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Postprint: Morphological Response of Heteroblastic Leaves of Abies faxoniana Seedlings to Long-term Simulated Warming in the Western Sichuan Timberline Ecotone

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Abstract

Global climate warming exerts strong effects on plant morphology in high-latitude, high-altitude regions. The forest-timberline ecotone in western Sichuan constitutes an important component of the alpine ecosystem in the eastern Qinghai-Tibet Plateau and is extremely sensitive to global change. Focusing on heteroaged leaves of Abies faxoniana seedlings in the forest-timberline ecotone of western Sichuan, this study employed in situ open-top chambers (OTCs) to simulate warming, investigated the responses of leaf morphological traits such as leaf length and leaf thickness in heteroaged leaves of A. faxoniana seedlings under long-term simulated warming, and analyzed the plasticity of leaf morphology using phenotypic plasticity index and coefficient of variation. The results showed that warming restricted leaf elongation, widening, and the expansion of leaf area and volume, causing reductions of 12.77%, 11.86%, 17.76%, and 11.49% in leaf length, leaf width, leaf area, and leaf volume, respectively, compared with the control; warming promoted an increase in leaf thickness, which increased by 7.27% relative to the control; except for the leaf length-to-width ratio, warming had significant effects on all other leaf morphological traits ($P < 0.05$). The response of leaf morphology to simulated warming exhibited significant age-dependent differences ($P < 0.05$). The interactive effects of temperature and leaf age had significant influences on leaf length and leaf area ($P < 0.05$), but non-significant effects on leaf width and leaf thickness ($P > 0.05$). The results from both phenotypic plasticity analyses indicated that, except for leaf length in 1-year-old leaves, warming increased the plasticity of various leaf morphological traits to varying degrees. Long-term warming induced xeromorphic tendencies and more divergent morphological values in fir seedling leaves. This study provides evidence for differential responses of A. faxoniana seedling leaves to long-term warming, offering fundamental data and theoretical refer-

ence for assessing the responses of dominant plants on the eastern margin of the Qinghai-Tibet Plateau to climate warming.

Full Text

Preamble

Long-term Warming Effects on Leaf Traits of Different-aged Needles of *Abies faxoniana* Seedlings in a Treeline Ecotone of Western Sichuan

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Abstract

Global warming has pronounced effects on plant morphology in high-latitude and high-altitude regions, which are extremely sensitive to global change. The treeline ecotone in western Sichuan represents a critical component of the alpine ecosystem on the eastern Tibetan Plateau. Using *Abies faxoniana* seedlings in this ecotone and employing in situ open-top chambers (OTCs), we investigated responses of leaf morphology (leaf length, width, thickness, etc.) and phenotypic plasticity in different-aged needles to long-term simulated warming. Warming constrained leaf elongation, expansion, and area in *A. faxoniana* seedlings, reducing leaf length, width, volume, and area by 12.77%, 11.86%, 17.76%, and 11.49%, respectively, while promoting increased leaf thickness (7.27% greater than control, $P < 0.05$). Leaf morphology responses to simulated warming exhibited significant age-related differences ($P < 0.05$). The interactive effects of warming and leaf age significantly influenced leaf length and area ($P < 0.05$) but not leaf width or thickness ($P > 0.05$). Both phenotypic plasticity analyses revealed that warming enhanced plasticity across most leaf traits, providing evidence for differential responses of *A. faxoniana* seedlings to long-term warming. Long-term warming induced xeromorphic tendencies and greater morphological divergence in fir seedling leaves. This study provides fundamental data and theoretical reference for assessing responses of dominant plants on the eastern Tibetan Plateau to climate warming.

Keywords: simulated warming; *Abies faxoniana* seedlings; different-aged leaves; leaf traits; plasticity

Introduction

Global climate warming is an undeniable reality. IPCC assessments indicate that global mean temperature increased by 0.85°C between 1880 and 2012 and is projected to rise by an additional 0.3–4.8°C by the end of the 21st century. Temperature controls the rate of nearly all biochemical reactions in ecosystems and represents a crucial driver of ecosystem processes. The ongoing expansion of the greenhouse effect directly and indirectly affects plant physiological processes, with even greater impacts on alpine regions. Leaves, as the primary photosynthetic organs, directly influence plant performance and function through their growth, development, and morphological characteristics. Leaf morphology serves as an important indicator of plant survival strategies adapted to environmental change. Leaf area correlates with photosynthesis and transpiration, representing a key metric for evaluating community net primary productivity and rational community structure, while leaf thickness affects resource acquisition and utilization.

During plant growth, leaves of different ages exhibit distinct environmental sensitivities due to differences in formation year, developmental stage, and biomass allocation, with strong correlations among morphological traits. Century-long global warming has significantly impacted plant phenology and physiological processes. Under changing environmental conditions, different-aged leaves likely exhibit varying plasticity due to differential sensitivity to warming. Investigating long-term simulated warming effects on leaf morphology across age classes is therefore critical for accurately predicting plant adaptation patterns to global warming.

The subalpine treeline ecotone in western Sichuan represents a typical ecologically sensitive and fragile area in the upper Yangtze River basin, serving as a critical transition zone from coniferous forest to alpine meadow. Its simple community structure, low plant height, and ubiquitous edge effects provide an ideal natural platform for understanding alpine ecosystem responses to climate change. Under global warming scenarios, the subalpine treeline ecotone will likely be colonized by dominant species from lower-elevation coniferous forests. *Abies faxoniana*, a key constructive species in northwestern Sichuan' s subalpine coniferous forests, is located below the treeline ecotone. Whether its leaves will exhibit morphological adaptation to long-term warming, and whether such adaptation shows interannual variation, remains uncertain. Previous studies demonstrated that warming advanced budburst and promoted leaf elongation and thinning in *A. faxoniana* seedlings, with significant increases in specific leaf area and positive responses in phenology and lateral growth. However, systematic investigation of leaf morphology in *A. faxoniana* seedlings has been lacking.

We hypothesized that different-aged leaves of *A. faxoniana* seedlings would respond differentially to long-term simulated warming. Using established open-top chambers (OTCs), we examined effects of long-term simulated warming on leaf length, width, area, and thickness across different-aged needles, analyzing plasticity changes to explore functional trait responses. This study aims to provide baseline data for understanding subalpine forest community functional changes under global warming.

1. Study Area Overview

This study was conducted in Wanglang National Nature Reserve, Pingwu County, Sichuan Province (32°59 N, 104°01 E, elevation 2600–3500 m). The region has a mountain monsoon climate with mean annual temperature of 1.5–2.9°C and January and July mean temperatures of -6.1°C and 12.7°C, respectively. Annual precipitation ranges from 801–825 mm, concentrated during the rainy season. Soils are dark brown forest soils. Soil organic carbon content is (180.2$±5.5)g/kgat0–15cmdepthand(180.2±3.7)g/kgat15–40cmdepth.Totalsoilnitrogenis(5.5±0.7)g/kgand(4 g/kg at these respective depths. *Abies faxoniana* forest is the main forest community type in the reserve.

2. Sample Plot Establishment

OTCs measuring 1.5 m × 1.5 m × 2.6 m were constructed from polycarbonate in gentle slope areas of the treeline ecotone on the outer slope of Dawodang. The base area measured 1.2 m × 1.2 m. Healthy, uniformly sized target species were transplanted from nearby sites into both OTCs and control plots (CK) of identical base area. Seedlings were approximately 12-15 years old, 50–60 cm tall, and spaced 30 cm apart. Temperature sensors (6507A, $±$0.1°C, Finland) were installed in each chamber. Warming was maintained year-round, with chamber air temperature averaging 2.9°C higher than ambient. The site was fenced, and chambers were kept clean with periodic maintenance. No artificial fertilization or watering was applied after establishment.

3. Research Methods

3.1 Plant Sampling

We sampled annual, biennial, and triennial needles of *A. faxoniana* seedlings. In 2016, healthy branches were collected from different directions of individual plants in each plot. Branch age was determined by bud scale scars. Intact, non-senescent needles were randomly collected, placed in labeled ziplock bags, and transported to the laboratory. For each treatment (OTC and CK), we ensured 30 valid samples per age class. Leaf length and width were measured with digital calipers ($±$0.01 mm). Single leaf area was determined using a leaf area meter (CI-203, CID, USA).

3.2 Statistical Analysis

Data were organized and analyzed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Split-plot ANOVA was performed with temperature as the main plot factor and leaf age as the subplot factor to test significance of leaf traits under both conditions. Duncan' s multiple range test was used for post-hoc comparisons. Phenotypic plasticity index (PPI) and coefficient of variation (CV) were calculated to assess plasticity: PPI = (MAX-MIN)/MAX and CV = standard deviation/mean. Box plots were generated using Origin 8.0 (Origin Lab, MA, USA) to display distribution and dispersion of morphological data, with upper and lower segments representing maximum and minimum values, and box boundaries representing upper and lower quartiles.

2. Results Analysis

2.1 Responses of Leaf Length, Width, and Length-Width Ratio

Simulated warming significantly reduced leaf length and width ($P < 0.01$) but had minimal effect on length-width ratio. Warming, leaf age, and their interaction all significantly affected leaf length ($P < 0.05$). Only leaf age significantly influenced leaf width; other factors showed no significant effects.

The range and dispersion of leaf morphological values in *A. faxoniana* seedlings differed in response to warming and leaf age. Warming reduced leaf length by 21.87% in 1-year-old needles ($P < 0.01$), 15.11% in 2-year-old needles ($P < 0.01$), and 13.48% in 3-year-old needles. Under OTC conditions, leaf length distributions for 2-year and 3-year needles were more concentrated, with no significant differences among age classes. In contrast, control plots showed significant differences. Leaf length ranges were 10.63–28.31 mm (OTC) versus 12.24–25.98 mm (CK).

Warming reduced leaf width by 18.82% in 1-year needles, 7.93% in 2-year, and 5.70% in 3-year needles. Width distributions were more dispersed under warming. The range was 1.331–2.527 mm (OTC) versus 1.801–3.112 mm (CK), with significant differences between 1-year and 3-year needles under warming ($P < 0.05$) but not in controls.

Warming did not significantly affect length-width ratio, though responses varied by age. Under warming, 1-year and 3-year needles showed significantly different ratios ($P < 0.05$), with more dispersed distributions (2-year: 6.02–20; 3-year: 5.35–12.44). Control plots showed no significant differences among age classes.

Effects of simulated warming on leaf traits of *A. faxoniana* seedlings

2.2 Responses of Leaf Area, Thickness, and Volume

Warming extremely significantly reduced leaf area by 17.76% ($P < 0.01$) and significantly reduced leaf volume by 11.49% ($P < 0.05$), while extremely significantly increasing leaf thickness by 7.27% ($P < 0.01$). Temperature and leaf age significantly affected leaf area ($P < 0.05$), while temperature extremely significantly affected thickness ($P < 0.01$) and leaf age significantly affected thickness ($P < 0.05$). Their interaction was not significant.

Leaf area and volume responses differed by age class. Warming inhibited area expansion, reducing 1-year leaf area by 28.41% ($P < 0.01$), 2-year by 12.97%, and 3-year by 8.81%. Under OTC, 1-year and 3-year leaf area changes were not significant, while 2-year leaves differed significantly from both ($P < 0.05$). Area ranges were 14–64 mm^2 (OTC) versus 28–64 mm^2 (CK), with increased dispersion under warming.

Warming promoted thickness increases of 7.84%, 3.51%, and 6.78% for 1-year, 2-year, and 3-year needles, respectively. Under warming, thickness values were concentrated near medians with expanded ranges: 0.31–0.74 mm, 0.41–0.63 mm, and 0.43–0.86 mm for 1-year, 2-year, and 3-year needles, respectively, compared to 0.41–0.63 mm and 0.45–0.69 mm in controls. One-year and 3-year needles differed significantly under warming ($P < 0.05$).

Warming inhibited volume expansion, reducing it by 11.49% overall. Volume ranges under warming were 4.4–32.4 mm^3, 7.92–37.76 mm^3, and 4.3–35.2 mm^3 for 1-year, 2-year, and 3-year needles, respectively, versus 7.74–28.42 mm^3, 11.76–40.6 mm^3, and 9.52–29.48 mm^3 in controls. Significant differences existed between 1-year and 3-year needles ($P < 0.05$).

[Figure 1: see original paper] Effects of warming on leaf length, width, and length-width ratio of *A. faxoniana* seedlings

[Figure 2: see original paper] Effects of warming on leaf area, thickness, and volume of *A. faxoniana* seedlings

2.3 Plasticity Responses of Leaf Length and Width

Phenotypic plasticity index analysis revealed that warming increased plasticity of leaf width and length in different-aged needles, except for 1-year leaf length plasticity, which decreased. The maximum increase reached 68.22% (3-year width). Results from both plasticity analysis methods were similar, confirming their utility for studying warming effects on leaf dimensions.

[Figure 3: see original paper] Phenotypic plasticity index (PPI) and coefficient of variation (CV) for leaf length and width of *A. faxoniana* seedlings

2.4 Plasticity Responses of Leaf Area and Thickness

Both plasticity measures indicated greater plasticity in leaf area and thickness under warming compared to controls, though the magnitude differed by method. Warming increased thickness plasticity most substantially (143.57% increase over control), while area plasticity increased by 69.02%. The 3-year needles showed the greatest plasticity enhancement across all age classes.

[Figure 4: see original paper] Phenotypic plasticity index (PPI) and coefficient of variation (CV) for leaf area and thickness of *A. faxoniana* seedlings

3. Discussion

3.1 Leaf Morphological Responses to Warming

Leaf expansive growth is highly sensitive to environmental factors, particularly temperature. In alpine regions where ambient temperatures are generally below optimal for plant growth, warming typically enhances vegetative growth in polar and high-mountain areas. However, this study found that warming significantly reduced leaf length, width, and area in different-aged *A. faxoniana* seedlings, contrasting with previous research. Xu et al. reported that simulated warming promoted leaf elongation and thinning in *A. faxoniana* seedlings, while Yang et al. found warming increased leaf area. These discrepancies may arise from differences in warming duration and soil moisture conditions. Xu et al. conducted their study during a year with higher-than-average rainfall, while Yang et al. irrigated to maintain adequate soil moisture. In natural warming scenarios, while occasional wet years may prevent significant soil moisture decline, the long-term trend of reduced soil moisture persists. OTCs exacerbate this by limiting rainfall input and intensifying water loss. This negative effect likely causes leaves to reduce area and develop xeromorphic traits to prevent excessive water loss and maintain optimal leaf temperature. Reduced leaf area also mitigates high transpiration damage, consistent with Yates et al.' s conclusion that smaller leaves serve as self-protection. This represents adaptation to hotter, drier conditions.

Long-term warming significantly increased leaf thickness ($P < 0.05$), enhancing water retention capacity. The shift toward smaller, thicker leaves in seedlings represents adjustment to relatively dry, hot habitats, further supported by our findings. The lack of significant effect on length-width ratio suggests differential responses of cell division and expansion to warming. Warming significantly reduced leaf volume, indicating constrained growth that may benefit carbon allocation to defense structures.

3.2 Interannual Variation in Leaf Morphology

Although warming altered leaf morphology overall, significant differences existed among age classes, indicating that interannual fluctuations in temperature and precipitation strongly influence warming responses. This aligns with findings on hybrid walnut (*Juglans nigra* × *J. regia*) and *Chenopodium album.* While continuous temperature monitoring was not provided, our previous research suggests clear interannual variation in warming effects, particularly between years with contrasting rainfall, which affects phenology and leaf development.

Short-term warming studies confirm this speculation. In 2007, abundant rainfall at our Wanglang site prevented substantial soil moisture reduction despite a 2.2°C temperature increase during the growing season, promoting *Salix eriostachya* growth and increasing individual leaf area. In contrast, 2009 had less rainfall, and warming reduced soil moisture by 2.4%, causing significant reductions in leaf length, width, and area with clear xeromorphic tendencies. Although individual leaf area decreased, total leaf number per plant increased, compensating for the reduction. Warming effects on phenology and leaf lifespan also showed interannual variation. Adaptive adjustments to annual microenvironmental conditions cause significant interannual differences in leaf morphology. Secondary metabolite accumulation in older needles may also contribute to thickening.

3.3 Phenotypic Plasticity and Morphological Parameter Responses

This study demonstrates that *A. faxoniana* seedling leaf morphology (area and thickness) adapted to simulated warming toward xeromorphism, maintaining optimal leaf temperature. Interannual climate variation significantly influenced these responses. The non-significant interactive effects on leaf width and thickness suggest counteracting trends under warming and age conditions.

Phenotypic plasticity, present in most plants, enhances species' ecological amplitude and tolerance, improving adaptation to changing environments and competitive ability. Our results show that warming generally increased plasticity of *A. faxoniana* leaf traits, indicating morphological adjustment as an adaptation mechanism. However, this adjustment was influenced by interannual microclimate variation, with different-aged leaves showing varying plasticity magnitudes. Notably, while warmed 1-year leaves had greater length values than controls, their plasticity was lower, further confirming interannual variation in adaptation. Smaller leaves with reduced transpiration better adapt to dry, hot environments for self-protection.

In summary, *A. faxoniana* seedlings adjusted leaf morphology in response to long-term warming, showing significant phenotypic plasticity that varied by leaf age due to interannual climate differences, fully supporting our initial hypothesis. Future studies should integrate microclimate data and investigate belowground ecological processes to better elucidate mechanisms of response to global warming.

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