Ecology / Ecología

Use of remote sensing and anatomical evidence at contrasting elevations to infer climate change sensitivity: preliminary results in *Pinus patula*

Uso de sensores remotos y evidencia anatómica a elevaciones contrastantes para inferir sensibilidad al cambio climático: resultados preliminares en *Pinus patula*

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Abstract
Background: Indicators of productivity could be useful to identify vulnerable species to climate change, stress and safeguarding sites, and early detection of climate change effects, but require to be developed and tested.
Species study: *Pinus patula* is a tropical Mexican mountain pine on which divergent opinions are available regarding its sensitivity to global warming. Local anecdotes indicate upslope shifts.
Methods: We use a space-for-time substitution to infer climate change sensitivity in *Pinus patula,* testing putative productivity indicators at different elevations: the normalized difference vegetation index (NDVI) and leaf anatomical traits, after following standardization protocols.
Results: As elevation increases, the NDVI, leaf thickness, and the mesophyll width increased, while the xylem-to-mesophyll ratio decreased, probably as different plant manifestations to higher productivity towards the mountain tops. These results concur with other studies showing evidence of more productivity toward higher elevations on the leeward side of the southern Mexico mountains based on NDVI, small mammal abundance, soil macrofungi carpophore cover, and tree basal area. Under global warming, high elevations in south Mexico appear to become more favorable because of their less extreme cold temperatures and higher rainfall.
Conclusions: Our results provide an explanation of previous findings suggesting that global warming could reduce the population size and the habitable area of *Pinus patula*, and the observed upslope shifts. After following standardization protocols, the NDVI, mesophyll width, and xylem-to-mesophyll ratio could be promising tools to assess climate change sensitivity in terrestrial plants and deserve further studies to test their validity in other situations and species.
Key words: ecological indicators, environmental monitoring, global warming, leaf anatomy, NDVI, standardization protocols.

Resumen
Antecedentes: Indicadores de productividad pueden ser útiles para identificar especies vulnerables al cambio climático, sitios adversos o seguros, y oportunamente detectar efectos del cambio climático, pero requieren desarrollarse y probarse.
Especie de estudio: Existen puntos de vista divergentes respecto a la vulnerabilidad del pino mexicano *Pinus patula* al cambio climático. Localmente, se reconocen desplazamientos de esta especie hacia mayores elevaciones.
Métodos: Usamos la sustitución espacio por tiempo y seguimos protocolos de estandarización para inferir la sensibilidad al cambio climático empleando posibles indicadores de productividad a elevaciones contrastantes: el índice diferencial de vegetación normalizado (NDVI) y atributos foliares.
Resultados: El NDVI y los espesores foliares y del mesófilo aumentaron con la elevación y la razón xilema-mesófilo disminuyó, probablemente debido a incrementos en productividad asociados con la elevación. Estos hallazgos coinciden con otros estudios que evidencian una mayor productividad hacia sitios elevados en las regiones de sotavento de las sierras del sur de México basados en el NDVI, abundancia de pequeños mamíferos, cobertura de carpóforos de macromicetos del suelo y área basal arbórea. Con el cambio climático, los sitios elevados parecen tornarse más favorables por sus temperaturas menos frías y mayor precipitación.
Conclusiones: Nuestros resultados pueden explicar estudios previos que sugieren que *P. patula* es sensible al cambio climático. Éste puede disminuir el tamaño poblacional, la superficie habitable y causar los desplazamientos observados. Después de seguir protocolos de estandarización, los indicadores usados pueden servir para estimar sensibilidad climática, pero ameritan estudios posteriores para probar su efectividad en otras situaciones y especies.
Palabras clave: anatomía foliar, calentamiento global, indicadores ecológicos, monitoreo ambiental, NDVI, protocolos de estandarización.

Climate change has been widely recognized as a significant threat to biodiversity (Dawson *et al*. 2011). Because global warming is a common consequence of climate change, range shifts towards higher latitudes (*e.g.,* Parmesan & Yohe 2003) or elevations in mountain species (Brusca *et al*. 2013, Colwell *et al*. 2008), are often observed. Thus, projecting species distributions based on the current species distribution and climate projections models is a preferred approach to infer climate change effects. However, this approach ignores possible alterations of species interactions (Davis *et al*. 1998) and assumes a lack of dispersal constraints or dispersal barriers that limit species occupancy (Normand *et al*. 2011). Furthermore, distributional shifts or local extinctions are the last effects of climate change on the species (Martinez-Meyer 2012). Since the response to climate change should differ among species, a pressing environmental task is assessing the climate sensitivity of the species, allowing us anticipating climate change effects (Aubin *et al*. 2016). This task could be tackled by comparing the species performance along an environmental gradient such as those observed in the mountains. Environmental gradients mimic global change effects, a space-by-time substitution (Araújo & Rahbek 2006, Blois *et al*. 2013), and upslope displacements are the most likely response of mountain species to global warming if higher-altitude sites are available (Colwell *et al*. 2008).

Mountain species are particularly vulnerable to climate change. High temperatures are expected to become more severe at low elevations while at high altitudes, temperatures are becoming milder, and such changes take place at very short distances (Brusca *et al*. 2013; Colwell *et al*. 2008). Furthermore, precipitation is expected to increase with elevation, and water availability is commonly the primary limiting factor for productivity (Churkina & Running 1998). Since individual performance depends in part upon the rate at which organisms take up resources from the environment (Brown *et al*. 2004), individuals in more-favored areas might show higher productivity rates. Thus, global-warming sensitive species should tend to increase their productivity towards higher elevations of their range. Contrastingly, if climate change has no effects, productivity should be lower at the extremes of their altitudinal range, where populations usually are more stressed by limited resources (see Brown 1984, Sexton *et al*. 2009). Alternatively, with adaptive phenotypic plasticity, little or no differences in productivity associated with elevation should be detected. These alternatives need to be explored but require testing putative indicators of plant productivity and developing standardization protocols to hold constant potential confounding factors.

We explored these alternatives in *Pinus patula* Schltdl. & Cham., a tropical mountain pine of southern Mexico abundant at 2,400-2,700 m. According to climate change projections, southern Mexico mountains are becoming drier and warmer (*e.g.*, Sáenz-Romero *et al*. 2010). Thus, the prevailing climatic conditions at a given point in the mountains will likely occur at higher elevations (del Castillo *et al*. 2009, del Castillo *et al*. 2013). Previous approaches to assess climate change effects on this species revealed conflicting results.

Gómez-Mendoza & Arriaga (2007), based on genetic algorithms for niche modeling, current knowledge of plant distributions, and climate change projections, predicted a reduction between 23.4 and 35.7 % relative to the current geographic distribution. Contrastingly, van Zonneveld *et al*. (2009) concluded that this species could withstand the environmental changes projected by climate change models due to its high phenotypic plasticity detected in plantations outside their natural distribution. Adaptive phenotypic plasticity involves constant fitness in different environments through physiological homeostasis (Thompson 1991). If *P. patula* is little affected by climate change, then this species should maintain relative constant productivity through its altitudinal range. Conversely, if this species is sensitive, productivity should increase towards high elevations relative to stressful environments at low altitudes. This study compared how putative indicators of plant productivity change at a low, mid, and high elevation of the altitudinal range of this species: the normalized difference vegetation index (NDVI) and leaf anatomical measures from young and adult individuals.

The NDVI is a productivity estimator based on remote sensing defined as (NIR - RED) / (NIR + RED), where RED and NIR are the red and near-infrared radiation reflected by the vegetation and detected by the multispectral satellite sensors. Most of the RED of solar radiation is absorbed by the plants' chlorophyll to conduct photosynthesis, whereas the adjacent NIR is reflected. The NDVI reveals this contrast and can be taken as an indicator of energy absorption and chlorophyll abundance (Myneni *et al*. 1995). Areas with high photosynthetic activity reflect lower levels of RED than regions with low photosynthetic activity. The NDVI ranges from -1 to +1, and it is strongly and positively correlated with the above-ground net primary productivity (Kerr & Ostrovsky 2003). The NDVI has been recommended as a tool for environmental change studies (Pettorelli *et al*. 2005). To be useful for monitoring the productivity of a given species, such estimates should be taken in monospecific stands of the focus species, in stands in which it is dominant, or at resolution levels at which the individuals can be discriminated. Seasonality is another factor that should be considered as productivity may vary widely among seasons. If moisture is limiting productivity,

the dry season should be the time at which plants are withstanding the highest moisture stress.

Furthermore, during this season, the images display a lower incidence of obstructing clouds. Cloud cover could be a significant source of noise for NDVI analysis (Myneni *et al*. 1995, Pettorelli *et al*. 2005). It can be very problematic in the mountains, particularly at high elevations as mountains generate orographic clouds resulting from water condensation of rising winds (see Banta 1990). Also, the NDVI taken during the dry season has been found useful for productivity comparisons in some cases (Meneses-Tovar 2012, Ramirez-Bautista & Williams 2018).

If high-elevation sites are becoming more suitable for *P. patula* within its altitudinal range, this should be evident in their leaf anatomical traits, which in several taxa tend to display a coordinated variation along with elevation gradients (*e.g.*, Grill *et al*. 2004, Jiménez-Noriega *et al*. 2017). However, the detected trends along resource gradients documented for several taxa in worldwide or regional-level studies, may not be the same to those at local conditions and infraspecific level (Niinemets 2015). The importance of factors that change with elevation and affect plant physiology, such as irradiance, partial pressures of O_2 and CO_2, temperature, and humidity, may depend on the mountain system. In Neotropical mountains whose peaks are far below the timberline, low humidity at lower elevations is probably more stressful for the organisms' physiology than low temperatures at high altitudes, particularly under global warming.

If productivity tends to increase with elevation in *P. patula*, leaves from plants at higher elevations should accumulate more photosynthates elicited by higher moisture levels and low respiration rates associated with lower temperatures. We expect that the photosynthetic leaf tissue (mesophyll) increases towards conditions of higher moisture availability. This increase should be proportionally more elevated than that of conductive tissues, as has been observed in *P. canariensis* (Grill *et al*. 2004). If this correct, leaf thickness should also increase in conditions of high moisture, as the mesophyll layer is a substantial component of the leaves (see below). Indeed, leaf thickness has long been associated with the capacity of the plant to acquire resources, including water (Helmers 1943, Witkowski & Lamont 1991), and with high photosynthetic rates (Niinemets 2001). However, confounding factors that also affect leaf anatomy such the tree age, developmental level, and leaf position in the trees should be considered to ensure meaningful results (see Lin *et al*. 2001, Apple *et al*. 2002, Niinemets *et al*. 2007, Azuma *et al*. 2016, Chin & Sillet 2016).

We examine how NDVI and leaf traits vary in plants of *P. patula* near the lowest (2,400), mid (2,750), and highest (3,100 m asl) altitudinal levels of their distribution range in the Sierra Norte in Oaxaca, southern Mexico. We expect that if climatic conditions are becoming more hospitable towards high elevations, plants at such elevations should show evidence of higher resource acquisition than plants at low- and mid-elevations of their range. We used leaf samples from young and adult individuals to consider possible effects on leaf traits associated with plant age and leaf position and suggest other standardization protocols.

Materials and methods

Study system. Pinus patula is a diploxylon Mexican pine, widely planted in many tropical areas for timber production, has a straight trunk, the height reaches 30-40 m (Farjon 2018, van Zonneveld *et al*. 2009). This pine occurs naturally in limited areas of southern Mexico mountains (Farjon 2018) and is classified as a "least concern species" (IUCN Red List of threatened species, Farjon 2013). Growth is relatively fast. Dominant or subdominant trees reach ~32 m in 25 years and produce ~3m³ of wood in a natural habitat, those figures are higher to those reported in several plantations of *P. patula* in southern Africa (Aguirre-Bravo & Smith 1986). In a seed orchard within its natural range, *P. patula* produced male and female cones 8 years after being planted (Hernández-Zaragoza *et al*. 2016). In the Sierra Norte mountain range, in the Oaxaca state, Mexico, *P. patula* usually occurs between 2,400-2,900 m asl in native temperate pine or pine-oak forests (del Castillo *et al*. 2004, and personal observation). For at least one-decade, local people have noticed more establishments of this species towards higher elevations in this mountain range.

Study sites. Our study was conducted on the leeward side of the Sierra Norte de Oaxaca, a mountain range of southern Mexico, in the municipality of Ixtlán de Juárez in Oaxaca state, Mexico, between 2,100-3,150 m asl (Figure 1). Based on the nearest meteorological stations ($\leq$ 6 km to our sampling sites), the climate is temperate subhumid. Rainfall is very seasonal. Nearly 85 % of the rainfall is concentrated between May and October, due to trade winds from the Gulf of Mexico, which generate copious amounts of rain. By contrast, winter rains are very scarce: less than 6 % of the annual rainfall (Fernandez-Eguiarte *et al*. 2020). Annual precipitation and temperature vary widely with elevation, ranging from 18.3 ºC and 759 mm at 2,150 m asl (Santa Catarina Ixtepeji) to 10 ºC and 1,124 mm at 3,150 m asl (Cuajimoloyas) near the tops of this mountain range. Linear regression analyses indicate that by every 100 m increase in elevation, mean annual rainfall increases 36.5 mm (Zacarías-Eslava & del Castillo 2010), and soil water content increases 7.8 % (Vázquez-Mendoza 2008) (Appendix 1). Thus, our highest elevation site should receive ~260 mm (24 %) more annual rainfall than the
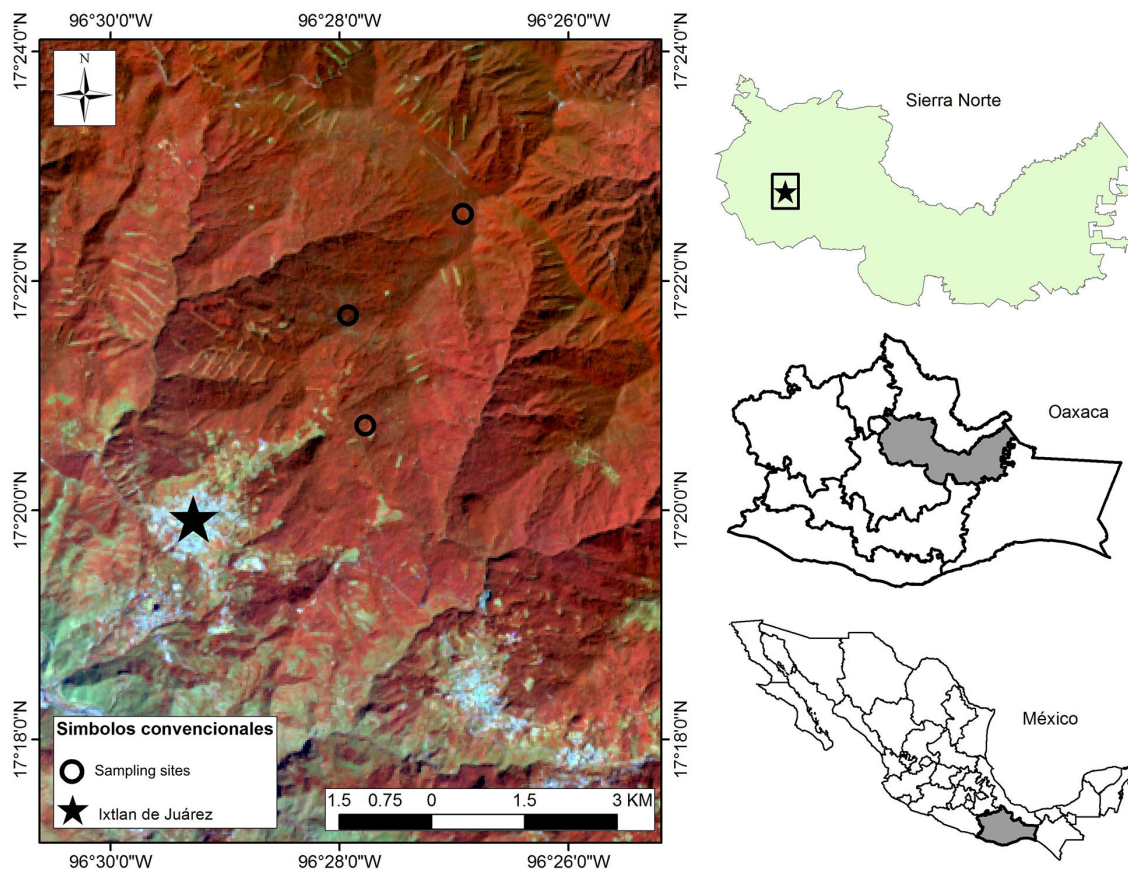
Figure 1. Distribution map of the study sites in Sierra Norte, Oaxaca, Mexico.

lowest-elevation site. Such amount of precipitation, together with its low annual temperature and the consequently low evaporative demand of the atmosphere, should contribute to explain the observed increase in soil water with elevation.

Standardization protocols. As discussed above, the putative indicators of plant performance explored in this study are likely affected by other factors. We took explicit account of other critical possible sources of variation to have meaningful comparisons and enhance result reproducibility. To achieve this goal, we suggested and followed the standardization protocols summarized in Appendix 2. The scientific principles of the indicators and the reasons for standardization are also shown.

NDVI estimations. NDVI estimations were obtained from seven Landsat images taken from 1990 to 2014 during the dry season at a low, mid, and high elevations of the distributional range of *P. patula*. The estimations covered a 1 ha area and were taken from the average of the NDVI values of all the pixels within this area (Appendix 3). Prior analysis, each image was geometrically, radiometrically,

and topographically corrected. Based on the images, the studied stands were nearly monospecific at the tree layer (> 95 % *P. patula*). The sites have been little disturbed as judging by the presence of few trails and cutting stumps and were relatively homogenous in microtopography.

Morphometric analyses. Mature needle samples were obtained from 50 randomly selected plants of which 25 were from young plants (< 10 cm diameter of the trunk at breast height, (1.30 m, dbh), and non-reproductive, and 25 were from adult trees (>15 cm dbh), all of which were reproductive. These two size groups were the most abundant in the study sites. The leaves were collected at the tip of the branches at 1-2 m height in young individuals; and 2.5-4.0 m in adult plants, which correspond to the lowest branches in adult trees. The leaves were kept cold in an ice cooler, brought to the lab, and immediately refrigerated at -20 ºC. For microscopic studies, two leaves per fascicle and plant from five randomly selected trees of each altitude and stage were used. Tissue samples were obtained each of the leaves and were fixed in FAA (formaldehyde, acetic acid, and distilled water, Ruzin 1999). The fixed samples were

vacuumed at 3.45 bar for 15 minutes and left in the vacuum pump for 24 h after that time. Then, the samples were stored in ethylic alcohol until sectioning. We only used the middle part of the leaf to do transverse free-hand sections with a single-edged blade (Appendix 2). Sections were bleached with 50 % commercial Clorox®, double stained with Safranin-fast green, and mounted with synthetic resin. Nine-leaf anatomical characteristics: length of hypodermal cells (µm), width of hypodermal cells (µm), number of resin ducts, length of endodermal cells (µm), width of endodermal cells (µm), endodermal cell wall thickness (µm), mesophyll width (µm), xylem area (µm^2), and phloem area (µm^2) were quantified using an image analysis program (Image-Pro Plus® version 6.1, Media Cybernetics Inc., Maryland, USA).

Statistical analyses. We studied the relationship of the NDVI with elevation (linear and quadratic components) and the stage of the plant (coded as -1 for juveniles and 1 for adults) with multiple regression analyses based on ranks testing the linear and quadratic component (mean - weighted) of altitude, stage and the two-way interactions of stage with the linear and quadratic components of elevation. Rank regression has been found to be a robust technique to address problems of normality and outliers that can be exacerbated with small sample sizes (Potvin & Roff 1993, Chen *et al*. 2014). We conducted a stepwise regression procedure based on the Akaike criterium to find the model that best explains the data using the function step of the library MASS of R (R Core Team 2014). The estimation of the regression coefficients of the best-fitting models were conducted following standard regression analysis, which provide an interpretable estimation of the magnitude of the effect analyzed.

Some of our leaf measurements were likely correlated, as some were taken from the same structure. Furthermore, measures of different leaf tissues in close contact between each other might also covary, as development depends on coordination between different cell layers (Bar & Ori 2014). Therefore, for handling linear dependence and multicollinearity, we analyzed the structure of the data with exploratory factor analysis, with the function principal of the package Psych of R, and the varimax rotation (Revelle 2017). This analysis identifies groups of correlated variables and generates a subset of explanatory variables. The varimax rotation provided an anatomical and straightforward interpretable structure of the data, as described below. We found seven factors that contributed to 88 % of the total variance of the anatomical traits analyzed (Appendix 4). The variables whose loadings has the highest absolute value of each of the seven factors were used as explanatory variables in multiple regression analyses, following Zuur *et al*. (2007). These variables cover the main

components of the leaf anatomy from the external to the inner layers including epidermis (epidermis cell width, factor 7), hypodermis (length of hypodermal cells, factor 5), resin ducts (number, factor 4), endodermis (thickness of endodermal cell wall, factor 2, and width of endodermal cells, factor 3), vascular tissue (xylem area, factor 1), and leaf area in cross-section (factor 6). A further reason for retaining seven factors and not a higher number is that the eigenvalues of such factors were > 1 while the eighth factor has a < 1 eigenvalue. Keeping factors with eigenvalues > 1 has been recommended as a cutoff point for factor retaining following the Kaiser criterion (Yong & Pearce 2013). The explanatory variables were previously standardized by subtracting the mean and dividing by the standard deviation (Z-transformation) to avoid scale-factor errors.

With the seven anatomical variables described above, stage, and the two-way interactions between each morphological variable with stage, we conducted a stepwise regression procedure based on the Akaike criterion, as described above. As in the NDVI statistical analysis, we conducted regression analyses between the explanatory variables that were significant in the best multiple regression analyses described above with elevation. Besides, we analyzed the relationship between mesophyll width with elevation because: (a) The mesophyll is the thickest layer of the leaf and the third more external layer, after the epidermis and the hypodermis; and (b) mesophyll tissue area comprises ~50 % of the total leaf area in cross-section. Because of the position in the leaf and its thickness, we expect that any size change at the mesophyll level will have a more significant effect than any other leaf tissue on leaf thickness. Indeed, (c) mesophyll thickness was the leaf trait to which leaf area in cross-section had the highest correlation (r = 0.61, P = 0.0004). Finally (d), photosynthesis is conducted in this layer. Therefore, any change in mesophyll width should have direct implications on plant growth. We also analyzed the root square of xylem area-to-mesophyll length ratio as an estimate of the ratio of supplied versus supplying tissues (Grill *et al*. 2004) with elevation to explore the possibility of coordinated changes at leaf level between tissues involved in water and mineral transport (xylem) and tissues involved in carbon fixation (mesophyll). We used the root square of the xylem area to have a dimensionless (and more straightforward) ratio since our mesophyll estimation is linear (width).

Results

The NDVI increased linearly 0.21 per 1 km increase in elevation, and the relationship was highly significant (r^2 = 0.73, P = 6.93 × 10^{-7}, rank regression) (Figure 2). Three anatomical traits rendered a significant relationship with elevation. These anatomical traits displayed a similar

pattern in both juveniles and adults. Leaf area showed maximum values at the highest-elevation site and similar values at the lowest and mid-elevation site ($r^2 = 0.43$, $P = 0.002$, rank regression) (Figure 3). Mesophyll width displayed a quadratic relationship with elevation, showing the highest values at the highest elevation ($r^2 = 0.46$, $P = 0.0003$, rank regression) (Figure 4). Finally, the square root of the xylem area-to-mesophyll width ratio significantly decreased with elevation ($r^2 = 0.52$, $P = 5.72 \times 10^{-5}$, rank regression) (Figure 5).
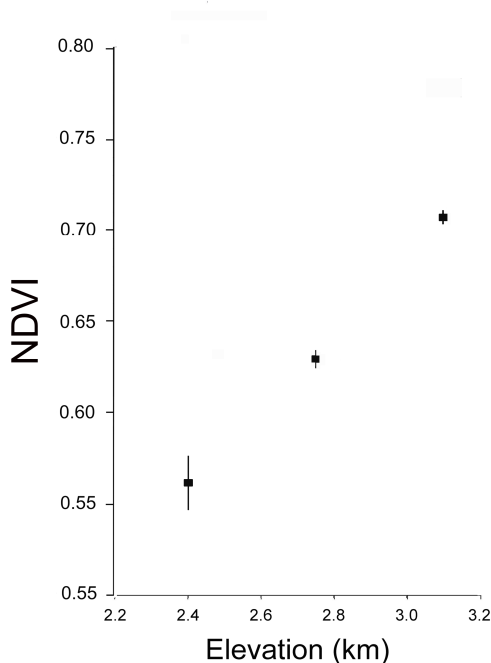

Figure 2. Relationship between the normalized difference vegetation index (*NDVI*) with elevation (*El*) in Sierra Norte, Oaxaca, Mexico. The best model resulted from the stepwise regression procedure and the Akaike criterion is: *NDVI = 0.060 + 0.208 El.* The fitted line and standard errors are also shown.

Discussion

We found remote sensing and anatomical evidence suggesting that *Pinus patula* is sensitive to altitudinal changes on the leeward side of the mountains in Southern Mexico. The highest site near the altitudinal limit of its distribution, and closer to the highest points in these mountains, appears to be more favorable for this species than lower elevation sites. First, NDVI in nearly monospecific stands of *P. patula* increased 20 % on average at the highest altitude site relative to the lowest elevation site and close to the lowest altitudinal limit of this species. Second, at the highest elevation, both adult and juvenile plants of *P. patula* displayed the thickest leaf areas, the

widest mesophyll, and the lowest xylem to mesophyll ratio. These results suggest that high-elevation sites are closer to the physiological optimum in this species. Furthermore, the warm and dry climate below 2,200 m in Sierra Norte could explain, at least in part, the absence of *P. patula* at low elevations on the leeward side of this massif.
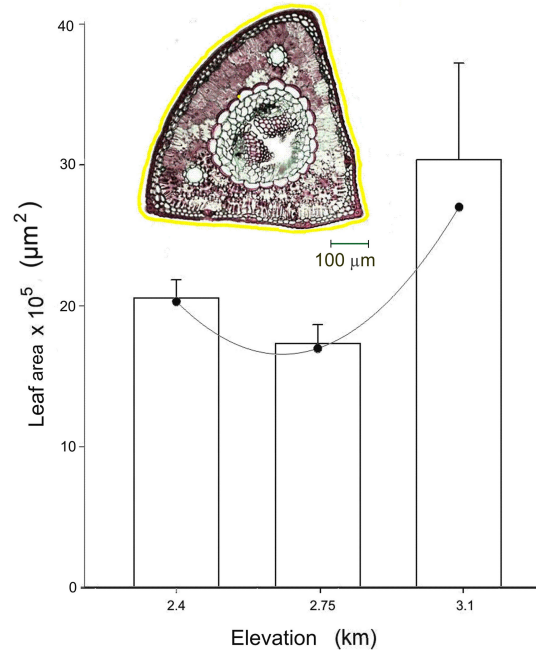

Figure 3. Relationship between leaf area (*LA*) and elevation (*El*). The best fitting regression model among the models including stage and elevation following a stepwise procedure and the Akaike criterion is $Log_{10}[LA] = 4.74 + 0.18 \; El + 1.14 \; \left(El - \overline{El}\right)^2$. The same pattern was found in both young and adult plants and the data of both groups of plants were combined. The fitted line and standard errors are also shown. The insert is a microphotography of the cross section of a leaf. The yellow line shows the contour of the leaf section whose area was estimated.

One possible explanation for the increase in leaf area observed at higher elevations in *P. patula* is a more significant accumulation of photosynthates as detected in other studies (Niinemets 2015). In particular, the higher humidity near the tops of the mountains and less extreme cold temperatures elicited by global warming could explain the highest leaf area. A reduced leaf area has long been found to be associated with water stress in conifers (Helmers 1943, Witkowski & Lamont 1991). We expect more humidity at higher elevation because of a higher rainfall, higher soil moisture content, and low temperatures that should reduce the evaporative demand of the atmosphere (Appendix 1). Indeed, leaf shrinkage is an indicator of water deficit (Chin & Sillet 2016). Increases in leaf thickness may result from a higher rate of carbon gain

and growth resulting from a larger surface for light interception (Lambers *et al*. 1998), and water caption, as conifer needles can take up water from fog and intercepted rainfall (Hacke *et al*. 2015). Water availability is likely the primary limiting factor for productivity over a wide range of conditions worldwide (Churkina & Running 1998). In conifers, water stress is considered the main limiting factor (Rouget *et al*. 2001, Alba-López *et al*. 2003, Eckert *et al*. 2010).
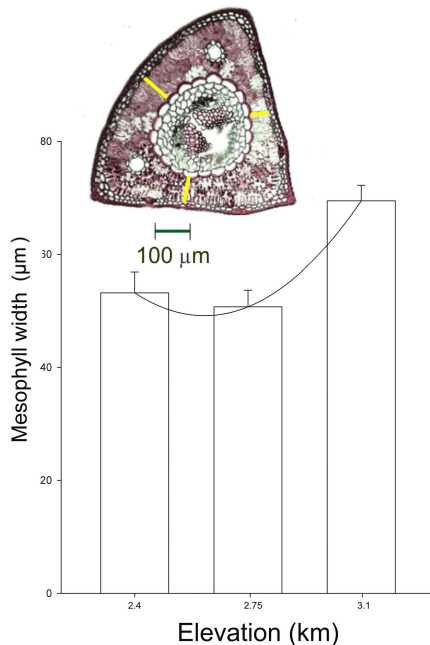

Figure 4. Relationship between mesophyll width (*WM*) and elevation (*El*). The best fitting regression model among the models including stage and altitude following a stepwise procedure and the Akaike criterion is $WM = -13.3 + 23.28 \; El + 86.71 \; \left(El - \overline{El}\right)^2$. The fitted line and standard errors are also shown. The insert is a microphotography of the cross section of a leaf. The yellow lines show the width of the mesophyll.

Irradiance, temperature, and humidity are factors often correlated with elevation, which probably account for changes in leaf thickness in other studies. However, only moisture and temperature appear to explain the observed results in *P. patula*. Increases in irradiance resulted in proportional increases in leaf thickness and the volume fraction of mesophyll in *P. patula* in branches at different heights of the same tree (Niinemets *et al*. 2007). To avoid this confounding factor, we took leaf samples at the same plant position within the two groups studied (adult and young plants, Appendix 2). Furthermore, irradiance levels should be lower at high elevations of Sierra Norte because of the higher rates of fog and cloud formation by the cooling effect typically found in mountain tops (see Banta

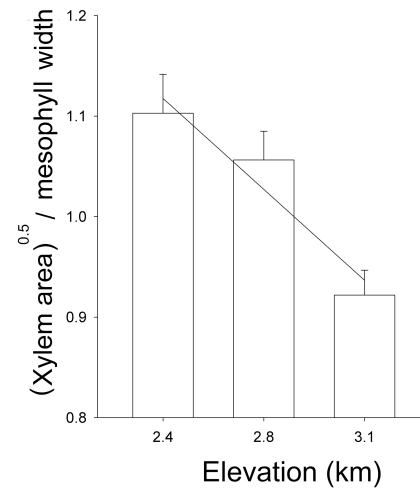1990). Yet, we found that the leaf area in cross-section increased at higher elevations.

Figure 5. Relationship between the square root of xylem area to mesophyll width ratio (*XM*) with elevation (*El*). The best fitting regression model among the models including stage and altitude following a stepwise procedure and the Akaike criterion is: $XM = -1.74 - 0.258 \; El$. The fitted line and standard errors are also shown.

The relationship between temperature and leaf thickness was found to be negative when sites reported as extreme cold are included (Mediavilla *et al*. 2012, González-Zurdo *et al*. 2016). Our most top elevation site cannot be considered as extremely cold and had the highest leaf area in cross-section of all the studied sites. Probably, in species such as *P. patula*, the relationship between leaf area and temperature had a maximum at temperatures close to that observed at our highest point. In other mountain systems with highest peaks and colder temperatures, leaf area could start to decline at some point as temperature decreases. Furthermore, different factors may induce similar changes in leaves, making it challenging to reach generalizations. The use of other pieces of evidence. such as the NDVI, may help to interpret the anatomical results.

The lower xylem-to-mesophyll ratio and the thickest mesophyll at high elevations are likely anatomical pieces of evidence supporting the conclusion that water availability and productivity tends to increase with altitude in *P. patula*. A decreasing ratio of photosynthetic to non-photosynthetic tissue has been associated with lower rates of carbon assimilation and growth (Carey *et al*. 1998, Poorter & Remkes 1990). The maintenance costs of the leaves should be more economical in conditions or high-water supply as their conductive tissues probably are smaller than the photosynthetic tissues and thus proportionately less

demanding of resources. Indeed, water limitation in conifers is associated with higher tracheid diameter (Maherali & DeLucia 2000), and leaf-level vascular investment decreases towards a gradient of water availability (Chin & Sillet 2016).

Therefore, the probabilities of survival and reproduction in *P. patula* should be higher near the tops of these mountains. This statement is in agreement with recent studies indicating that mid-elevation plants are finding better growth conditions at higher elevations owing to global warming in species in tropical mountain areas (Colwell *et al*. 2008, Wilson & Gutiérrez 2012). If climate variations in the mountains associated with elevation mimic global change effects (Araújo & Rahbek 2006, Blois *et al*. 2013), global warming could negatively impact the populations of *P. patula*. The increases in productivity of *P. patula* toward high elevations suggested in our study may explain local anecdotes indicating upslope displacements. It is unlikely that *P. patula* will disappear soon at the lowest elevational sites, at least not because of climate change, as *P. patula* is adapted to wide variability of climates (van Zonneveld *et al*. 2009). But in the mid to long term, global warming could be a severe threat to this species.

Our study suggests that global warming could be a significant threat to *P. patula* by increasing drought risks associated with warm temperatures. Other species appear to be in a similar situation, even in different ecosystems. In boreal tree species, for instance, climate warming may reduce water availability to plants and can be more important than temperature in affecting carbon gain (Reich *et al*. 2018 and reference therein).

On the other hand, the lower temperatures expected at higher elevations do not seem to be a significant constraint for productivity in these mountains, as their tops are far below the altitude above which trees cannot grow because of low temperatures (timberline). In the volcanoes of the Trans-Mexican Volcanic Belt, > 280 km NW of our study site, the timberline is located at ~ 4 km elevation (Holtmeier 2009). The scarcity of high-elevation sites in mountain chains of southern Mexico (see Trejo 2004), could be a significant constraint for species such as *P. patula* affected by climate change, as there are few options for upslope shifts. Conversely, at low elevations of its range, high temperatures and low humidity could be critical factors increasing local extinctions. The evidence of higher productivity towards higher elevation as provided by the NDVI and anatomical features shown in this study offer an ecophysiological explanation to the projections by Gómez-Mendoza & Arriaga (2007), suggesting that global warming could shrink the habitable area of *P. patula* if the current trends in climate continue.

It is not possible to make a general statement of the relationship between productivity and altitude in southern Mexico mountain chains given their complex topography (see Trejo 2004). However, productivity appears to increase at higher elevations in the rain shadow areas of these mountain chains not only in *P. patula* but in a wide variety of organisms. Between 2,100 to 3,100 m asl total plant cover and tree basal area increased with elevation in Sierra Norte in Santa Catarina Ixtepeji (Zacarías-Eslava & del Castillo 2010). The NDVI and the abundance of small mammals increased monotonically between 300 to 2,600 m asl in Cerro Piedra Larga (Ramirez-Bautista & Williams 2018). Furthermore, high elevations on the leeward side of the mountains appear to be more favorable for other organisms with high water requirements. The total cover of carpophores of soil macromycetes increases significantly with elevation in Santa Catarina Ixtepeji Sierra Norte, between 2,100 to 3,120 m asl (Appendix 1). It is well known that the number of carpophores per unit area tends to increase with rainfall (*e.g*., Salerni *et al*. 2001), and that a relatively high soil moisture content is necessary for fruit body initiation (Boddy *et al*. 2014). Higher precipitation and low evaporative demand could explain these results, as precipitation and soil water content tend to increase with elevation in dry areas of the mountains (see Appendix 1).

None of the estimators used in this study are exempt from caveats, and all require standardization protocols, as summarized in Appendix 2. The NDVI based on remote sensing can be very useful in monospecific stands or stands dominated by the focus species but can be useless for understory or rare species. Studies of leaf anatomical traits can be benefited by the wealth of literature studying their associations with environmental factors, which can be very valuable to interpret the data. The relationship with commonly used leaf features such as leaf thickness or specific leaf area with ecological factors is complex (Wilson *et al*. 1999). Nevertheless, the combined use of anatomical attributes with other estimators such as the NDVI can provide a robust conclusion regarding situations of stress or safeguarding in the species, providing they concur with the conclusions derived from the findings they provide.

In conclusion remote sensing and anatomical evidence in both juvenile and adult plants suggest that productivity in *Pinus patula* tends to increase towards higher elevations and that such an increase can be ascribed to a great extent to a higher humidity and less extreme cold temperatures at the highest elevations of the studied mountains. This conclusion can explain local anecdotes indicating upslope shifts in this species, and its absence at altitudes lower than 2200 m asl. Based on the indicators of productivity obtained, we conclude that *P. patula* is sensitive to climate change. Both the limited surface at high elevations and the increasingly drier and warmer climate at low altitudes of its range are factors that could jeopardize the persistence of this species if global warming continues. Leaf anatomical traits

associated with photosynthesis and hydraulic conductivity, such as leaf mesophyll width and xylem-to-mesophyll ratio, and the NDVI could be promising tools as indicators of productivity and climate change sensitivity but require more studies to test their validity in other situations and species.

Acknowledgments

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Appendix 1. Linear regressions between climatic, edaphic and biological variables with elevation (*x,* km) in temperate forest areas of Sierra Norte, Oaxaca, Mexico from 2.1 to 3.2 km elevation.

Response variable (*Y*)	equation	r^2	*P*
Mean annual precipitation (mm) [2]	$Y = 9.1 + 351\ x$	0.98	< 0.03
Mean annual temperature (ºC) [2]	$Y = 32.3 - 7.0\ x$	0.97	< 0.02
Soil water content (%) [1]	$Y = -35.377 + 7.8\ x$	0.999	< 0.005
Tree basal area (m² ha⁻¹) [2]	$Y = -8.152 + 4.7\ x$	0.43	0.001
Carpophore cover area per year in 0.08 ha (m²) [1]	$Y = -1.936 + 1.5\ x$	0.924	< 0.005

[1] Vázquez-Mendoza (2008); [2] Zacarías-Eslava & del Castillo (2010).

Appendix 2. Indicators of plant performance used in this study, their scientific principle, standardization protocols, and reasons for standardization

indicator	scientific principle	standardizations	reasons for standardization
NDVI	Productivity estimations based on a strong light absorption contrast between the red (highly absorbed) and the adjacent near infrared (not absorbed) regions by photosynthetic structures [1]	1 Using images taken during a single season, preferably the dry season for NDVI comparisons	1.1 Minimize time-based atmospheric differences that may alter NDVI.
			1.2 Reduce the probabilities of cloudiness.
			1.3 Reveal stress conditions associated with water limitation.
			1.4 Productivity has been associated with the NDVI in dry season in some studies [2]
			1.5 Allow comparisons with other studies that use dry season images for productivity estimations [3]
		2 Use monospecific spots or spots dominated by the focus species	2.1 Nullify or minimize the effects of light absorbed by other species.
leaf anatomical properties	Anatomic leaf properties such as leaf thickness are correlated with the plant's capacity to acquire resources (see text for details and references)	3. Samples taken from fully developed leaves, at the same height in the plant, and with individuals of similar age.	3.1 Reduce variations in leaf properties that may change with the degree of development of the leaves such as vacuolar content.
			3.2 Cellular components vary with tree or shoot age [4]
			3.3 Leaf properties such as leaf thickness change by irradiance variations such as those caused by self-shading associated with tree height in trees [4,5]
		4. Samples taken at the mid-section of the leaf	4.1 The probabilities a getting samples with different degrees of elongation is higher at the proximal side of the needles, because most of the cell elongation takes place there in plants such as conifers or grasses.
			4.2 The distal section of the needles is more prone to damage by mechanical, desiccation or temperature stresses

[1] Myneni *et al*. (1995), [2] Ramirez-Bautista & Williams (2018), [3] Meneses-Tovar (2012), [4] Chin & Sillet (2016), [5] Niinemets *et al*. (2007)

Appendix 3. Dates of the Landsat images and mean values of NDVI at three elevation intervals in Sierra Norte, Oaxaca Mexico

	Elevation (km)		
Date (d-m-y)	2.30-2.50	2.65-2.85	3.00-3.12
29-04-1990	0.637	0.627	0.671
26-01-1994	0.510	0.628	0.676
26-03-1998	0.575	0.568	0.671
13-03-2002	0.530	0.632	0.657
08-03-2006	0.532	0.624	0.675
22-01-2010	0.608	0.630	0.684
02-02-2014	0.561	0.629	0.707

Appendix 4. Results of the exploratory factor analyses of the morphological variables used in this study, in which the first seven factors were retained. The numbers in bold correspond to the variables with the highest loadings. See text for details

	Factor						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Leaf area in cross section (ln)	0.32	-0.04	0.14	-0.04	0.22	**0.83**	0.09
length of epidermal cells (ln)	-0.02	0.03	0.19	0.13	0.8	0.08	0.37
width of epidermal cells (ln)	-0.04	0.01	0.19	-0.05	0.11	0.2	**0.93**
number of hypodermal cell layers	0.46	0.55	0.13	0.05	-0.05	-0.42	-0.07
length of hypodermal cells (ln)	0.19	-0.2	-0.08	-0.06	**0.84**	0.27	-0.08
width of hypodermal cells (ln)	0.08	-0.57	0.33	0.25	0.23	-0.13	0.59
number of resin ducts	-0.03	0.09	0.06	**0.98**	0.03	0.05	0
length of endodermal cells	0.32	0.13	0.83	0.02	0.09	0.14	0.2
width of endodermal cells	0.04	0.13	**0.93**	0.03	0.01	-0.09	0.11
Thickness of the cell wall of endodermal cells (ln)	0.14	**0.87**	0.29	0.14	-0.08	-0.07	0.02
Mesophyll width	0.2	-0.18	-0.24	0.37	0.3	0.64	0.29
Xylem area (ln)	**0.93**	0.07	0.13	-0.04	0.04	0.13	0.03
Xylem area (ln)	0.9	0.12	0.13	0.01	0.14	0.25	-0.04
	Eigenvalues						
	1	3	5	6	2	7	4
SS loadings	2.19	1.96	1.58	1.53	1.51	1.50	1.20
Proportion variance	0.17	0.15	0.12	0.12	0.12	0.12	0.09
Cumulative variance	0.17	0.32	0.44	0.56	0.68	0.79	0.88
Proportion Explained	0.19	0.17	0.14	0.13	0.13	0.13	0.10
Cumulative Proportion	0.19	0.36	0.50	0.63	0.76	0.90	1.00