their germination significantly decreased when exposed to the warmest temperature (30/20°C), and this response was similar for both geographical distribution groups (**Figure 5**). The germination rate and the time for germination (MTG) showed a similar pattern in response to temperature. Seeds in the mild temperature (25/15°C) showed higher germination rates and shorter germination times (**Table 2, Figure 5**) than seeds in the other two treatments.

The reduction in FPG in response to the warmest temperature, when the germination at the two extremes (20/10°C and 30/20°C) was compared, shows a higher reduction in the widespread group with a decrease of 37% in comparison to a 29% reduction in the restricted group ( $z=3.57, p=0.001$ , **Table 2, Figure 5**). The germination rate also decreased more in the widespread group, with a 34% reduction compared to the 18% reduction observed in the restricted group ( $z=1.92, p=0.05$ ).

**Table 2.** Summary of the Scheirer-Ray-Hare test and post-hoc Dunn test for final percentage of germination, germination rate and mean time germination. Statistically significant values are shown in bold.

Parameters	<i>H</i>	Df	<i>p</i> -value	Pairwise comparison	<i>Z</i>	<i>P</i> .adj
<b>Final percentage of germination</b>						
Distribution range	10.49	1	<b>0.001</b>	20/10 - 25/15	0.004	0.99
Temperature treatment	17.10	2	<b>&lt;0.001</b>	20/10 - 30/20	3.57	<b>0.001</b>
Interaction	0.07	2	0.96	25/15 - 30/20	3.56	<b>0.0005</b>
<b>Germination rate</b>						
Distribution range	17.56	1	<b>&lt;0.001</b>	20/10 - 25/15	-2.16	<b>0.046</b>
Temperature treatment	16.73	2	<b>&lt;0.001</b>	20/10 - 30/20	1.92	0.06
Interaction	0.12	2	0.94	25/15 - 30/20	4.08	<b>0.0001</b>
<b>Mean time germination</b>						
Distribution range	0.001	1	0.97	20/10 - 25/15	2.35	0.06
Temperature treatment	6.249	2	<b>0.04</b>	20/10 - 30/20	0.41	0.68
Interaction	1.37	2	0.50	25/15 - 30/20	-1.95	0.07



**Figure 5.** Final percentage germination (A), germination rate (B) and mean time of germination (C) under three temperature treatments for species with restricted and widespread geographical and altitudinal distribution. Different color indicates distributional range group, green widespread distribution, and orange for restricted distribution. Statistical differences as determined by a post-hoc Dunn test and S-R-H test. \*\* indicates  $p < 0.01$  and \*\*\*  $p < 0.001$

### 3.4 DISCUSSION

Given the dire predictions of rising temperatures in páramo ecosystems, we need to understand the thermal requirements for páramo seed germination in some depth if we want to understand the future that páramo vegetation will face in the future. In this study, we investigated how rising temperatures affect germination in seventeen páramo plants with different elevational ranges to evaluate whether the distribution range of the species in the mountains could function as a proxy for thermal vulnerability at this stage of the life cycle. To accomplish this, we carried out germination trials in the laboratory at three different temperatures (the optimal germination temperature, 20/10°C, one mimicking mild warming, 25/15°C, and one mimicking extreme warming event, 30/20°C) for nine species with restricted distribution and eight species with widespread distribution. Our result indicates that future mild warming would not negatively affect any of the studied species, in fact, when we consider species grouped by distribution range, both groups (restricted and widespread) had the best germination rates (higher GR) and germinated faster (lower MGT) in this mild warming scenario. Moreover, the treatment simulating extreme future events had no effect in about 50% of the species studied and it had positive effects in two of the species considered in this analysis (*G. hypocarpium* and *P. goudotiana*). For six of the studied species (35%) under extreme warming conditions (30/20°C) there were no effect in more than one germination parameter. These species were *O. chimboracensis*, *V. floribundum*, *A. cylindristachya*, *M.*

*summa*, *N. granadensis*, and *P. santosii*. Moreover, this treatment had the worst adverse effects on the seed germination of all species from both distribution groups, especially on species with widespread distribution. Overall, the results indicate that the thermal germination responses will be species-specific, that future warming will not be as catastrophic for páramo plants at their initial stages, and that species with restricted ranges will be less affected by the extreme warming scenario, in contrast to what was expected.

Understanding the thermal requirements of páramo seeds for optimal germination is critical because this is the most susceptible stage during the life cycle of these plants. In high mountain ecosystems, the greatest losses in plant populations occur during germination and seedling establishment, (Körner, 2003). Due to the expected warming trend in the páramo, the success of germination and seedling establishment could be further reduced. Although there are some studies on seed dispersal (Frantzen & Bouman, 1989; Melcher et al., 2000; Posada, 2013; Velasco-Linares & Vargas, 2008), seed banks (Cardenas et al., 2002; Guariguata & Azocar, 1988), and on light and moisture requirements (Calderón-Hernández & Pérez-Martínez, 2018; Guariguata & Azocar, 1988; Mancipe-Murillo et al., 2018; Pannier, 1969; Vadillo et al., 2004), there are few reports on temperature requirements in seeds of páramo species (Vargas et al., 2014). Here we find a diversity of possible temperature responses associated with each species. Some species are not affected by the increased temperature. Other species are affected by the extremely warm conditions in which their seeds germinate slowly and in fewer numbers. Few species seem to perform better under warmer temperatures.

Species that seem not to be much affected by increased temperature include *B. glutinosa*, *E. humboldtii*, *V. pilosa*, *L. orbiculata*, *G. myrsinoides*, and *E. grandiflora*. This lack of response to temperature could have different explanations depending on the species. For example, in the case of *E. grandiflora*, we found parasitized seeds and wide variability in response among replicates within the same treatment. This has been reported for the subtribe Espeletinae, known locally as frailejones, to which this species belongs (Pérez-Martínez & Calderón-Hernández, 2017; Pérez-Martínez & Velasco-Linares, 2021), and could be masking the effect of temperature on such germinating seeds. In the case of *B. glutinosa* and *V. pilosa*, we also found low seed viability and germination, which may indicate vulnerability of these species at this stage regardless of the temperature (Vargas et al., 2014). In the case of *E. humboldtii*, some dormancy could be occurring since this species has high seed viability but low germination. It is possible that the seeds have other germination requirements and should be treated pre-germination to attain high germination and then determine the effect of temperature on the germination of these seeds.

Besides these few species with no clear germination response to temperature, most species had a range of responses. For example, there were no significant differences between the standard and the mild warm temperature in any germination trial, except for *G. hapalotricha* and *S. formosoides*, for which 25/15°C is the best germination temperature. For *G. hypocarpium*, 30/20°C treatment is the optimal germination temperature, as this widespread herb with low seed viability (33.3%) and low germination percentages in 20/10°C and 25/15°C treatments (1%) strongly increases its FPG (32%) and GR (from 0.02 to 0.16 seeds germinated/day) in higher temperatures while for *P. goudotiana*, there was also a positive effect at 30/20°C treatment because this treatment decreased its mean germination time by half and doubled its germination rate. Vargas et al., (2014) previously reported this treatment as the optimal temperature for three of 13 tropical alpine páramo species (*M. summa*, *P. santosii*, and *Halenia major*, (Vargas et al., 2014)). However, we observed that this temperature had negative consequences for *M. summa*, *P. santosii*, and *N. granadensis*, with lower germination rates and a doubled mean time of germination at these temperatures, as well as profound adverse effects for *O. chimboracensis*, *V. floribundum*, and *A.*

*cylindristachya* due to an excessive reduction in their germination percentages on this treatment that mimic future extreme events.

Seed viability is one of the aspects of germination that offers most information (Calderón-Hernández & Pérez-Martínez, 2018; González, 2015; Mancipe-Murillo et al., 2018; Mora et al., n.d.; Vadillo et al., 2004; Vargas et al., 2014). Our findings broadly agree with previous reports, which show wide ranges of variability in the genus *Puya* (Calderón-Hernández & Pérez-Martínez, 2018; González, 2015; Mora et al., 2007; Vadillo et al., 2004). Likewise, our result coincides with those reported by Vargas et al., (2014) that shows low seed viability for *B. glutinosa* and intermediate values for *G. myrsinoides* and *M. summa*. However, here we found 93.3% of viable seeds for *D. phyllicoides* while Vargas et al., (2014) found 0% viability in this species. This species did have low seed germination percentages, but given the high viability detected it is possible that some physiological dormancy is in place for this species. Our findings, on the other hand, support the findings of Mancipe-Murillo (2018), in which it is suggested that different types of tests should be used to evaluate the viability of each species, because the tetrazolium test not always reveals all the species' germination potential, and this type of test requires specific evaluation conditions for each species (Mancipe-Murillo et al., 2018). Here, for *A. cylindristachya* and *V. floribundum* we found low viability percentages and high germination percentages, implying that other methods would be more appropriate to test seed viability for these species.

Tropical species are always assumed to be more vulnerable to warming because there are no seasons in the tropics, and temperatures are relatively constant throughout the year (Janzen, 1967; Moles et al., 2014). However, not all the tropics are the same, and in the high elevation ecosystems, the daily variation in temperature is tremendous. Previous studies have shown that tropical alpine species are not as vulnerable to warm conditions as expected and that many páramo species seem to tolerate higher temperatures quite well (Cruz & Lasso, 2021; Lasso et al., 2021; Leon-Garcia & Lasso, 2019; Rada et al., 2019). The heat tolerance of the photosynthetic machine was not correlated with their distribution range (Leon-Garcia & Lasso, 2019), as we see in their response to different incubation temperatures. Here we demonstrate that various responses exist within each distribution range group. Moreover, contrary to our initial expectation, we found that species endemic to the alpine ecosystem and with a more restricted distribution range are not as thermal specialists as we thought. Therefore, they seem to be the least vulnerable to higher temperatures. Our result corroborates the importance of studying species at different phenological stages, given that the limiting factors at each stage may be different, and the plant's response too.

The páramo flora is thought to be threatened by high temperatures. However, physiological studies suggest that paramo species can tolerate temperatures a few degrees higher without problems (Lasso et al., 2021; Leon-Garcia & Lasso, 2019). There are not many studies on the effect of high temperatures on germination but the few that exist and ours suggest that this stage and the germination process are not particularly affected by higher temperatures. However, given the variety of responses seen in this group of 17 species, we recommend expanding this type of research and including information on other factors that could be affecting seed germination, such as physical attributes of the seed, the dispersal mode of the species, and phylogenetic background (Mancipe-Murillo et al., 2018) to understand how temperature may affect germination. We also propose to investigate further seed dormancy mechanisms in these tropical high-elevation habitats, where it may be common given nutrient-poor soils (Cavieres, 1999; Vargas et al., 2014). Considering the importance of germination in the persistence of species populations over time and the maintenance of ecosystem structures (Baskin & Baskin, 1998; Fenner, 1995) we need to fully

understand the effect of temperature and other factors that have a significant impact on this process so we can store seeds *ex-situ* and define conservation plans of vulnerable species of the páramo ecosystem.

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## REFERENCES

- Baskin, C. C., & Baskin, J. M. (1998). *Seeds: Ecology, biogeography, and, evolution of dormancy and germination*. Elsevier.
- Bradley RS, Keimig FT, Diaz HF. (2004). Projected temperature changes along the American cordillera and the planned GCOS network. *Geophysical Research Letters* 31: 1-4.
- Bradley RS, Vuille M, Diaz HF, Vergara W. (2006). Climate change. Threats to water supplies in the tropical Andes. *Science* 312: 1755–1756.
- Billings, W. D., & Mooney, H. A. (1968). THE ECOLOGY OF ARCTIC AND ALPINE PLANTS. *Biological Reviews*, 43(4), 481–529. <https://doi.org/10.1111/j.1469-185X.1968.tb00968.x>
- Buytaert W, Cuesta-Camacho F, Tobón C. 2011. Potential impacts of climate change on the environmental services of humid tropical alpine regions. *Global Ecology And Biogeography* 20: 19–33.
- Calderón-Hernández, M., & Pérez-Martínez, L. V. (2018). Seed desiccation tolerance and germination of four Puya (Bromeliaceae) high-andean tropical species from Colombia. *Caldasia*, 40(1), 177–187. <https://doi.org/10.15446/caldasia.v40n1.67740>
- Cardenas, C., Posada, C., & Vargas, O. (2002). Banco de semillas germinable de una comunidad vegetal de paramo humedo sometida a quema y pastoreo (Parque Nacional Natural Chingaza, Colombia). *Ecotropicos*, 15(1), 51–60.
- Cavieres, L. A. (1999). Bancos de semillas persistentes: Modelos de germinación retardada y su aplicación en ambientes alpinos. *Revista Chilena De Historia Natural*, 72, 457–466.
- Cavieres, L. A., & Arroyo, M. T. K. (2000). Seed Germination Response to Cold Stratification Period and Thermal Regime in *Phacelia secunda* (Hydrophyllaceae): Altitudinal Variation in the Mediterranean Andes of Central Chile. *Plant Ecology*, 149(1), 1–8. JSTOR.
- Cruz, M., & Lasso, E. (2021). Insights into the functional ecology of páramo plants in Colombia. *Biotropica*, 53(5), 1415–1431. <https://doi.org/10.1111/btp.12992>
- Curriel-Yuste, J., Hereş, A. M., Ojeda, G., Paz, A., Pizano, C., García-Angulo, D., & Lasso, E. (2017). Soil heterotrophic CO<sub>2</sub> emissions from tropical high-elevation ecosystems (Páramos) and their sensitivity to temperature and moisture fluctuations. *Soil Biology and Biochemistry*. <https://doi.org/10.1016/j.soilbio.2017.02.016>
- Chevallier P, Pouyaud B, Suarez W, Condom T. (2010). Climate change threats to environment in the tropical Andes: glaciers and water resources. *Regional Environmental Change* 11: 179–187.
- Fenner, M. (1995). Ecology of seed banks. In *Seed development and germination*. (pp. 507–528). Marcel Dekker.
- Frantzen, N. M. L. H. F., & Bouman, F. (1989). *Dispersal and growth form patterns of some zonal páramo vegetation types*. 38(4), 449–465.

- González Insuasti, M. S. (2015). Patrones de biología reproductiva de *Puya clava-herculis* y *Puya cryptantha* en ambientes paramunos contrastantes en el departamento de Nariño. *REVISTA DE LA ASOCIACION COLOMBIANA DE CIENCIAS BIOLÓGICAS*, 1(22).  
<https://www.revistaaccb.org/r/index.php/accb/article/view/94>
- González-Zertuche, L. (1996). Métodos de análisis de datos en la germinación de semillas, un ejemplo: *manfreda brachystachya*.
- Greenpeace. (2009). El cambio climático: Futuro negro para los páramos. *Distrito Federal, México. Consejo Nacional Para La ...*, 18–18.
- Guariguata, M. R., & Azocar, A. (1988). Seed Bank Dynamics and Germination Ecology in *Espeletia timotensis* (Compositae), an Andean Giant Rosette. *Biotropica*, 20(1), 54–59. JSTOR.  
<https://doi.org/10.2307/2388426>
- Hofstede, R. (1999). El páramo como espacio para la fijación de carbono atmosférico. In G. Medina, P. Mena, & C. Josse (Eds.), *El páramo como espacio de mitigación de carbono atmosférico* (1st ed., pp. 57–57). Abya Yala.
- Hofstede, R., Hofstede, R., Segarra, P., Mena Visconez, P., & Union Internacional para la Conservacion de la Naturaleza y de los Recursos Naturales. (2003). *Los paramos del mundo: Proyecto Atlas Mundial de los Paramos 2003*. UICN ;
- Janzen, D. H. (1967). Why Mountain Passes are Higher in the Tropics. *The American Naturalist*, 101(919), 233–249. <https://doi.org/10.1086/282487>
- Holt RD. 1990. The microevolutionary consequences of climate change. *Trends in ecology & evolution* 5: 311–315.
- Kelly A.E. and Goulde M.L. (2008). Rapid shifts in plant distribution with recent climate change. *PNAS* 105: 11823-11826.
- Körner, C. (2003). Alpine Plant Life: Functional Plant Ecology Of High Mountain Ecosystems. In *Alpine Plant Life, 2nd Edn*. <https://doi.org/10.1007/978-3-642-18970-8>
- Lasso, E., Matheus-Arbeláez, P., Gallery, R. E., Garzón-López, C., Cruz, M., Leon-Garcia, I. V., Aragón, L., Ayarza-Páez, A., & Curiel Yuste, J. (2021). Homeostatic Response to Three Years of Experimental Warming Suggests High Intrinsic Natural Resistance in the Páramos to Warming in the Short Term. *Frontiers in Ecology and Evolution*, 9, 55.  
<https://doi.org/10.3389/fevo.2021.615006>
- Leon-Garcia, I. V., & Lasso, E. (2019). High heat tolerance in plants from the Andean highlands: Implications for paramos in a warmer world. *PLoS ONE*, 14(11), 1–14.  
<https://doi.org/10.1371/journal.pone.0224218>
- Luteyn, J. (1999). Paramos: A checklist of plant diversity, geographical distribution, and botanical literature. Costa Rica and Panama. *Memoirs of the New York Botanical Garden*, 84, 138–141.
- Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics*, 4(192).  
<https://doi.org/10.3389/fgene.2013.00192>
- Maguire, J. D. (1962). Speed of Germination—Aid In Selection And Evaluation for Seedling Emergence And Vigor1. *Crop Science*, 2(2), crops1962.0011183X000200020033x.  
<https://doi.org/10.2135/crops1962.0011183X000200020033x>
- Malcolm JR, Liu C, Neilson R, Hansen L, Hannah L. (2006). Global Warming and Extinctions of Endemic Species from Biodiversity Hotspots. *Conservation Biology* 20: 538–548.
- Mancipe-Murillo, C., Calderón-Hernández, M., & Pérez-Martínez, L. V. (2018). Evaluación de viabilidad de semillas de 17 especies tropicales altoandinas por la prueba de germinación y la prueba de tetrazolio. *Caldasia*, 40(2), 366–382. <https://doi.org/10.15446/caldasia.v40n2.68251>

- Melcher, I. M., Bouman, F., & Cleef, A. M. (2000). Seed dispersal in paramo plants: Epizoochorous and hydrochorous taxa. *Plant Biology*. <https://doi.org/10.1055/s-2000-9146>
- Milbau, A., Graae, B. J., Shevtsova, A., & Nijs, I. (2009). Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany*, *104*(2), 287–296. PubMed. <https://doi.org/10.1093/aob/mcp117>
- Moles, A. T., Perkins, S. E., Laffan, S. W., Flores-Moreno, H., Awasthy, M., Tindall, M. L., Sack, L., Pitman, A., Kattge, J., Aarssen, L. W., Anand, M., Bahn, M., Blonder, B., Cavender-Bares, J., Cornelissen, J. H. C., Cornwell, W. K., Díaz, S., Dickie, J. B., Freschet, G. T., ... Bonser, S. P. (2014). Which is a better predictor of plant traits: Temperature or precipitation? *Journal of Vegetation Science*, *25*(5). <https://doi.org/10.1111/jvs.12190>
- Mora, F., Chaparro, H. A., & Vargas, O. (2007). Dinámica de la germinación, latencia de semillas y reclutamiento de plántulas en *Puya cryptantha* y *P. trianae*, dos rosetas gigantes de los páramos colombianos. *Ecotropicos*, *20*(1), 31–40.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. <https://doi.org/10.1038/35002501>
- Pannier, F. (1969). Untersuchungen zur Keimung und Kultur von *Espeletia*, eines endemischen Megaphyten der alpinen Zone (“Páramos”) der venezolanischen-kolumbianischen Anden. *Berichte Der Deutschen Botanischen Gesellschaft*, *82*(9), 559–571.
- Pérez-Martínez, L. V., & Calderón-Hernández, M. (2017). Researching ex situ conservation of ‘frailejón’ seeds in Colombia. *Samara*, *30*.
- Pérez-Martínez, L., & Velasco-Linares, P. (2021). *Viveros de paramo para la restauracion ecologica*. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.
- Posada, J. (2013). *El rol de la dispersión de semillas en la regeneración de la vegetación de páramo en un paisaje fragmentado (Tesis de Maestría)*. [Tesis de maestría]. Universidad de los Andes.
- Rada, F., Azócar, A., & García-Núñez, C. (2019). Plant functional diversity in tropical Andean páramos. *Plant Ecology & Diversity*, 1–15. <https://doi.org/10.1080/17550874.2019.1674396>
- Rangel, J. (2000). *La región paramuna y franja aledaña en Colombia* (pp. 1–23).
- Schmidt-Mumm, U., & Vargas Ríos, O. (2012). Comunidades vegetales de las transiciones terrestre-acuáticas del páramo de Chingaza, Colombia. *Revista de Biología Tropical*, *60*(1). <https://doi.org/10.15517/rbt.v60i1.2361>
- Schütz, W., Milberg, P., & Lamont, B. B. (2002). Germination requirements and seedling responses to water availability and soil type in four eucalypt species. *Acta Oecologica*, *23*(1), 23–30. [https://doi.org/10.1016/S1146-609X\(01\)01130-4](https://doi.org/10.1016/S1146-609X(01)01130-4)
- Vadillo, G., Suni, M., & Cano, A. (2004). Viabilidad y germinación de semillas de *Puya raimondii* Harms (Bromeliaceae). *Revista Peruana de Biología*, *11*, 71–78.
- Vargas, O., Pérez-Martínez, L., Insuasty, J., Rodríguez Castillo, N. A., & Melgarejo, L. M. (2014). *Semillas de plantas de páramo: Ecología y métodos de germinación aplicados a la restauración ecológica*.
- Velasco-Linares, P., & Vargas, O. (2008). La dispersión por aves y la restauración de los ecosistemas altoandinos. *Restauración ecológica del bosque altoandino. Estudios diagnósticos y experimentales en los alrededores del Embalse de Chisacá*, 217–228.

## SUPPORTING INFORMATION

**Table S1.** Summary statistics results for germination parameters: Final percentage germination (FPG); germination rate (GR); mean time germination (MTG) in 17 different páramo species. Statistically significant values are shown in bold.

Species	20/10			25/15			30/20			F-test for homocedasticity		Kruskal Wallis-test		Pos hoc test				
	Mean	Se	n	Mean	Se	n	Mean	Se	n	F	p- value	w	df	p- value	20/10	25/15	30/20	p- value
<i>Puya santosii</i>																		
FPG (%)	92.00	6.46	5	82.67	11.85	5	84.00	4.99	5	0.45	0.65	1.94	2	0.40	a	a	a	ns
GR (Germinated seeds/day)	0.99	0.08	5	1.36	0.22	5	0.57	0.05	5	2.73	0.11	10.5	2	0.005	ab	a	b	<b>0.004</b>
MTG (days)	14.15	0.23	5	11.53	1.10	5	24.61	2.44	5	1.69	0.23	10.5	2	0.005	ab	a	b	<b>0.004</b>
<i>Puya goudotiana</i>																		
FPG (%)	65.33	4.90	5	73.33	8.16	5	65.33	6.80	5	0.41	0.68	0.66	2	0.72	a	a	a	ns
GR (Germinated seeds/day)	0.37	0.03	5	0.59	0.05	5	0.70	0.09	5	2.85	0.1	8.84	2	0.01	a	ab	b	<b>0.02</b>
MTG (days)	29.46	2.08	5	21.42	1.59	5	17.35	1.53	5	0.33	0.76	9.4	2	0.009	a	ab	b	<b>0.007</b>
<i>Espeletia grandiflora</i>																		
FPG (%)	55.00	18.53	4	50.00	16.89	4	5.33	3.89	5	1.36	0.3	5.03	2	0.08	a	a	a	ns
GR (Germinated seeds/day)	0.13	0.05	4	0.26	0.10	4	0.03	0.02	5	7.40	0.01	5.65	2.00	0.06	a	a	a	ns
MTG (days)	48.25	16.21	4	31.79	3.67	4	17.33	10.86	5	0.55	0.60	3.12	2.00	0.21	a	a	a	ns
<i>Acaena cylindristachya</i>																		
FPG (%)	82.67	8.06	5	84.00	1.63	5	28.00	7.12	5	1.03	0.39	9.62	2	0.008	a	a	b	<b>0.04</b>
GR (Germinated seeds/day)	0.42	0.04	5	0.52	0.03	5	0.11	0.03	5	0.37	0.7	10.8	2	0.004	a	ab	b	<b>0.003</b>
MTG (days)	33.55	3.07	5	28.37	1.98	5	49.89	8.81	5	3.33	0.07	3.66	2	0.16	a	a	a	ns
<i>Diplostephium phyllicoides</i>																		
FPG (%)	34.67	8.54	5	30.67	3.40	5	22.67	4.52	5	2.13	0.16	1.9	2	0.39	a	a	a	ns
GR (Germinated seeds/day)	0.28	0.07	5	0.34	0.03	5	0.15	0.04	5	2.17	0.16	4.74	2	0.09	a	a	a	ns
MTG (days)	20.12	0.67	5	17.66	2.73	5	27.97	4.21	5	2.3	0.14	4.56	2	0.1	a	a	a	ns
<i>Gaultheria hapalotricha</i>																		

FPG (%)	46.67	7.60	5	60.00	4.71	5	53.33	10.54	5	0.99	0.40	1.59	2	0.45	a	a	a	ns
GR (Germinated seeds/day)	0.15	0.02	5	0.39	0.03	5	0.31	0.07	5	1.17	0.34	8.06	2	0.02	a	b	ab	<b>0.01</b>
MTG (days)	47.44	0.62	5	24.34	1.12	5	30.01	2.51	5	3.43	0.06	10.2	2	0.006	a	b	ab	<b>0.006</b>

Species	20/10			25/15			30/20			F-test for homocedasticity		Kruskal Wallis-test		Pos hoc test				
Parameters	Mean	Se	n	Mean	Se	n	Mean	Se	n	F	p-value	w	df	P-value	20/10	25/15	30/20	P-value

*Miconia summa*

FPG (%)	81.33	4.42	5	81.33	4.42	5	54.67	5.73	5	0.24	0.79	8.12	2	0.02	a	a	b	<b>0.04</b>
GR (Germinated seeds/day)	0.35	0.03	5	0.40	0.03	5	0.18	0.02	5	0.22	0.81	9.98	2	0.007	ab	a	b	<b>0.007</b>
MTG (days)	37.20	2.46	5	35.67	1.77	5	49.98	3.12	5	0.27	0.77	7.98	2	0.02	ab	a	b	<b>0.03</b>

*Bucquetia glutinosa*

FPG (%)	25.33	3.89	5	13.33	6.32	5	12.00	5.33	5	0.54	0.6	3.32	2	0.19	a	a	a	ns
GR (Germinated seeds/day)	0.17	0.02	5	0.11	0.06	5	0.09	0.04	5	1.34	0.30	3.04	2	0.22	a	a	a	ns
MTG (days)	24.47	1.40	5	11.96	4.96	5	18.32	8.14	5	4.02	0.05	3.75	2	0.15	a	a	a	ns

*Senecio formosoides*

FPG (%)	86.67	2.11	5	82.67	4.52	5	80.00	4.22	5	0.47	0.63	1.31	2	0.52	a	a	a	ns
GR (Germinated seeds/day)	0.62	0.03	5	0.81	0.05	5	0.71	0.09	5	0.5	0.62	3.66	2	0.16	a	a	a	ns
MTG (days)	22.39	0.87	5	16.96	0.42	5	21.16	1.34	5	1.04	0.38	9.64	2	0.008	a	b	a	<b>0.01</b>

*Eryngium humboldtii*

FPG (%)	16.00	4.52	5	22.67	5.42	5	20.00	6.67	5	0.54	0.6	0.73	2	0.7	a	a	a	ns
GR (Germinated seeds/day)	0.07	0.02	5	0.13	0.03	5	0.10	0.03	5	0.26	0.77	2.06	2	0.36	a	a	a	ns
MTG (days)	41.28	7.63	5	26.36	0.53	5	25.12	6.78	5	1.86	0.2	3.77	2	0.15	a	a	a	ns

*Gaultheria myrsinoides*

FPG (%)	28.00	15.97	5	41.33	12.00	5	33.33	9.43	5	0.39	0.68	0.88	2	0.64	a	a	a	ns
GR (Germinated seeds/day)	0.14	0.10	5	0.37	0.16	5	0.34	0.13	5	0.34	0.72	2.2	2	0.33	a	a	a	ns
MTG (days)	30.08	13.78	5	26.95	6.30	5	19.67	6.19	5	2.42	0.13	0.38	2	0.83	a	a	a	ns

*Vaccinium floribundum*

FPG (%)	78.67	6.46	5	82.67	5.81	5	5.33	3.89	5	0.45	0.65	9.74	2	0.008	a	a	b	<b>0.04</b>
GR (Germinated seeds/day)	0.32	0.03	5	0.36	0.03	5	0.02	0.01	5	2	0.18	9.69	2	0.008	a	a	b	<b>0.01</b>
MTG (days)	42.92	2.15	5	40.98	1.96	5	20.20	14.27	5	1.41	0.28	4.01	2	0.14	a	a	a	ns

*Nertera granadensis*

FPG (%)	96.00	1.63	5	94.67	5.33	5	70.67	8.06	5	1.73	0.22	7.4	3	0.02	ab	a	b	<b>0.03</b>
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GR (Germinated seeds/day)	1.09	0.03	5	1.09	0.07	5	0.50	0.09	5	0.5	0.62	9.38	2	0.009	a	a	b	<b>0.02</b>
MTG (days)	13.80	0.43	5	14.68	0.62	5	24.88	1.62	5	1.48	0.27	9.62	2	0.008	a	a	b	<b>0.04</b>

Species	20/10			25/15			30/20			F-test for homocedasticity		Kruskal Wallis- test		Pos hoc test					
	Parameters	Mean	Se	n	Mean	Se	n	Mean	Se	n	F	p- value	w	df	P- value	20/10	25/15	30/20	P- value
<i>Lachemilla orbiculata</i>																			
FPG (%)	69.33	6.18	5	57.33	6.18	5	65.33	8.27	5	0.2	0.82	2.06	2	0.36	a	a	a	ns	
GR (Germinated seeds/day)	0.37	0.04	5	0.32	0.03	5	0.35	0.05	5	0.44	0.65	0.62	2	0.73	a	a	a	ns	
MTG (days)	30.39	1.95	5	30.84	2.64	5	31.31	2.60	5	0.48	0.63	0.01	2	0.95	a	a	a	ns	
<i>Galium hypocarpium</i>																			
FPG (%)	1.33	1.33	5	1.33	1.33	5	32.00	6.80	5	3.06	0.08	11.1	2	0.004	a	a	b	<b>0.01</b>	
GR (Germinated seeds/day)	0.02	0.02	5	0.01	0.01	5	0.16	0.04	5	2.5	0.12	10.2	2	0.006	a	a	b	<b>0.02</b>	
MTG (days)	2.40	2.40	5	4.80	4.80	5	32.25	1.47	5	0.18	0.84	11	2	0.004	a	a	b	<b>0.01</b>	
<i>Orthrosantus chimboracensis</i>																			
FPG (%)	90.67	1.63	5	78.67	3.89	5	14.67	4.42	5	1	0.4	11.9	2	0.003	a	ab	b	<b>0.0005</b>	
GR (Germinated seeds/day)	0.22	0.01	5	0.42	0.04	5	0.04	0.02	5	1.85	0.2	12.5	2	0.002	ab	a	b	<b>0.001</b>	
MTG (days)	64.57	1.41	5	30.87	0.83	5	52.00	14.58	5	3.7	0.06	6.19	2	0.05	a	a	a	ns	
<i>Valeriana pilosa</i>																			
FPG (%)	6.67	2.11	5	22.67	7.18	5	2.67	1.63	5	3.5	0.06	5.39	2	0.07	a	a	a	ns	
GR (Germinated seeds/day)	0.09	0.03	5	0.19	0.06	5	0.01	0.01	5	2.9	0.09	5.79	2	0.06	a	a	a	ns	
MTG (days)	9.80	2.80	5	14.94	3.76	5	12.40	7.65	5	0.88	0.44	1.39	2	0.50	a	a	a	ns	

#### 4. CONCLUSIONS

Unique, sky islands in the Northern of South America, or páramos, are strongly threatened by several factors as mining, land-use change and especially, climate change. In the higher elevations of the tropical Andes, climatic projections indicate significant warming by the end of this century which can affect biodiversity, ecosystem services and community resilience of páramos. Although these ecosystems are recognized as hotspots of biodiversity, where high species richness, significative endemism and high rates of speciation are found, nevertheless, páramos are considered especially vulnerable to rapid environmental changes. In all ecosystems, plants can respond to climate change by adaptation, migration or extinction, but in páramo plants the probability of species extinction is higher due to their narrow distribution range, and the overall evidence suggests that most of species are going to migrate to higher altitudes, and as a consequence, the area of páramos will shrink due to warmer environment conditions in the coming years. Due to the great diversity of páramo plants, one might think that vegetation could have very divergent responses to global warming, as, perhaps, the high diurnal variation in temperature and large annual variation in precipitation could shape very different physiological response mechanisms to cope with these stressful environments, in addition that previous ecophysiological research in other páramos has shown that different growth forms may be more or less susceptible to higher temperatures and lower precipitation. However, there have not been enough studies on the different responses that plants may have, i.e., on the capacity of páramo plants to adapt or migrate in response to climate change and it is very important to improve our understanding and ecological predictions about this valuable ecosystem, as well as to increase efforts to understand the effect of higher temperatures on its flora.

These ecosystems characterized by high biological diversity and abundance of plant adaptations to specific stresses in response to páramo unique conditions, seems to have a high degree of convergence between plant ecological strategies and functional groups. This study was conducted including more functional traits than in previous studies in páramos, but confirmed previous results, i.e., a limited number of plant functional types (PFTs) and plant strategies. Based in the functional classification of this study, the pattern presented by different growth forms in previous studies was corroborated, shrubs have different functional adaptations, while the other growth forms are distinctively grouped together. Thus, now there is solid evidence that growth forms can be used as a proxy of PFTs, but special caution must be kept with shrubs which are the growth form with less originality combination traits and that may belong to different PFTs. In the other hand, since most of the páramo plant species belong to S and SC strategies, that means that the range of ecological strategies are limited by páramos' harsh conditions; in other words, although plant adaptations respond to environmental drivers, the functional response converge. Therefore, páramos could be considered areas of high functional redundancy in which resource acquisition processes and species competition respond or affect the ecosystem in more or less the same way, regardless of their adaptations or plant architecture. This implies that, in a future global warming scenario, vulnerability could increase due to the limited range of responses that páramo plants may have, but at the same time, high redundancy could function as a buffer for ecosystem functionality.

The purpose of this functional classification was to simplify the enormous challenge of determining which of the nearly four thousand vascular plant species found in the páramo will be most affected by climate change. After the great diversity of páramo plants was reduced to few PFTs, some species belonging to each PFT were chosen to evaluate the effect of climate change on them and their capacity to adapt or migrate in warmer scenarios.

To assess adaptation response in páramo plants, this study evaluated thermal acclimation of photosynthesis and dark respiration in 5 páramo species: *Puya goudotiana* and *Espeletia grandiflora*, two rosettes belonging to PFT2; two forbs, *Orthrosanthus chimboracensis* and *Valeriana pilosa*, from the PFT1 and one shrub from the same group, *Diplostegium phyllicoides*. The absence of PFT3 representatives was due to the difficulty in establishing healthy and sufficiently strong seedlings of this group for the experiment; however, the results confirmed that physiological responses of plants to high temperatures were species-specific. From the results obtained, it can be concluded that páramo plant species have a limited acclimation capacity for both metabolic processes, photosynthesis and respiration. But there is still promising news, although it seems that páramo plants have little capacity to acclimate their photosynthetic apparatus or respiration to high temperatures, the effect was not negative either. It seems that in a warmer future, the páramos will unfortunately become carbon sinks, but at least the losses of biodiversity and other ecosystem services will be maintained.

To evaluate migration response in páramo plants, this study analyzed the response of seed germination on 17 species belonging to different functional groups and different altitudinal distribution ranges under three temperature treatments, and again the response was found to be species-specific. The results confirm that in a mild warming scenario of 5°C above current páramo temperatures, no changes in the germination capacity would be observed, while in an extreme simulating scenario of +10°C there would be a positive effect in two species and adverse effects in almost 40% of the páramo species, with different intensity of the effect depending on the species. These results could explain the reason for the frequent upward migration observed and projected by various previous research studies, because even if soil temperature were to increase greatly due to global warming, this would have no effect on 50% of the species and would benefit about 10% of them. However, species with negative effects in germination traits, under extreme temperature conditions, would practically go to extinction. Furthermore, these experiments also reveal that for one-third of the species there was no effect of temperature on any of the germination traits and also that species with restricted distribution ranges had a better germination performance. All germination results seem to suggest that most páramo plants will not be dramatically affected under warming scenarios, but for those species that will, the result would be catastrophic.

In conclusion, the rigorous conditions that characterize the páramo ecosystem are the drivers of a large number of morphological adaptations, but at the same time, of a reduced number of functional responses, which are more linked to growth forms. In addition, it was found that páramo plants have a low capacity for thermal acclimation, which was to be expected, since in the páramo, most plants decouple the temperature of the leaf from the ambient temperature. Although páramo plants cannot adapt to the new thermal conditions, these temperature changes neither do not negatively affect the correct performance of plants. And finally, most páramo species can germinate over a slightly wide range of temperatures, so for most organisms, reproductive success is a given. Studying the future of the flora of this ecosystem will require further efforts to break through the current lack of knowledge about the physiology and ecology of paramo plant diversity and to assess the resilience of the ecosystem to global changes.

## 5. APPENDIX

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ORIGINAL ARTICLE

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# Insights into the functional ecology of páramo plants in Colombia

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### Abstract

Páramos are exceptionally species-rich tropical alpine ecosystems that provide several important ecosystem services, including carbon storage and water provision. The responses of páramo plant species to climatic change are largely unknown, but classifying species by functional type and ecological strategy can help reduce complexity for modeling and conservation efforts. We measured 22 traits in 42 plant species with various growth forms in the Colombian Andes. We classified plant ecological strategies using CSR (C: competitor, S: stress tolerator, and R: ruderal) analysis and identified plant functional types (PFTs) using hierarchical clustering of principal components. Additionally, we calculated the functional originality of each species to identify species with unique trait combinations. We identified a high degree of convergence among traits and strategies, with most species characterized by the S and SC strategies. Considering the full set of traits, only three PFTs were identified: one composed of forbs and shrubs with tender leaves (PFT1), another composed only of species with rosettes form (PFT2), and a third composed of shrubs with tough leaves (PFT3). Values of functional originality were generally low, suggesting high redundancy in plant traits. PFT2 species, mostly rosettes, exhibited the highest functional originality of all the groups. Our results imply the existence of a limited set of ecophysiological strategies in the páramos, likely driven by the extremely stressful conditions in these systems. This functional redundancy may indicate heightened vulnerability to environmental changes; however, the presence of multiple species with shared functional traits could provide some resilience to disturbance.

Abstract in Spanish is available with online material.

### KEYWORDS

andes, functional originality, functional traits, high elevation grassland, specific leaf area, tropical alpine ecosystem

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**Appendix A.** Online publication on Biotropica of paper “Insights into the functional ecology of páramo plants in Colombia”

**Seed Science Research**  
**Thermal requirements for Páramo's seed germination**  
 --Manuscript Draft--

<b>Manuscript Number:</b>	SSR-D-22-00063
<b>Full Title:</b>	Thermal requirements for Páramo's seed germination
<b>Article Type:</b>	Research Paper
<b>Keywords:</b>	Climate change; northern Andes; optimum temperature; páramo; seed germination; tropical alpine ecosystems
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<b>Abstract:</b>	The páramos, tropical alpine ecosystems of the northern Andes, are considered biodiversity hotspots, hosting more than 60% of endemic species. Climate models predict that global temperatures will increase by 2-5°C by the end of this century. Yet, little is known about the consequences of rising temperatures on the seed germination of páramo species, a critical stage in the life cycle of plants that could determine the survival and distribution of species in the future. We examined seed germination responses of 17 páramo species under three temperature treatments: the optimum germination temperature for many alpine species (20/10°C) and other two incubation temperatures with 5°C and 10°C above those temperatures to simulate mild (25/15°C) and extreme future events (30/20°C). We found that seed germination thermal responses were specie specific. For some species, germination may improve in a nearby future warmer scenario, while for others may be reduced. For páramo species, as for other alpine species, relatively high temperatures (20-25°C) are required for optimal germination, suggesting that species are not particularly vulnerable to warming at the germination stage. When species were pooled by their distribution range, we found that contrary to our initial expectation, seeds of species with widespread distribution have lower percentage germination and slower germination rates than seeds of species with restricted distribution and are more affected by the highest temperatures.

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**Appendix B.** Online submission to Seed science research of paper “Thermal requirements for páramos seed germination”