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Evaluating Leaf Trait Variation in High Elevation Bristlecone Pine (*Pinus longaeva*) Under Increasing Water Stress: Insights from Needle Length, Stomatal Density, and Cambial Growth

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Evaluating Leaf Trait Variation in High Elevation Bristlecone Pine (*Pinus longaeva*) Under Increasing Water Stress: Insights from Needle Length, Stomatal Density, and Cambial Growth

By

Audrey Salerno

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

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Audrey Salerno

July 31st, 2023

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A Thesis
Presented to
the Faculty of
Western Washington University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Audrey Salerno
July 2023

Abstract

Increasing aridification caused by climate change is altering growth patterns in trees. There is revived attention on how foliar traits respond to climate and the relationship of these traits to ring width. Bristlecone pine (*Pinus longaeva*, DK Bailey), a long-lived conifer found at high elevations in the cool and dry intermountain west of America, is used in paleoclimate reconstructions by measurement of their annually resolvable tree rings. The species also has annually datable needles retained on their branches for an average of 45 years making it the ideal subject for research on foliar trait and growth relationships under contemporary changes in hydroclimate. To explore this relationship, we sampled six individuals: three from the alpine treeline ecotone and three from the subalpine forest, on Mt. Washington in Nevada. From each tree, we measured ring width using tree cores and measured length and stomatal density of needles from a branch. Analysis included generalized-least squares regression followed by an ANOVA. For individual trees, needle length and ring width had variable growth trajectories over time compared with an inconsequential response of stomatal density to time. By site, stomatal density was higher and ring width was wider at the alpine treeline. The greater stomatal density of ATE trees explains the wider rings at this location by increasing the number of stomates per needle as mean needle length did not differ by site. Ring width and needle length were positively correlated and each trait reported higher first-order autocorrelation. Stomatal density, on the other hand, had a weak negative relationship to ring width and low first-order autocorrelation implying a minimally plastic response to climate conditions. The varied response of these traits by individual and site shows that the response to climate is not uniform within sites or between traits likely due the topoclimatic conditions experienced by each individual. By expanding beyond ring width, interannual variation in needle physiology allows us to understand the response of the bristlecone pine to recent climate change and the relationship of these traits to one another.

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1. Introduction

Global climate change is increasing global surface temperature and exacerbating drought stress enhancing aridification across environments (IPCC 2023). Historically, the field of dendrochronology used tree ring-width measurements to understand the response of trees to climate over their lifespan (LaMarche 1974). The response in tree growth to anthropogenic climate change across different environments is variable, with some studies finding improved growth with higher temperatures and others recording a decrease in growth due to drought stress from increased evaporative demand (Adams and Kolb 2004, Bunn et al. 2005, 2018, Boer et al. 2019, Dudley et al. 2023). In environments restrictive to growth, like alpine or boreal ecosystems, temperature and moisture availability are reflected more strongly in ring width (Cook et al. 2018, Oleksyn et al. 2020). However, changes in those factors cause differing physiological growth responses (Cook et al. 2018, Williams et al. 2021, Dudley et al. 2023). There is a transition in growth response between temperature and moisture limitation across elevational and latitudinal gradients linked to photosynthetic capacity and is explained, in part, by changes in foliar traits (Jankowski et al. 2017, Dudley et al. 2023). Consequently, exploring the relationships between hydroclimate, foliar traits, and changing cambial growth can enhance our understanding of how species respond and survive in such environments under future drought conditions.

For conifers, the relative plasticity of different foliar traits in response to changing climate conditions and interannual climatic variation likely mitigate the direct and indirect effects of warming on plant growth by mediating water loss (Gornish and Prather 2014, Tyukavina et al. 2019, Dudley et al. 2023). Marek et al. (2022) used the wide latitudinal range of *Pinus sylvestris* to identify changes in stomatal density of needles in response to temperature

finding that stomatal density decreased with increasing latitude and decreasing mean annual temperature. Shorter needles were also found in colder, less productive environments and these colder environments were more responsive to temperature change (Marek et al. 2022). Grossiord et al. (2017) found precipitation to have the strongest effect on piñon pine (*Pinus edulis*) growth and needle length in an area limited by soil moisture. The main limiting factor, be it temperature or moisture, has the strongest effect on growth and foliar traits (Grossiord et al. 2017, Guérin et al. 2018, Dudney et al. 2023). However, increased global temperatures reduce cold-limitation on growth highlights the importance of understanding the influence of drought stress on growth and tree physiology. In typically cold-limited ecosystems, increasing temperatures induce drought stress earlier in the season and possibly increase drought severity (Tyukavina et al. 2019, Dudney et al. 2023). In response to drought, trees use a water-saving strategy reducing the area of transpiration surfaces of their foliage, like needle length, and by reducing growth (Grossiord et al. 2017).

Measurements of leaf traits such as needle length and stomatal density offer a reflection of a tree's balance between transpiration and CO₂ absorption for photosynthesis, which directly influence tree growth through carbon fixation (Qiang et al. 2003). Stomates are the gas exchange port of the leaves facilitating CO₂ flux for photosynthesis and balancing stomatal closure to limit excess water loss and potential hydraulic failure from transpiration (Franks and Farquhar 2007, Wang et al. 2019). Together, stomatal density and needle size thus relate to tree photosynthetic capacity as they impact the degree of light interception, carbon assimilation, and rate of water loss (Guérin et al. 2018, Wang et al. 2019). Photosynthetic capacity, by extension, affects growth. There are diverse carbon allocation strategies across different *Pinus* species resulting in differences in the interaction of leaf phenology and physiology, the allocation of stored

carbohydrates, and the annual growth of the cambium and roots (Guérin et al. 2018, Wang et al. 2019). The variation in carbon allocation strategies and timing of growth likely results in a range of growth responses in leaves and sapwood (Guérin et al. 2018). Consequently, knowledge of the variable plastic response of needles within and between species to changing climate can enhance our understanding of forest productivity and species resilience (Oleksyn et al. 2020).

Due to the relatively short longevity of leaves compared to the lifespan of the entire tree, there are limited insights into the physiological impacts of climate on foliar traits. However, bristlecone pine (*Pinus longaeva*, DK Bailey) have annually datable needles which the trees retain on their branches for approximately 45 to 55 years making it the ideal subject for this research (LaMarche 1974, Ewers and Schmid 1981). Bristlecone pines are a well-known species in paleoclimatology with extended lifespans of upwards of 4,000 years (LaMarche 1974). Much of the existing research on this species revolves around understanding past temperature and precipitation signals found in their annually resolvable growth rings although the relationship between ring width and climate contains a mix of these climate signals (LaMarche 1974, Salzer et al. 2009, Salzer et al. 2014, Bunn et al. 2018 and sources therein). The growth signals present in the bristlecone pine's tree rings are dependent on their topoclimatic conditions over a narrow elevational range (LaMarche 1974, Salzer et al. 2014, Bunn et al. 2018). Subalpine forest trees show a moisture limitation in their rings (LaMarche 1974, Salzer et al. 2014). Trees at the alpine treeline experience cold limitation through most of the year while experiencing elevated drought stress during the summers (Salzer et al. 2009). Since the early 2000's, trees at the alpine treeline are likely exhibiting drought stress similar to trees in the subalpine forest (Salzer et al. 2014) following abnormally wide growth rings in the second half of the 20th century. Salzer et al.

(2009) associated this growth habit with increased temperature exacerbating drought stress in the area.

The prior knowledge of the limiting factors on growth of bristlecone pines opens the ability to relate that knowledge to patterns in physiological traits found in their needles.

LaMarche (1974) was one of the first researchers to relate a climatic variable to the needles of the bristlecone pine noting that needle length positively corresponded to summer temperature in alpine treeline trees. More recently, Barber (2013) investigated the stomatal frequency of bristlecone pine only finding a weak inverse relationship between stomatal frequency and atmospheric CO₂, identifying variation within individuals as the driver of stomatal frequency changes. Individual variation is expected from the exertion of topoclimate on growth and potential genetic factors (Bunn et al. 2011, 2018, Tran et al. 2017, Jankowski et al. 2019). To our knowledge, bristlecone pine needle length has not been reassessed since LaMarche (1974) and stomatal density since Barber (2013).

Given the extensive longevity of bristlecone pine needles and this species' overall utility for research in paleoclimatology, there is an opportunity to quantify the relationship between its needle traits and cambial growth response under contemporary changes in hydroclimate.

Additionally, the switch between temperature and moisture-limitation on growth based on elevation allows us to view variation within and between these traits, thus allowing for gaining new insights into this species' performance under future hydroclimatic conditions.

Therefore, the objectives of this study are as follows:

1. Address how three physiological traits: needle length, stomatal density, and ring width, vary over time and by individual in trees growing near and at the upper elevational limit of the species.
2. Address how three physiological traits: needle length, stomatal density, and ring width vary between their two respective locations on slope: ATE – alpine treeline ecotone and SAF – subalpine forest.
3. Address whether stomatal density and needle length are explained by hydroclimate conditions using ring width as a proxy.

2. Methods

2.1 Study area

Bristlecone pines grow in parts of the American west at high elevations in dry and cool climates. Branches and cores were sampled from private land on Mt. Washington in the Snake Range, NV. The sampling location was chosen as a function of accessibility, past research, and timing of sampling. Samples were taken over an elevational gradient between 3370m and 3460m. Treeline at Mt. Washington is located at approximately the highest elevation of our sampling transect.

On Mt. Washington, bristlecone pines are interspersed within a mixed conifer forest at lower elevations but are monotypic as elevation increases in an open canopy forest. Our lowest elevation sample, SAF01, demarcated the lower bound of the monotypic range of the bristlecone pine beginning the mixed conifer forest (Table 1). Our highest elevation sample, ATE03, was at approximate treeline (Table 1).

2.2 Field sampling

Field sampling took place in mid-August 2022. We sampled six trees for the analysis. Three samples were from an alpine treeline ecotone (ATE) site and the remaining three were from a subalpine forest site (SAF) to enable testing trait differences by site (Table 1). For location and elevation of each sample refer to Table 1. Samples were all located on a south-west facing slope on Mt. Washington with branches cut from the northern side of each tree to maintain consistency and ease of sampling. GPS coordinates were taken for each tree, and each was labeled with an aluminum tag for future reference. Branches were clipped from each sample two to 2.5 meters off the ground. Branches selected for sampling were pollen cone branches for ease of dating the needles (Ewers and Schmid 1981). Two to three branches were sampled from each tree, but only one was used for further analysis based on the number of years present on the branch. Branch length ranged from 30 cm to 50 cm. Cut and labeled branches were placed in a plastic bag with a moist paper towel surrounding the cut end to reduce moisture loss before processing and preservation. In addition, a tree core was taken using an increment borer from each tree for subsequent ring-width measurements. The cores were placed into labeled straws corresponding to the branch labels.

2.3 Field processing

Field processing lasted for two days following the initial sampling. For use in stomatal density and needle length measures, each branch was sectioned at internodes and placed young end toward the lid in labeled 50ml centrifuge tubes filled with 70% ethanol to reduce the effects of dehydration (Marek et al. 2022). The samples were refrigerated in the lab to reduce the risk of desiccation.

2.4 In-lab processing

For stomatal density and needle length measures, we imaged individual needles tree by tree. We processed each branch starting with the youngest needles and worked to the oldest removing each tube from the refrigerator as necessary. We used the whorls from the branch tip using pollen cone gaps and needle length as a guide to remove and record yearly needle variation. From each fascicle, we randomly selected three needles and used a calibrated electronic caliper to measure the length of each needle to the nearest hundredth of a millimeter.

2.4.1 Stomatal density

Using an Olympus CZ61 Stereoscope equipped with an Olympus EP50 camera, we imaged a minimum of three needles per year per tree on a 1mm x 1mm microscope grid. The needles imaged were usually the same as those measured for needle length. The selected needles had a waxy coating making the stomata easier to identify. A few years (<10 out of all measured) had only two needles with visible stomates and for those years, only two needles were used to calculate stomatal density. Each needle was imaged within the central fifty percent of its length to reduce the effects of needle tapering on the stomatal counts (Hultine and Marshall 2000, 2001, Marek et al. 2022). An image was taken of each surface for measurement: each (2) adaxial surface, needle width, and needle depth. Stomatal counts and surface measurements were reported on the three needles with the clearest stomates when calculating stomatal density.

Stomatal counts were completed manually using ImageJ (Schneider et al. 2012). For each image, the scale was reset and checked against the 1mm microscope grid. For bristlecone pine, only the adaxial surfaces contain stomates (Barber 2013). On the adaxial surfaces, we measured and marked the width of the surface and a 2mm long section of the needle containing the width measurement (Marek et al. 2022). Stomatal counts were taken within the delineated 2mm

section. The width of the needle and the depth of the needle were measured on their respective images.

The stomatal density calculation used each needle's raw stomatal count and needle dimension measurements. Because each adaxial surface was imaged and measured, stomatal density was calculated using the area of each surface, which we refer to as the direct method. We opted for the direct method rather than the commonly used method by Hultine and Marshall (2001). Hultine and Marshall (2001) use needle width multiplied by a constant to estimate the adaxial surface widths and we measured the adaxial surfaces directly and used those in our calculations.

2.4.2 Tree cores

The tree cores were air dried, mounted and sanded using progressively finer grits using methods laid out in Stokes and Smiley (1968). To properly cross date and confirm chronologies, the widths beginning in 1800 of all the samples were measured and recorded. The crossdated ages were confirmed by referencing the master chronology and using 'dplR' (Bunn 2010). Due to the modern time focus of this project, we were concerned with most recent 50-75 years of growth as it relates to concurrent needle traits.

2.5 Analysis

We used R 4.13 for analysis (R Core Team 2022) via R Studio Software version '2021.9.0.351' (RStudio Team 2021). The analysis used six total samples with three from the SAF (SAF01, SAF02, SAF03) and three from the ATE (ATE01, ATE02, ATE03). To maintain a complete matrix with equality of measurements across samples, we trimmed the data to the years after 1977 and excluded sample year 2022 as needle elongation had not ceased at the time of

sampling (44 years for all samples). For use in statistical models, we calculated the mean of the replicates within year for needle length and stomatal density for each individual tree, respectively.

We used generalized least square (GLS) models for these analyses from the ‘nlme’ package (Pinheiro et al. 2023), adding the appropriate correlation structures to the residuals. We ran an ANOVA from the ‘car’ package (Fox et al. 2023) on each of the GLS models to assess the significance among all interacting factors for each relationship. For the first objective, time and treeID predicted the mean values of each physiological trait. To test the second objective, we created a mean chronology by site and ran the mean values for each trait as the response variable to site. We tested the third objective using ring width to predict needle length and stomatal density. To further assess the relationship between needle length and ring width, we compared first-order autocorrelation to view how the strength of the autocorrelation varied between trait and tree and to see if the trees share similar autocorrelative memory in relation to their own growth and foliar traits. To view how ring width and needle length cross-correlate, we ran a cross-correlation function on the two traits for each tree.

3. Results

3.1 Temporal and individual variability

Individual trees had significantly different mean ring width and needle length ($\text{Chisq}_{5,264}=50.7$, $p<0.001$ & $\text{Chisq}_{5,264}=52.8$, $p<0.001$, respectively; Table 2, Fig. 1&2). Ring width had a marginally significant relationship to year across all trees combined, but individual trees were significantly different from one another in terms of their growth trajectories over time ($\text{Chisq}_{1,264}=3.64$, $p=0.056$ & $\text{Chisq}_{5,264}=12.1$, $p=0.034$, respectively; Table 2, Fig. 1). Needle length was not significantly different over time for all the trees combined, but individual trees

were significantly different from one another in terms of their growth over time ($\text{Chisq}_{1,264}=0.69$, $p=0.40$ & $\text{Chisq}_{5,264}=15.33$, $p=0.009$, respectively; Table 2). Time did not significantly explain stomatal density for all trees combined or across individuals ($\text{Chisq}_{1,264}=0.86$, $p=0.35$ & $\text{Chisq}_{5,264}=2.05$, $p=0.84$; Table 2). For stomatal density, only individuals exhibited significantly different mean stomatal densities ($\text{Chisq}_{5,264}=154.29$, $p<0.001$; Table 2, Fig. 3A).

3.2 Plasticity of traits with elevation

Needle length did not differ between the ATE and the SAF samples ($\text{Chisq}_{1,264}=0.061$, $p=0.80$; Table 3). Alpine treeline ecotone samples had wider rings compared to that of the subalpine forest ($\text{Chisq}_{1,264}=40.83$, $p<0.001$; Table 3, Fig. 3B). Stomatal density was also higher at the ATE site ($\text{Chisq}_{1,264}=313.02$, $p<0.001$; Table 3, Fig. 3A).

3.3 Covarying of traits

3.3.1 Ring width as an indicator of growing conditions

The interaction of ring width and treeID was not significant for either stomatal density or needle length ($\text{Chisq}_{5,264}=6.14$, $p=0.29$ & $\text{Chisq}_{5,264}=9.53$, $p=0.089$, respectively; Table 4, Fig. 4 & 5), so the effect of ring width does not differ by individual. Accounting for the autocorrelation in the residuals of this analysis reduced or removed the significant effects interaction between ring width and tree ID in predicting needle length. The slope for stomatal density was not different between individuals regardless of autocorrelation structure. Ring width significantly predicted needle length and stomatal density across all trees with wide rings predicting long needles and reduced stomatal density ($\text{Chisq}_{1,264}=146.26$, $p<0.001$ & $\text{Chisq}_{1,264}=8.15$, $p=0.004$, respectively; Table 4, Fig. 4 & 5). Needle length and stomatal density significantly differed in response to ring width among individual trees ($\text{Chisq}_{5,264}=94.47$, $p<0.001$ & $\text{Chisq}_{5,264}=189.88$, $p<0.001$, respectively; Table 4, Fig. 4 & 5).

3.3.2 Autocorrelation

To assess the relationship between needle length and ring width, reported are the autocorrelation values at a lag of one year. The first order autocorrelation of each trait varied by individual. For ring width, all samples, except for SAF02 and SAF03, had high autocorrelation with a mean of 0.51 (Table 5). SAF02 and SAF03 fell below those at 0.21 and 0.05, respectively (Table 5). The ATE samples averaged higher autocorrelation in their ring width than those in the SAF site; however, SAF01 retained a high first order autocorrelation similar to that of the ATE samples. Autocorrelation within needle length was strongest in SAF02, ATE01, and ATE03 with a mean of 0.57 (Table 5). The remaining ATE sample, ATE02, had the weakest autocorrelation at 0.29, similar to the two remaining SAF samples, SAF01 and SAF03 (Table 5).

Autocorrelation within stomatal density remained overall lower than the other traits; however, two of the ATE samples showed medium strength autocorrelation with an average of 0.36 (Table 5). ATE02 differed from the other two high elevation samples with a near zero autocorrelation. Aside from ATE02 in the ATE samples, the SAF samples had lower autocorrelation with SAF01 being most similar to ATE01 and ATE03 of the treeline samples.

3.3.3 Cross-correlation

Cross-correlation between ring width and needle length was strongest at a lag of zero for all samples (Fig. 6). The cross-correlation coefficient generally rose with an increase in elevation at lag zero (Fig. 6). ATE03 had the highest CCF value at lag zero followed by SAF01 and then the remaining ATE samples (Fig. 6). ATE03 is the only ATE sample with significant correlations at lags other than zero. SAF01 and SAF02 show distinct long-term covariation in their cross-correlation correlation (Fig. 6). The long-term covariation is not clear in samples higher in elevation than those two including the third SAF sample, SAF03 (Fig. 6).

4. Discussion

4.1 Temporal and individual variability

Individual trees had significantly different mean trait values, but for ring width and needle length, differences were driven by time and dependent on the individual (Table 2; Fig. 1&2). Variation of stomatal density had no relationship to time rather individual variability drove differences in stomatal density (Barber 2013). Foliar traits, specifically stomatal density, are less likely to be plastic as they are not always responsive to climate and photosynthetic change due to the ability to close stomates rather than temporally altering needle traits (Grossiord et al. 2017).

Looking at the relationship of ring width to time, trees at the ATE have a stronger trend than those at the SAF (Fig. 1). Ring width responded negatively over time for both ATE01 and ATE03, but ATE02 exhibited a positive growth response over the period of study (Fig. 1). The timespan used in the analysis (44-years) limits our ability to detect responses similar to that in Salzer et al. (2014), where the authors detected a switch in growth of the highest elevation trees from a positive growth response to increasing temperature to a reduction in growth in the early 2000s. Rather, two-thirds of the ATE trees exhibited a reduction in growth potentially linked to increasing drought stress normally mitigated by cooler temperatures (Salzer et al. 2009, 2014). ATE02's positive growth response to time might be due to its location in a cooler topoclimate compared to the other two high elevation samples (Fig. 1). Bunn et al. (2018) harkened back to the finding of LaMarche (1974) of 'apparent altitude' where small changes in distance between individual trees can cause substantially different growth responses based on topoclimate. The SAF samples have a weak ring width response over time between and amongst individuals. This weaker response indicates that changes in climate have minorly impacted growth response and

moisture-limitation is still likely the limiting factor for the SAF site (Fig. 2). SAF01 and SAF02 growth both slightly decreased over time and SAF03 growth weakly improved over time (Fig. 2).

As with ring width, the response of needle length to time is dependent on the individual (Table 2). Individual response is more variable for needle length than ring width with a weak pattern by site (Fig. 2). SAF02, SAF03 and ATE03 have the strongest response over time with variable length trajectories over time (Fig. 2). ATE03 has a strong negative response mirroring that of its ring width (Fig. 2). The response in needle length of SAF02 and SAF03 is the inverse of their temporal trend in ring width (Fig. 2). Needle length of the remaining individuals also have inverse temporal relationships to their ring growth measures, but the relationship to time is minor. ATE03, the highest elevation sample, likely faces some of the harshest adaptive conditions due to its treeline location (Fig. 2). The harsh environmental conditions likely link the growth responses of needle length and ring width in this sample (Tyukavina et al. 2019, Marek et al. 2022).

The inconsistent temporal response among individuals and between sites indicates there is not a unified response to changing climatic conditions, but for needle length and ring width, trees are responding plastically to these changes over time (Fig. 1 & 2). Our findings align with Bunn et al. (2018) that topoclimatic conditions can cause variable responses among trees in close proximity to one another given the differences in samples within each site. Individual variability is expected as growth and physiological data is prone to noise from other interacting factors (Barber 2013, Bunn et al. 2018). Annual biomass accumulation reflects plant growth performance, because plants tend to allocate new resources to where they are more resource-limited (Fan et al. 2019). In energy-limited systems, we would expect an increased allocation to

the needles; however, nutrient and other resource limitations might increase allocation to the roots which we did not measure here (Fan et al. 2019).

Additionally, the date range analyzed was used to ensure a complete set of data for all trees across all years; however, the longevity of the needles on each branch varied. SAF03 and ATE02 had the longest sample depths with SAF03 holding needles for approximately 75 years. Restrictive environmental conditions and nutrient availability like those of the subalpine and alpine treeline ecotones are linked to increased needle retention, shorter needles, and decreased photosynthetic rates leading to slower carbon assimilation and growth (Gornish and Prather 2014, Oleksyn et al. 2020, Marek et al. 2022). Although we did not observe the same trend in SAF03 and ATE02 as stated in those studies, we did not account for possible influence of needle longevity on tree response. There is also a potential genetic relationship to the plasticity of these traits. Selective pressures on younger trees might result in reduced trait plasticity in response to different conditions (Jankowski et al. 2019). The necessity to restrict the date range of interest to 44-years to enable proper leaf trait analysis did not inform us about age or genetic variation among samples.

4.2 Plasticity of traits with elevation

Ring width and stomatal density differed between the ATE and SAF with the high elevation trees having wider rings and greater stomatal density (Table 3; Fig. 3). Needle length, however, did not differ by site (Table 3). The difference in ring width by site is consistent with the growth noted in bristlecone pines in Salzer et al. (2009) where high elevation samples had larger raw ring width measures compared to lower elevation samples (Fig. 3B). As seen with the relationship with time, ring width between sites appears to become more similar over time (Fig. 1); however, they were significantly different in this analysis.

Given the difference of ring width by site, the same would be expected of needle length given that organs typically estimate the changes in growth of one another and the entire tree (Tyukavina et al. 2019). Our finding is inconsistent with research into other conifer species over an elevational gradient (Qiang et al. 2003, Tyukavina et al. 2019, Dudney et al. 2023). The moisture-limited subalpine forest samples would be expected to have shorter needles than the alpine tree line ecotone samples as water stress has been more strongly linked to shorter needles (Guérin et al. 2018, Dudney et al. 2023). The lack of difference in mean needle length between site indicates that either there is no difference in stressor on needle length or that the tradeoff in leaf size for each stressor leads to converging needle length.

The alpine treeline ecotone samples are historically cold temperature-limited and beginning in the 21st century exhibited a stronger response to drought conditions similar to the moisture-limited trees tens of meters below the ATE (Salzer et al. 2014, Bunn et al. 2018). Grossiord et al. (2017) found drought stress to drive needle length to a greater extent than temperature as trees will reduce water use by reducing surface area for transpiration potentially explaining the similarity in needle length between sites. Despite the finding for needle length, the greater stomatal density of ATE trees increases the gas exchange capacity of the higher elevation trees by increasing the total number of stomates per needle explaining the wider rings of the higher elevation trees (Fig. 3A) (Grossiord et al. 2017, Dudney et al. 2023). Increased stomatal density allows for higher rates of carbon assimilation as the trees might respond to drought by closing stomates to avoid hydraulic failure rather than reduce their stomatal density at higher elevations (Grossiord et al. 2017, Dudney et al. 2023).

4.3 Covarying of traits

4.3.1 Ring width as indicator of growing conditions

Ring width as a proxy for climate had a strong positive relationship to needle length and a weak negative relationship to stomatal density (Table 4; Fig. 4 & 5). Individual variation, as seen in the previous analyses, had strong predictive power for both stomatal density and needle length, respectively (Table 4). There was no significant interaction between ring width and tree ID for either needle length or stomatal density; however, the interaction was only marginally insignificant in predicting needle length (Table 4). Removing the autocorrelation in the residuals removed the significant effects in the results of our analysis in this interaction warranting further modeling to explain the contrasting results.

The positive relationship between ring width and needle length is indicative of the response LaMarche (1974) noted in the high elevation trees. The direct positive relationship between ring width and needle length is likely associated with climatic variation by time and position. This creates a reciprocal relationship whereby needle growth is influenced by ring growth and previous needle growth influences future ring growth (Fritts 1966, Tyukavina et al. 2019, Oleksyn et al. 2020). Wider rings are indicative of a better climatic year and our results show the needle length and ring width having a similar plastic response to interannual variation. In warm conditions with adequate moisture, trees tend to grow faster and increase needle length improving carbon assimilation and updating their water balance (Oleksyn et al. 2020). However, global climate change causes rising temperature and precipitation deficits increasing evapotranspiration altering stomatal conductance and reducing carbon storage leading to narrower rings and shorter needles (Fritts 1966, IPCC 2023). The relationship between needle

length and ring width appears to be parallel across individuals even though the traits for some individuals differed in their trajectory over time (Fig. 1,3 & 4).

The lack of difference between the relationship of needle length and ring width among trees is indicative of how the sensitivities of individuals at the ATE might have changed as observed by Salzer et al. (2014). The warming enhanced growth response found in Salzer et. al (2009) might be overtaken by a drought response in the highest elevation samples shaping the indifferent response of needle length to the interaction of individual and ring width. Additionally, ring width might not be the most accurate predictor of needle length, as cambial growth and needle elongation differ in their timing of growth and therefore have a reliance on different factors to initiate and sustain growth throughout the growing season (Fritts 1966, Han et al. 2019). However, ring width is our proxy for climate as wider rings are indicative of warm and moist climatic conditions throughout the growing (Fritts 1966). By extension, in relatively warm and wet years, trees can make larger needles and fix more carbon making wider rings. The addition of different needle metrics like width and depth might help to tease out why the relationship between these two growth structures is not stronger.

The minor, but significant, negative relationship between stomatal density and ring width, with the non-significant interaction of individual trees, has a less clear explanation (Table 4; Fig. 5). The weak relationship might be indicative of a minimally plastic response of stomatal density to climate conditions which is seen in our time analysis. If the trait responded plastically, we would expect a stronger relationship to ring width as seen with needle length. However, the negative response of stomatal density to ring width is not necessarily indicative of reduced gas exchange potential in better climatic years. Stomates per needle, a measure of needle length and stomatal density, might still increase with a decrease in stomatal density (Guérin et al. 2018).

Needle length is positively responsive to ring width, so there is an opportunity for photosynthetic capabilities of an individual to increase due to an increase in the number of stomates per needle or timing of stomatal conductance during the day (Rogiers and Clarke 2013, Guérin et al. 2018).

4.3.2 Autocorrelation

There was not a consistent trend in autocorrelation within or between sites for needle length or stomatal density (Table 5). Salzer et al. (2014) found first-order autocorrelation to increase in ring width with elevation and our findings for ring width generally followed that trend, except for ATE02. ATE02 has responded differently from the other ATE samples across most of the analyses indicating a different factor influencing its habits possibly based on topographic position (Bunn et al. 2018). The alpine treeline ecotone is known to be, albeit previously, cold-limited with shortened growing seasons exhibiting drought stress minorly as evaporative pressures mounted leading to higher first-order autocorrelations in ATE ring width (Fritts 1966, LaMarche 1974, Salzer et al. 2014, Dudney et al. 2023). Autocorrelation tends to be stronger in less productive environments, like that of the bristlecone pine, as previous year conditions enable adequate carbon storage for the following year to initiate and sustain growth (Fritts 1966, Gazol et al. 2022).

SAF01, located at the lower limit of the monotypic zone at our site for bristlecone pines, exhibited higher autocorrelation reminiscent of the ATE trees (Table 5). SAF01 might typify the drought stress experienced by the lower elevation trees and potentially be influenced by other competitive factors given its proximity to the mixed conifer zone. SAF02 and especially SAF03 have drastically low autocorrelation in their ring widths, which does not correspond to low needle length autocorrelation (Table 5). First-order autocorrelation in ring width is typically

calculated over longer timespans and the lack of first-order autocorrelation in ring width for SAF02 and SAF03 might explain this finding (Table 5).

By extension of the positive relationship between ring width and needle length (Fig. 4), needle length should follow a similar trend in autocorrelation. Autocorrelation, however, in needle length is higher for all the samples but is not clearly differentiated by site. The samples with the lowest needle length AR1 had the oldest needles on their branches, SAF03 and ATE02 (Table 5). Increased longevity of needles is typically associated with increased reliance on prior year conditions from poorer growing conditions (Fritts 1966, Ewers and Schmid 1981, Oleksyn et al. 2020). Conjunctively, researchers found reduced autocorrelation in low stress environments with more abundant resources and greater growth (Fritts 1966). SAF03 exhibited narrow, temporally stable ring-width with negligible ring-width autocorrelation, but needle length increased over time, which is not in agreement with either of those findings (Table 5).

The indifference of stomatal density to time and its minor relationship to ring width suggests a reduced plasticity and potentially weak autocorrelation, which aligns with our findings. The pattern of autocorrelation in the samples follows that of ring width but with overall weaker autocorrelation. ATE samples, ATE01 and ATE03, show similarly high autocorrelation across all the traits, with stomatal density being the weakest (Table 5). The autocorrelative results further demonstrate how differences between individuals drive variation in growth response and foliar traits. Variation in autocorrelation might be linked to specific topoclimatic conditions that alter the moisture and temperature experienced by individuals (Bunn et al. 2018, Dudney et al. 2023).

The utility of autocorrelative traits in understanding individual responses enhances ones understanding of ecological resilience. Gazol et al. (2022) found higher autocorrelation is linked

to lower a resilience to harsh conditions because the recovery relies on the conditions and carbon storage of the previous. Therefore, in a dry and cool environment, individuals with low autocorrelation in their traits might be more resilient to increasing drought stress. Moreover, as mentioned above, selective pressures might play a role in autocorrelative response given younger trees might be less plastic and less reliant on previous conditions (Oleksyn et al. 2020, Gazol et al. 2022).

4.3.3 Cross-correlation

The cross-correlation of needle length and ring width does not have a clear explanation. SAF01 and SAF02 showed long-term covariation in their cross-correlation while the other low elevation sample SAF03 did not exhibit that same response (Fig. 6). SAF03, as observed in our other analyses, does not behave similarly to the other SAF samples (Fig. 6). Covariation is weakly visible for ATE01, but the other trees have correlation between needle length and ring width within the same year, and this response is stronger up slope (Fig. 6).

The long-term covariation present in SAF01 and SAF02 between needle length and ring width reflects how the photosynthetic capacity of prior growth can influence future growth in individuals (Fig. 6) (Grossiord et al. 2017). This correlative relationship between needle traits and growth is linked more strongly to precipitation and drought conditions than to temperature, which is in alignment with our two lowest elevation and potentially most moisture-limited samples exhibiting this relationship (Grossiord et al. 2017, Guérin et al. 2018, Dudney et al. 2023). Moving up in elevation, the cross-correlation patterns are muddled. The relationship of current ring width to the photosynthetic capacity of previous needles as measured by needle length mostly disappears. The strength of the correlation at a lag of zero, on the other hand,

improves with elevation signifying the reliance of both needle length and ring width on climate conditions in the current year.

5. Conclusion

Needle length and ring width changed over time, and this change varied by individual. Individual variation explained stomatal density whereas time did not explain stomatal density. Although both needle length and ring width differed over time, these trends were not consistent across all the trees nor within sites. Alpine treeline ecotone samples had wider rings and higher stomatal densities compared to the subalpine forest, but needle length did not differ by site. A combination of higher stomatal density and indifferent needle length at the ATE could still lead to increased growth through a greater gas exchange capacity of those needles with higher stomates per needle (Guérin et al. 2018). Other studies have shown that lower stomatal density, like identified in the SAF samples, mitigates drought conditions by reducing leaf water potential leading to hydraulic failure (Grossiord et al. 2017, Guérin et al. 2018).

The autocorrelative structure of each trait further showed the individual variability and reliance on previous conditions for sustained and continued growth. The variability within site and between site was not wholly expected given that cold and precipitation-limited environments, respectively, are more apt to experience higher first-order autocorrelations in ring width and other growth organs (Fritts 1966, LaMarche 1974, Salzer et al. 2014, Dudney et al. 2023).

Cross-correlation between needle length and ring width was strong in the same year, confirming that both traits respond directly to climate conditions in the same direction. The breakdown of long-term cross-correlation with increased elevation between the traits support differing stressors limiting these trees and can be unique to each trait response. Recent research

found the relationship of ring width and needle length to climate related to the initial limiting factor on growth (Dudney et al. 2023); however, the temperatures experienced by individuals based on their topoclimatic position dictates the moisture or temperature-limited response (Bunn et al. 2018, Dudney et al. 2023).

To better understand the relationship between the three observed traits and how they explain the bristlecone pine's responses to changing hydroclimate, additional knowledge of the climatic conditions at each sample position is needed. This might help explain some of the divergent patterns between sites and individuals for measurements of ring width, needle length, and stomatal density. Understanding an individual tree's response to climate moderated by its topographic position would enable researchers to determine the conditions required for survival, the variation in plasticity to similar and differing climatic stress, and the identification of specific trees that will continue to best serve as adequate proxies for instrumental climate reconstructions. Overall, the varied response of these traits by individual shows that there is a strong plastic response in certain traits in response to climate. By expanding beyond ring width, interannual variation in needle physiology and ring width enables us to understand the response of the bristlecone pine to recent climate change and the relationship of these traits to one another through the lens of this change.

6. Tables

Table 1. Sample ID and location on slope for the six tree samples.

Tree ID	Elevation (m)	Latitude (°)	Longitude (°)
ATE01	3452	38.91010	-114.30775
ATE02	3454	38.91019	-114.30728
ATE03	3461	38.91034	-114.30833
SAF01	3366	38.90694	-114.30878
SAF02	3373	38.90734	-114.30838
SAF03	3380	38.90736	-114.30845

Table 2. ANOVA table of generalized least squares results using Year, Tree ID, and Year \times Tree ID to predict ring width, needle length, and stomatal density. Data from a 44-year dataset (1977 - 2021). Stars represent significant p-values. ** = $p < 0.01$, * = $p < 0.05$, . = $p < 0.10$.

Response Variable	Predictor Variable	dF	Chisq	P-Value
Ring Width	Year	1	3.64	0.056.
	Tree ID	5	50.73	<0.001**
	Year \times Tree ID	5	12.08	0.034*
Needle Length	Year	1	0.69	0.404
	Tree ID	5	52.78	<0.001**
	Year \times Tree ID	5	15.33	0.009**
Stomatal Density	Year	1	0.86	0.353
	Tree ID	5	154.29	<0.001**
	Year \times Tree ID	5	2.05	0.842

Table 3. ANOVA table of generalized least squares results using site to predict ring width, needle length, and stomatal density. Data from a 44-year dataset (1977 - 2021). Stars represent significant p-values. ** = $p < 0.01$, * = $p < 0.05$, . = $p < 0.10$.

Response Variable	Predictor Variable	dF	Chisq	P-Value
Ring Width	Site	1	40.83	<0.001**
Needle Length	Site	1	0.061	0.80
Stomatal Density	Site	1	313.02	<0.001**

Table 4. ANOVA table of generalized least squares results using ring width to predict needle length and stomatal density. Data from a 44-year dataset (1977 - 2021). Stars represent significant p-values. ** = $p < 0.01$, * = $p < 0.05$, . = $p < 0.10$.

Response Variable	Predictor Variable	dF	Chisq	P-Value
Needle Length	Ring Width	1	146.26	<0.001**
	Tree ID	5	94.47	<0.001**
	Ring Width × Tree ID	5	9.53	0.089.
Stomatal Density	Ring Width	1	8.15	0.004**
	Tree ID	5	189.88	<0.001**
	Ring Width × Tree ID	5	6.14	0.29

Table 5. First-order autocorrelation (AR1) reported for ring width, needle length, and stomate density for each sample tree of interest separated by site ran on a 44-year dataset (1977 - 2021).

Tree ID	Ring Width	Needle Length	Stomate Density
SAF01	0.50	0.45	0.32
SAF02	0.21	0.61	0.20
SAF03	0.05	0.38	0.10
ATE01	0.54	0.59	0.34
ATE02	0.48	0.29	-0.02
ATE03	0.50	0.51	0.37

7. Figures

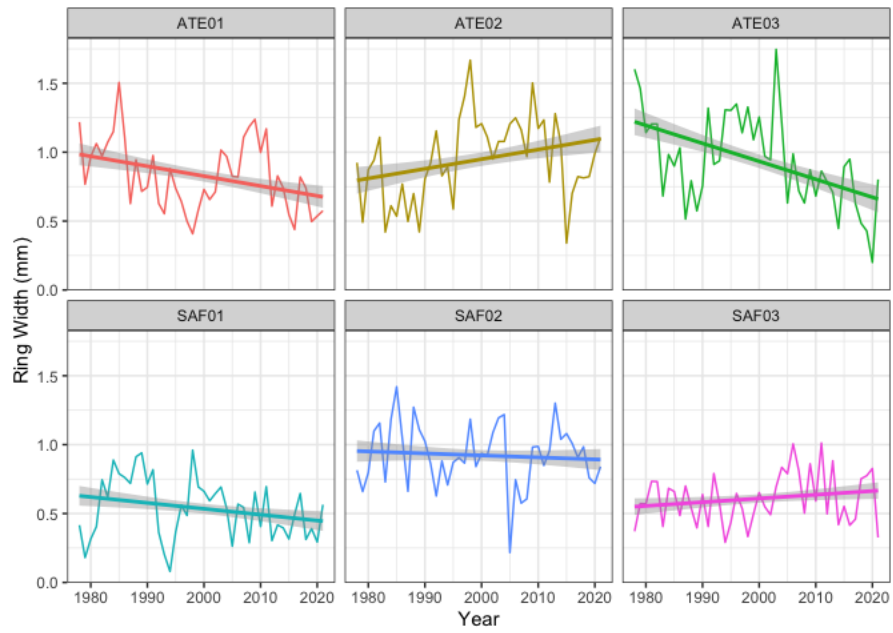


Figure 1. Raw ring-width chronologies from each tree labeled by site over 1977–2021 with a linear model individually applied to each tree.

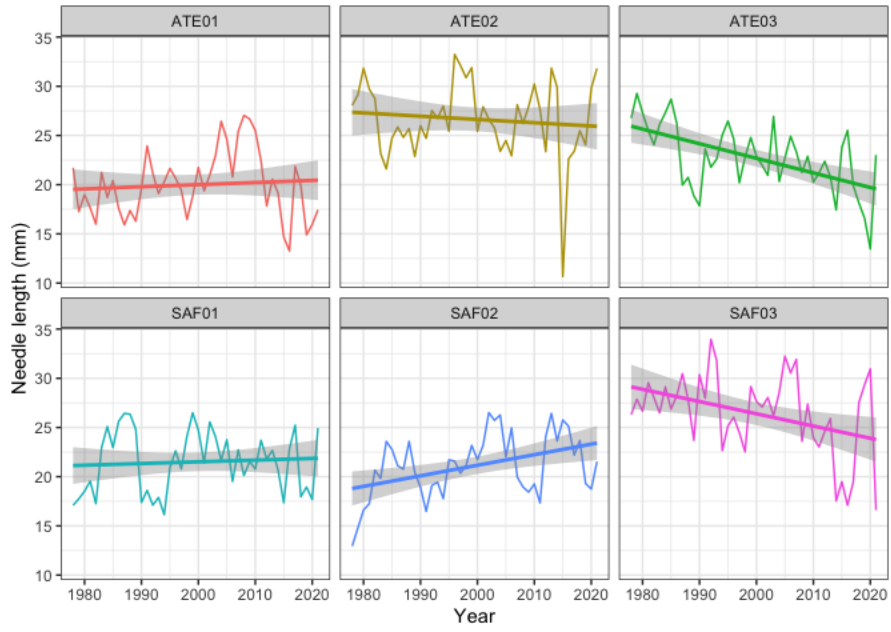


Figure 2. Needle length chronologies from each tree labeled by site over 1977–2021 with a linear model individually applied to each tree.

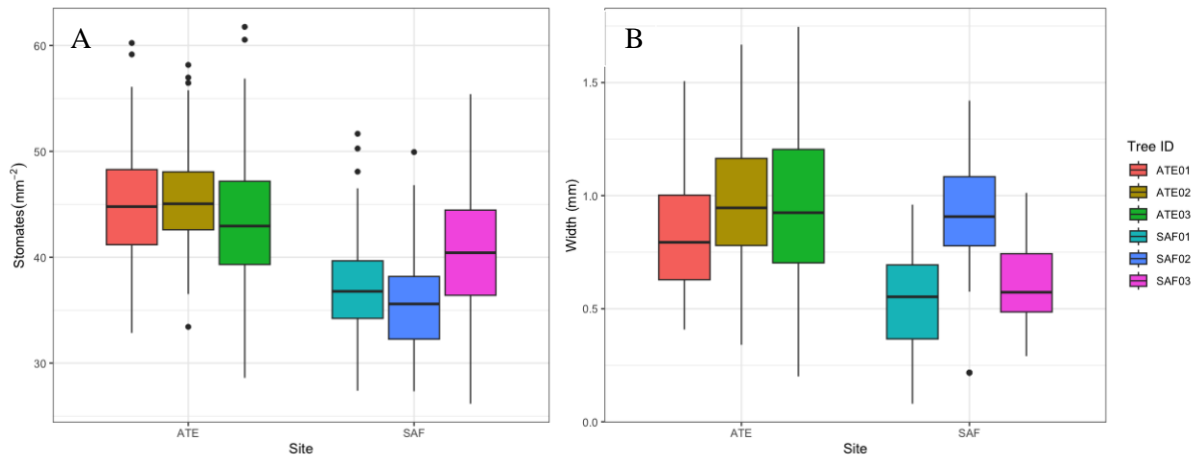


Figure 3. A) Stomatal density of each tree separated by site. B) Ring width of each tree separated by site. ATE – Alpine treeline ecotone. SAF – Subalpine forest.

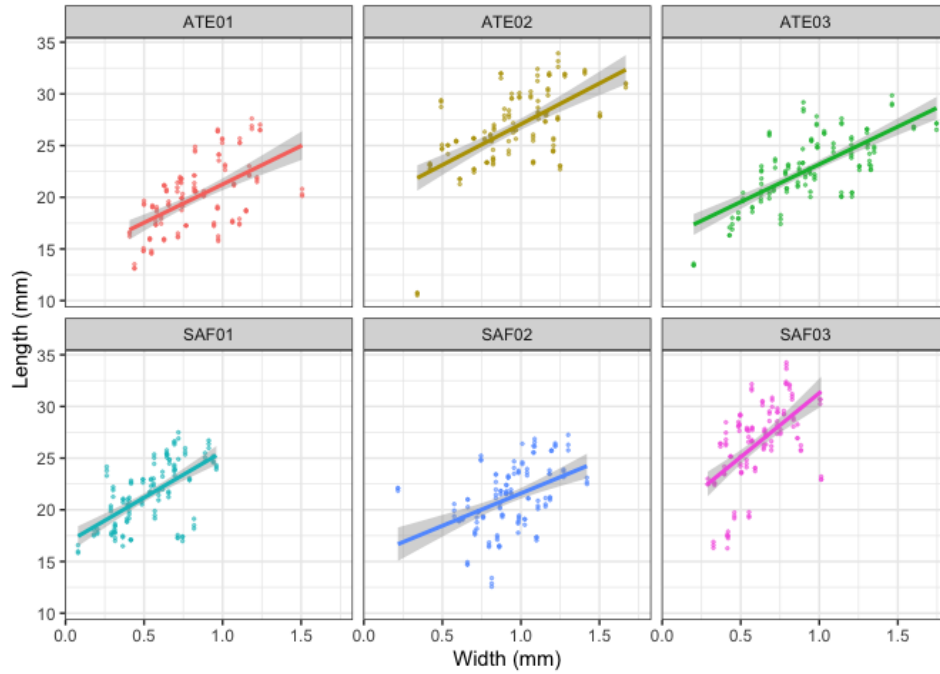


Figure 4. Needle length predicted by ring width by individual tree with a regression applied individually across trees separated by site.

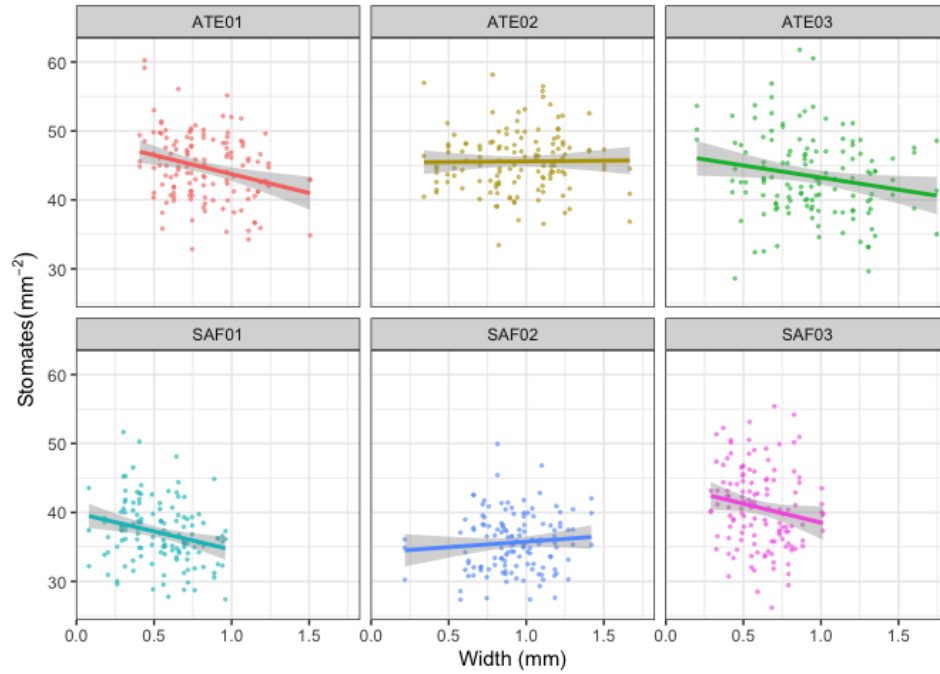


Figure 5. Stomatal density predicted by ring width by individual tree with a regression applied individually by tree separated by site.

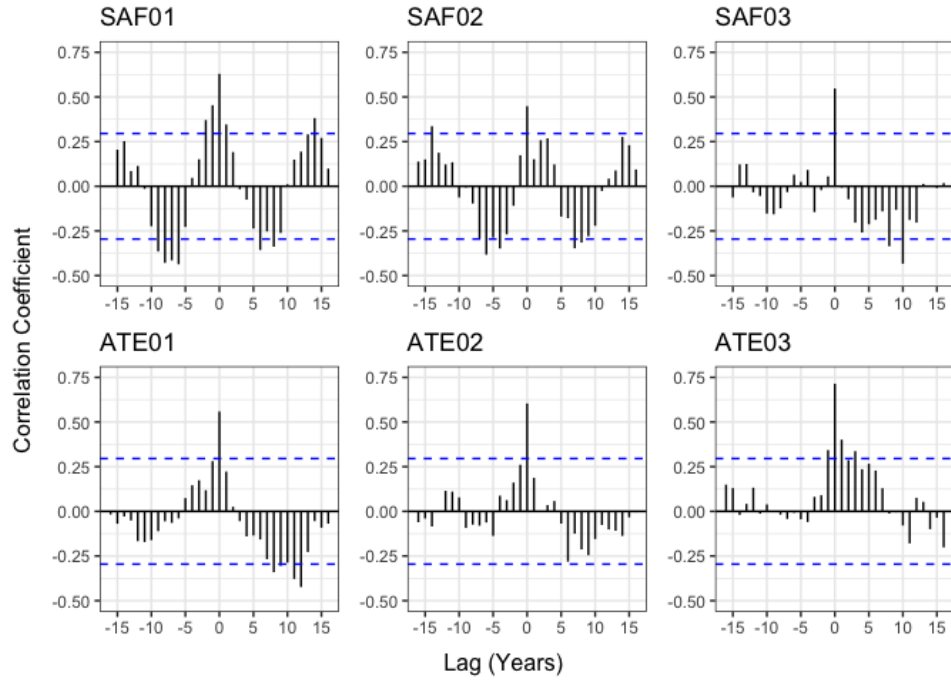


Figure 6. Cross-correlation between ring width and needle length. Dashed lines are representative of boundaries of statistically significant autocorrelations. Lag is in years.

8. References

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