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Research paper

Photosynthetic phenological variation may promote coexistence among co-dominant tree species in a Madrean sky island mixed conifer forest

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Across much of western North America, forests are predicted to experience a transition from snow- to rain-dominated precipitation regimes due to anthropogenic climate warming. Madrean sky island mixed conifer forests receive a large portion of their precipitation from summertime convective storms and may serve as a lens into the future for snow-dominated forests after prolonged warming. To better understand the linkage between physiological traits, climate variation, and the structure and function of mixed conifer forests, we measured leaf photosynthetic (A) responses to controlled variation in internal CO_2 concentration (C_i) to quantify interspecific phenological variation in A/C_i-derived ecophysiological traits among ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson), southwestern white pine (*Pinus strobiformis* Engelm.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Species had similar, positive responses in net photosynthesis under ambient conditions (A_{net}) to the onset of summertime monsoonal precipitation, but during the cooler portions of the year *P. ponderosa* was able to maintain greater A_{net} than *P. menziesii* and *P. strobiformis*. Moreover, *P. ponderosa* had greater A_{net} in response to ephemerally favorable springtime conditions than either *P. menziesii* or *P. strobiformis*. Monsoonal precipitation was associated with a sharp rise in the maximum rates of electron transport (J_{max}) and carboxylation (V_{Cmax}) in *P. menziesii* in comparison with *P. ponderosa* and *P. strobiformis*. In contrast, species shared similar low values of J_{max} and V_{Cmax} in response to cool winter temperatures. Patterns of relative stomatal limitation followed predictions based on species' elevational distributions, reinforcing the role of stomatal behavior in maintaining hydraulic conductivity and shaping bioclimatic limits. Phenological variation in ecophysiologial traits among co-occurring tree species in a Madrean mixed conifer forest may promote temporal resource partitioning and thereby contribute to species' coexistence. Moreover, these results provide a physiological basis for predicting the ecological implications of North American mixed conifer forests currently transitioning from snow- to rain-dominated precipitation regimes.

Keywords: A/C_i analysis, ecophysiology, niche partitioning, rain–snow transition.

Introduction

Anthropogenic climate warming and concurrent shifts in precipitation threaten the composition, structure and distribution of forest ecosystems worldwide (Allen et al. 2010). In the southwestern United States, well-documented regional forest decline is driven by drought and heat stress coupled with insect outbreaks and wildfire (Allen and Breshears 1998, Weiss and Overpeck 2005, Adams et al. 2009). The sensitivity of these ecosystems to climate variability and disturbance creates uncertainty for the potential of western North American forests to act as a sink for atmospheric carbon and to provide other ecosystem goods and services in predicted future climates (Archer and Predick 2008, Bentz et al. 2010, Hurteau et al. 2014). However, regional variation in hydroclimate, physiography, forest management, species composition and disturbance regimes suggest that the rate and trajectory of western North American forest responses to climate change may vary widely.

The role of temperature and water limitation in shaping forest community composition has long been recognized (Shreve 1915, Whittaker and Niering 1965, 1975). However, revealing how predicted climatic conditions of warmer temperatures, longer inter-storm periods of moisture stress and shifting seasonality of rainfall (Garfin et al. 2014) might influence western North American forests requires a fuller understanding of plant physiological responses to abiotic stress. While the physiological responses of conifers to abiotic stress in snow-dominated, montane ecosystems has become more readily available to parameterize ecosystem function models (Marshall et al. 2001, Nippert et al. 2004, Coops and Waring 2011), there is a paucity of similar data for Madrean sky island mixed conifer forests—a spatially extensive region that receives a large portion of their precipitation from convective storms associated with the North American Monsoon (Adams and Comrie 1997) and is predicted to experience a shift towards less snow and more rain (Seager et al. 2007, Klos et al. 2014, Melillo et al. 2014).

Madrean sky island mixed-conifer forests are distributed across the highest elevations of isolated mountain ranges south of Arizona's Mogollon Rim and north of the Sierra Madre in Sonora, Mexico (Warshall 1995). Unlike the snowmelt-dominated hydroclimate of more northern mountainous regions, the hydroclimate of the Madrean sky islands is characterized by a combination of snowmelt and convective storms associated with the North American Monsoon (Brown-Mitic et al. 2007, LaMalfa and Ryle 2008). These mid-summer monsoon storms trigger biological activity and are important drivers of primary productivity in these diverse ecosystems (Weiss et al. 2004, Poulos et al. 2007, Crimmins et al. 2008). At the highest elevations where mixed conifer forests occur, ecosystem carbon balance is influenced by winter precipitation as well as monsoon rains (Brown-Mitic et al. 2007). The distinctive hydroclimatology of the Madrean sky islands creates the potential for seasonally dynamic, species-specific patterns of physiological activity among co-occurring tree species in mixed conifer forests. One reflection of the seasonally dynamic nature of moisture availability in this ecosystem may be temporal resource partitioning, which could contribute to maintaining conifer diversity in these forest communities (McGill et al. 2006, Meinzer et al. 2007) as well as shape ecosystem-scale functional responses to intra- and inter-seasonal climate variability (Diaz and Cabido 2001). Examining physiological responses to abiotic drivers in this setting may serve as a lens into the future of snow-dominated montane systems projected to experience prolonged warming and a transition to more precipitation as rainfall.

Linking trade-offs in resource capture, use and productivity through time with patterns of community composition remains an active research area, and measures of photosynthetic assimilation (A) response to controlled variation in internal CO_2 concentration (C_i) has proven to be a useful tool in gaining insights into these physiological traits (Huxman et al. 2008, Barron-Gafford et al. 2013). Analysis of leaf-level responses of A vs C_i (A/C_i response curves) yields insights into within-leaf electron transport, rates of carboxylation, respiration and stomatal limitation, but their interpretation can extend well beyond the constraints of leaf function. Increasingly, these traits have been linked to patterns of plant nutrient status over large geographic scales (Walker et al. 2014), ecosystem-scale carbon fluxes measured using eddy covariance techniques (Kuppel et al. 2012), remotely sensed spectral vegetation indices (Serbin et al. 2012, 2015, Ainsworth et al. 2013) and in Earth system models (Bonan et al. 2012, Rogers 2014). Still, measures of the phenology of A/C_i-derived physiological traits and their interpretation in the context of species coexistence remains limited.

The objective of this research was to characterize phenological variation in leaf photosynthetic carbon assimilation of three widely distributed species in Madrean sky island mixed conifer forests: ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson), southwestern white pine (*Pinus strobiformis* Engelm.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Our predictions about the phenological dynamics of photosynthesis in these species are informed by their respective elevational distributions, which overlap one another in mixed conifer forests of the Madrean sky islands (Whittaker and Niering 1965, Niering and Lowe 1984, Adams and Kolb 2004). We predicted that warm and dry conditions would favor the physiological performance of *P. ponderosa*, which is dominant in warmer and drier lower elevation stands. Conversely, cool wet conditions associated with winter precipitation would favor the physiological performance of *P. menziesii*, which dominates cooler, more mesic, high elevation sites. Finally, we predicted that *P. strobiformis*, a much less studied species, but one whose elevational distribution overlaps both *P. ponderosa* and *P. menziesii*, would have intermediate physiological responses to seasonally dynamic temperatures and moisture availability (Looney and Waring 2013, Goodrich et al. 2016).

Materials and methods

Site description

Our research was conducted in the immediate vicinity of the Mt Bigelow eddy-covariance tower in the Santa Catalina Mountains north of Tucson, AZ (32° 25′ 00′N, 110° 43′ 31′W, elevation 2573 m). Brown-Mitic et al. (2007) provide a complete description of the research site's physiographic setting and climate. Briefly, the site is a mature, second growth stand of *P. menziesii*, *P. ponderosa* and *P. strobiformis*. Tree-ring analyses of *P. menziesii* from the same stand suggest that overstory trees established in the late 1930s (J. Minor, pers. comm.). Thin and rocky soils accentuate the site's dry climate, which averages ~750 mm annual precipitation (Mt Lemmon Climate Station, NCDC Co-op ID

025,732; elevation 2374 m). Early summer (May–June) is warm and dry while mid- to late summer (July–Sept) experiences higher humidity and convective storms associated with the North American Monsoon which account for ~50% of annual precipitation (Figure 1). Autumn (October–December) is characterized by a return to warm, dry conditions which are followed by cooler winter (January–April) temperatures and occasional frontal storms associated with rainfall and occasional snow. Occasional snowfall and mild temperatures allow soils to remain unfrozen below 4 cm depth throughout the winter (Brown-Mitic et al. 2007).

Temperature, precipitation and soil moisture

Above-canopy measurements of daily average air temperature for the period 1 April 2012 to 31 May 2013 were provided by a research standard platinum resistance thermometer (HMP60, Campbell Scientific, Logan, UT, USA) housed in a 6-plate solar radiation shield (41303-5 A, Campbell Scientific) and associated with the eddy-covariance tower located at our research site. Likewise, we report daily precipitation measured by a tipping bucket rain gauge (TE525MM-L, Campbell Scientific) also associated with the eddy covariance tower. Due to instrument failure, daily precipitation measurements did not begin until 7 June 2012. To capture seasonal trends in soil moisture, we recorded 30-min averaged volumetric water content of soil at 36-cm depth, measured at a nearby representative location as the dielectric constant of the soil by a Decagon soil moisture probe (EC-20 Soil Moisture Smart Sensor S-SMA-M005, Onset Computer Corporation, Bourne, MA, USA).

Leaf gas exchange

To characterize species-specific photosynthetic phenology, we measured the net photosynthesis rate across five sampling periods representing distinct temperature and soil moisture conditions throughout the year (Figure 1). Physiological performance during the warm and dry early summer (pre-monsoon) was represented by data collected during the week of 15 June 2012; warm and humid late summer (monsoon) conditions were characterized with measurements during the week of 26 July 2012, following the onset of monsoonal storms. Measurements during the week of 17 October 2012 characterized cool and dry autumn conditions. Data collected during the first week of February 2013 characterized the cool and wet winter conditions. The spring growing season of warm and wet conditions were represented by measurements during the third week of April 2013. We identified four to five representative, mature individuals from each of the three canopy co-dominant evergreen conifer species present at the research site. Among the sampled individuals, average height and diameter at breast height did not significantly vary between species (Table 1; ANOVA, $F = 1.22_{2,11}$, $P = 0.33$; $F = 0.29_{2,11}$, $P = 0.75$, respectively). On each sampling date, we used an extension ladder and telescoping tree pruner to collect south-facing, sun-exposed branches from each individual. This approach limited our sampling to branches less than ~6.5 m above the ground. These branches were then re-cut underwater, and kept hydrated in water filled buckets during leaf gas exchange measurements as described by Huxman et al. (2003). To address our concern about leaf age-mediated shifts in physiological performance, we selected

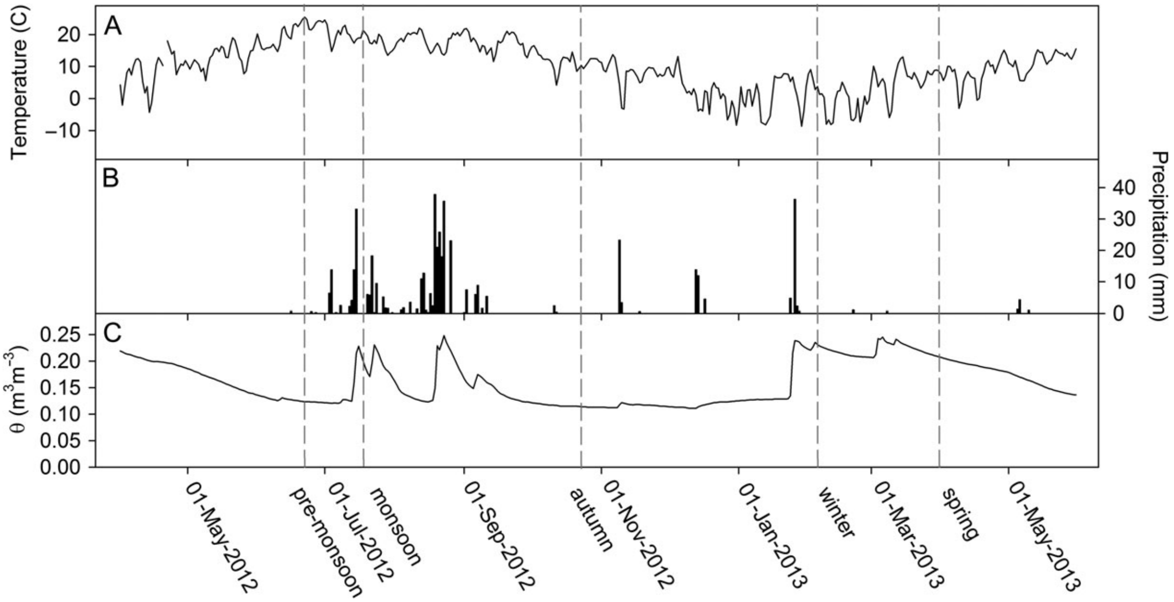

Figure 1. Seasonal variation in (A) daily mean air temperature (°C; 1 April 2012 to 31 May 2013), (B) daily precipitation (mm; 7 June 2012 to 31 May 2013) and (C) daily mean volumetric soil moisture at 36 cm depth ($m^3 m^{-3}$; 1 April 2012 to 31 May 2013) in a mixed conifer forest in the Catalina Mountains of southern Arizona. Vertical dashed lines indicate sampling days and are labeled according to the season which that sampling date was intended to represent.

Table 1. Diameter at breast height (DBH) and height of measured trees expressed as mean ± SD in a mixed conifer forest in the Catalina Mountains of southern Arizona.

Species	DBH (cm)	Height (m)
Pinus ponderosa ($n = 5$)	36.89 ± 14.94	14.08 ± 3.35
Pinus strobiformis ($n = 4$)	26.20 ± 5.61	13.40 ± 1.57
Pseudotsuga menziesii ($n = 5$)	28.44 ± 9.45	12.64 ± 3.34

groupings of representative, 1-year-old needles for leaf gas exchange measurements.

We measured leaf area-adjusted photosynthetic assimilation (A) rate versus internal [CO_2] response (A/C_i) curves using portable photosynthesis analyzers (LI-6400XT, Li-COR, Lincoln, NE, USA) configured with opaque conifer chambers (model 6400-22) connected to RGB light sources (model 6400-18 A). The sample cuvette light intensity was set to 1500 μmol photons m^{-2} s^{-1}, vapor pressure deficit was maintained between 1 and 2 kPa, and measurements were made at ambient environmental temperature. The initial measurement of leaf gas exchange was made at a cuvette [CO_2] of 400 ppm, which allowed us to measure the rate of net photosynthesis under ambient atmospheric conditions (A_{net}; μmol CO_2 m^{-2} s^{-1}). Subsequent measurements were made at cuvette [CO_2] concentrations of 300, 200, 100, 50, 200, 400, 600, 800, 1200 and 2000 ppm. At each cuvette [CO_2], leaf gas exchange parameters were monitored closely to ensure that the cuvette reached stable and steady-state conditions before measurements were made. Following the measurements, leaves from inside the cuvette were removed from the stem, placed in re-sealable plastic bags and stored on ice. Leaves were returned to the laboratory and their area was determined as described by Fites and Teskey (1988).

The resulting A/C_i response curves were analyzed according to Sharkey et al. (2007) to estimate the maximum rate of electron transport (J_{max}; μmol electrons m^{-2} s^{-1}), the maximum rate of carboxylation (V_{Cmax}; μmol CO_2 m^{-2} s^{-1}), mitochondrial respiration (R_d; μmol CO_2 m^{-2} s^{-1}) and triose phosphate utilization (TPU; μmol CO_2 m^{-2} s^{-1}). Triose phosphate utilization represents the rate of starch and sucrose synthesis and limits photosynthesis by constraining the supply of free phosphate (Sharkey 1985). Seasonal variation in TPU may reflect phenological variation in the strength or availability of carbon sinks (Herold 1980). In addition, we used A/C_i response curves to calculate relative stomatal limitation according to Farquhar and Sharkey (1982) (RSL; %). We calculated the ratio of the maximum rate of electron transport (J_{max}) to the maximum rate of carboxylation (V_{Cmax}; J:V) to quantify species and phenological differences in the coordinated investment in photosynthetic electron transport and the Calvin–Benson cycle.

Physiological response variables met the assumptions of normality and were analyzed using a repeated-measures analysis of variance (ANOVA) and post-hoc Tukey's HSD tests to examine the effects of species, season, and their interactive effect using JMP 7.0 (SAS Institute Inc., Cary, NC, USA). Finally, inspired by the use of V_{Cmax} to estimate photosynthesis in coupled vegetation and climate models (Bauerle et al 2012), we used least squares linear regression to examine the influence of species and season on the correlation between the maximum rate of carboxylation (V_{Cmax}) and net photosynthesis (A_{net}).

Results

While seasonal patterns of precipitation for the period May 2012–May 2013 were representative of the region's distinctive summer and winter rainfall regimes, total precipitation at during that period was just 440 mm, well below the historic average of ~750 mm (Figure 1). Seasonal variation in temperature and rainfall are reflected in species-specific patterns in the response curves of net photosynthesis rate versus internal [CO_2] (A/C_i; Figure 2). For example, across the three species, flattened A/C_i curves during the pre-monsoon and winter are consistent with strong limitations on the rate of net photosynthesis during these dry seasons. Conversely, autumn and spring were characterized by greater convexity in the A/C_i response curves suggesting more amenable environmental conditions for photosynthesis. A more quantitative analysis of the seasonal and species-specific patterns of physiological performance is provided by subsequent analyses of the various physiological performance metrics we derived from these response curves.

Patterns of A_{net} under ambient atmospheric [CO_2] were characterized by species-specific responses to seasonal environmental variability (Table 2; species × season). Across species, hot and dry conditions during the pre-monsoon were associated with very low A_{net} (Figure 3A). The onset of monsoonal storms triggered a similar positive A_{net} response across all species, which persisted through a gradual decline in air temperatures and soil moisture into the autumn sampling period in *P. strobiformis* and *P. menziesii*. In contrast, *P. ponderosa* A_{net} increased following the monsoon and was greater than *P. strobiformis* and *P. menziesii* during the autumn (Tukey's HSD, $P < 0.05$). Whereas the cool and wet conditions of winter were associated with a decline from autumnal A_{net} values across species, rates of A_{net} in *P. ponderosa* remained significantly higher than the other co-occurring species (Tukey's HSD, $P < 0.05$). We observed an increase in A_{net} associated with increasingly warm temperatures during spring. Consistent with a statistically significant interaction in our repeated measures analysis, this springtime A_{net} increase was variable among species and most apparent in the *P. ponderosa* (Tukey's HSD, $P < 0.05$).

The maximum rate of electron transport (J_{max}) followed a phenological pattern similar to that of A_{net} (Figure 3B). Among the three species, J_{max} was least in *P. menziesii* during the pre-monsoon and greatest during the monsoon (Table 2; species × season). In contrast, the J_{max} of *P. ponderosa* and *P. strobiformis*

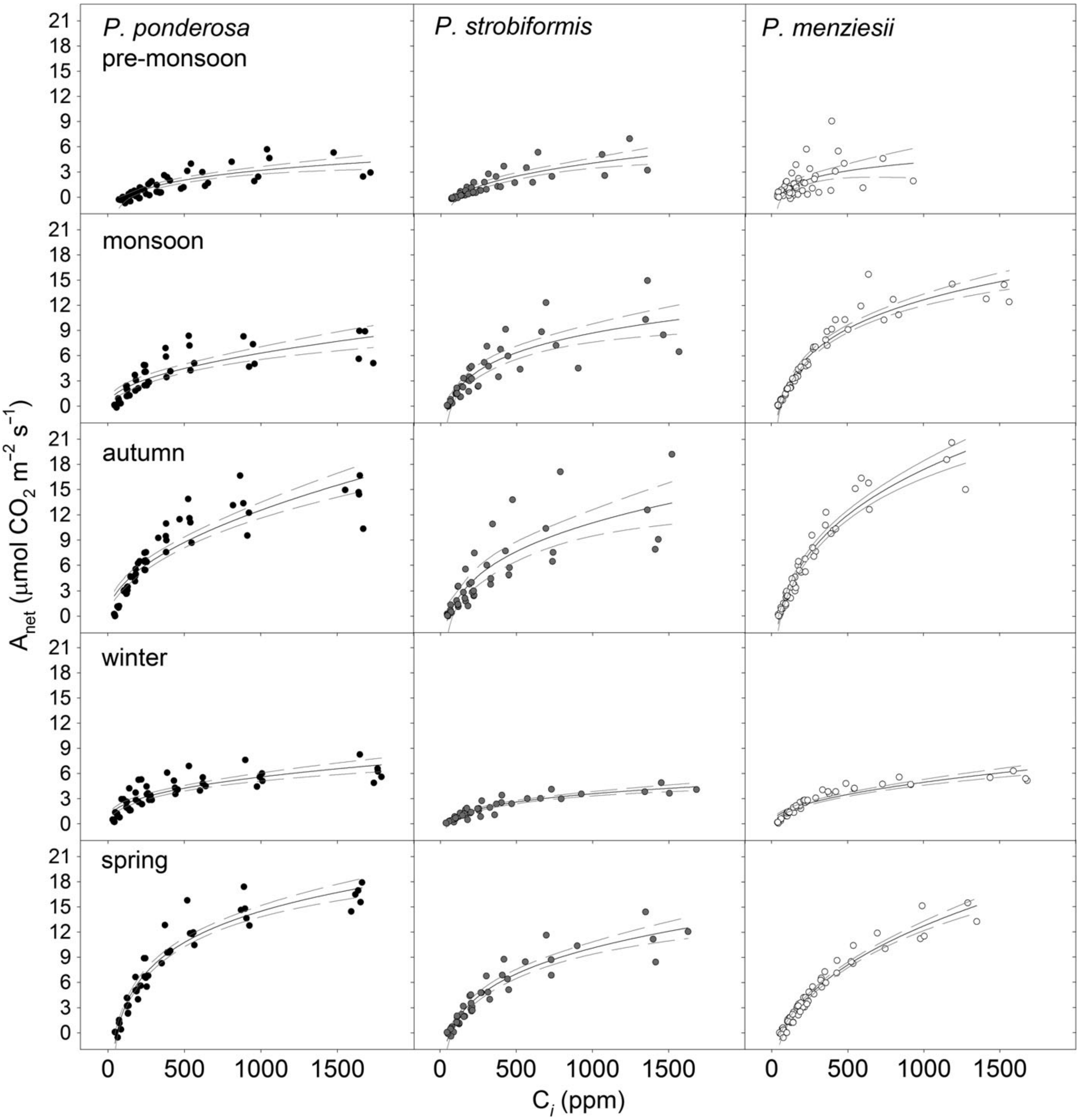

Figure 2. The rate of net photosynthesis (A_{net}; μmol CO_2 m^{-2} s^{-1}) versus internal [CO_2] (C_i; ppm) response curves among three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona. Seasons are illustrated by row; data are shown for *P. ponderosa* (left columns), *P. strobiformis* (middle columns) and *P. menziesii* (right columns). Dashed lines indicate the 95% confidence interval for the fitted C_i response of A_{net} across the range of measured C_i.

shared a common, muted phenological response to the monsoon (Tukey's HSD, $P > 0.05$). Spring conditions were associated with an increase in J_{max} among all three species. Reflecting close coordination of the two photosynthetic cycles, the maximum rate of carboxylation (V_{Cmax}) closely tracked J_{max} across seasons (Figure 3C) and had similar patterns of seasonal specific species responses including contrasting responses of *P. menziesii* and *P. ponderosa* to the monsoon (Table 2; species × season).

Patterns of relative stomatal limitation of photosynthesis (RSL) were species-specific and seasonally dynamic (Table 2; species × season). In addition to exhibiting greater overall RSL than

Table 2. Degrees of freedom (df) and *F*-statistics from repeated-measures ANOVA of net photosynthesis at ambient atmospheric [CO_2] (A_{net}), the maximum rate of electron transport (J_{max}), the maximum rate of carboxylation (V_{Cmax}), relative stomatal limitation (RSL), daytime respiration (R_d), triose phosphate utilization (TPU) and the ratio of J_{max} to V_{Cmax} (J:V) from three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona.

Factor	df	A_{net}	J_{max}	V_{Cmax}	RSL	R_d	TPU	J:V
Species	2,9	13.6**	2.39	0.086	30.6**	5.96*	1.83	2.37
Season	4,6	101**	87.4**	205**	23.0**	350**	58.4**	27.08**
Species × season	8,12	4.69**	3.02*	2.85*	2.88*	9.29**	2.88*	1.46

$^*P < 0.05$; $^{**} P < 0.01$.

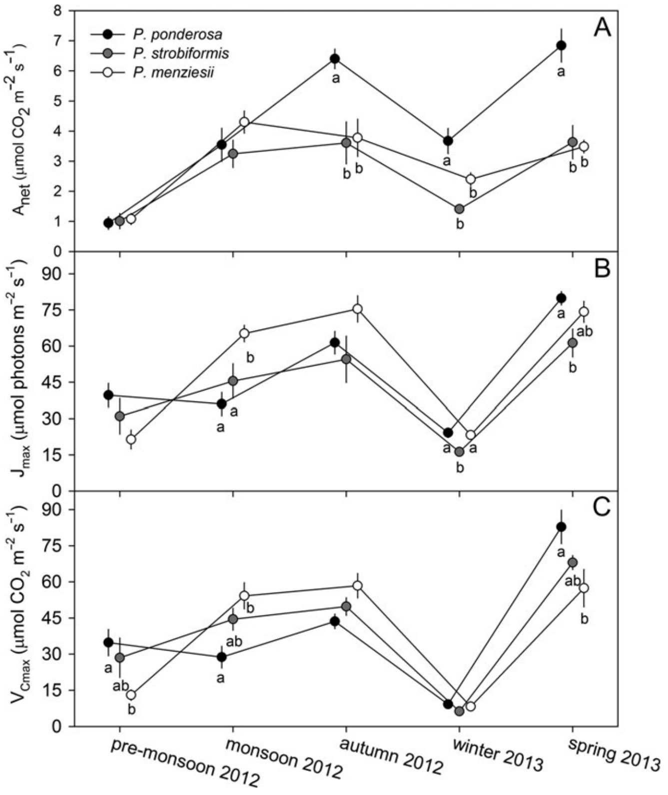

Figure 3. Phenological variation in (A) mean ± SE net photosynthesis at ambient [CO_2] (A_{net}; μmol CO_2 m^{-2} s^{-1}), (B) mean ± SE of the maximum rate of electron transport (J_{max}; μmol electrons m^{-2} s^{-1}) and (C) mean ± SE of the maximum rate of carboxylation (V_{Cmax}; μmol CO_2 m^{-2} s^{-1}) among three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona. *Pinus ponderosa* are illustrated as black symbols, *P. strobiformis* are illustrated as gray symbols, *P. menziesii* are illustrated as open symbols; lower case letters indicate significant, same-day differences in means (Tukey HSD, $P < 0.05$). For clarity, same day treatments are slightly offset from one another on the *X*-axis.

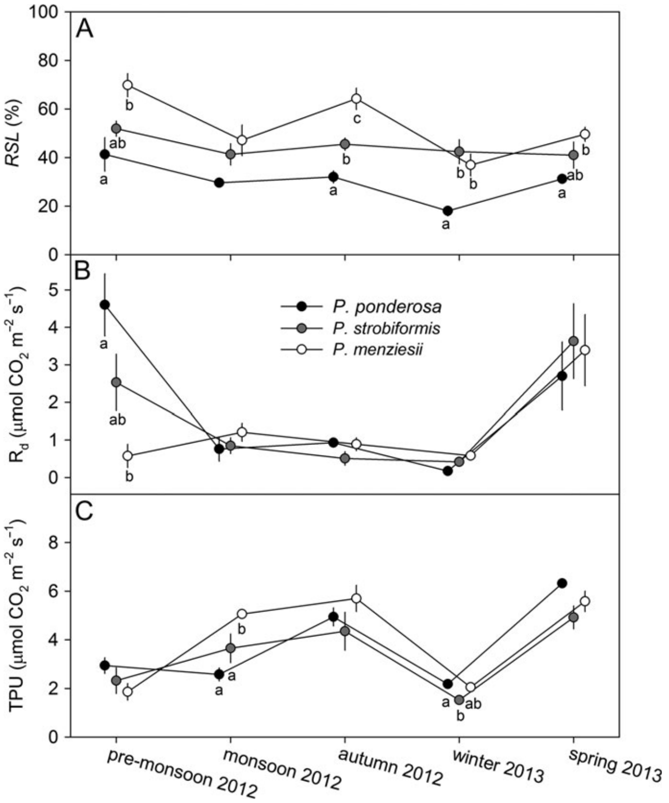

Figure 4. Phenological variation in (A) mean ± SE relative stomatal limitation (RSL; %), (B) mean ± SE respiration (R_d; μmol CO_2 m^{-2} s^{-1}) and (C) mean ± SE triose phosphate utilization (TPU; μmol CO_2 m^{-2} s^{-1}) among three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona. Symbols follow Figure 3.

P. strobiformis or *P. ponderosa*, *P. menziesii* was particularly dynamic in its response to seasonal moisture availability. In contrast, *P. strobiformis* maintained seasonally constant and intermediate values of RSL relative to the other species (Figure 4A). Similarly, the effect of season on daytime respiration (R_d) was species-specific (Table 2; species × season). Species differences in R_d were most apparent during the warm and dry pre-monsoon season when *P. ponderosa* had R_d much greater than *P. menziesii* (Figure 4B; Tukey's HSD, $P < 0.05$). The onset of the monsoon was associated with a convergence among the three species as R_d in the pines declines and as R_d in *P. menziesii* increased. From the monsoon on through to the winter season, species had similar, low R_d. The spring season was associated with a general increase in R_d.

We observed autumn and spring peaks in TPU, a seasonal pattern distinctive from A_{net} and R_d (Figure 4C). Moreover, species responded differently to seasonal environmental variability (Table 2; species × season). For example, species had individualistic, positive responses of TPU to the onset of monsoonal storms, with *P. menziesii* having the most pronounced increase followed by a gradual rise through the autumn. In comparison,

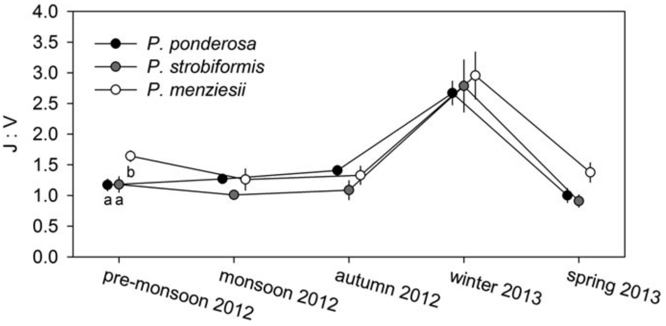

Figure 5. Phenological variation in the mean ± SE ratio of the maximum rate of electron transport (J_{max}; μmol electrons m^{-2} s^{-1}) to the maximum rate of carboxylation (V_{Cmax}; μmol CO_2 m^{-2} s^{-1}; J:V) among three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona. Symbols follow Figure 3.

TPU of *P. ponderosa* did not respond to the monsoon but increased later during the autumn. Species had similar positive increases in TPU in response to spring growing conditions.

Overall, we detected similar J:V responses to dynamic environmental conditions throughout the year among the species (Figure 5; Table 2; season). From the pre-monsoon through to the autumn, J:V remained very low, and cool, wet winter conditions were associated with a rise in J:V. During the pre-monsoon and spring seasons, *P. menziesii* tended towards greater J:V than either *P. ponderosa* or *P. strobiformis*, however this pattern was not statistically significant (Table 2; species × season; $P = 0.14$) Likewise, A_{net} was positively correlated with V_{Cmax} (Figure 6). This positive correlation was consistent across species and seasons (species × V_{Cmax}: $F = 0.21$; df = 2, 38; $P = 0.81$ and season × V_{Cmax}: $F = 1.93$; df = 4, 38; $P = 0.12$).

Discussion

Taken together, these results demonstrate the seasonally dynamic and interrelated nature of photosynthetic traits among three species of conifers to improve our understanding of the mechanisms that will shape community composition and ecosystem function in response to future climate change. For example, whereas all three species had similar, positive responses in A_{net} to the onset of monsoonal precipitation, species varied strongly in rates of photosynthesis during the cooler portions of the year (Figure 3A)—those which are predicted to experience the greatest climatic change. This seasonal pattern of interspecific functional variability stands in contrast to more seasonally consistent patterns of relative stomatal limitation across species (RSL; Figure 4A).

Species-specific patterns of RSL were consistent with our initial predictions based on elevational distributions of the three species. For example, at our site near the lower boundary of its elevational range, *P. menziesii* experienced persistent RSL (Figure 4A), which in turn limited A_{net} (Figure 3B) despite relatively large investments in electron transport (J_{max}; Figure 3B) and carboxylation rate (V_{Cmax}; Figure 3C). Conversely, near the upper boundary of its elevational range at our site, *P. ponderosa* experienced consistently lower RSL and correspondingly greater rates of A_{net}, particularly during autumn, winter and spring, than *P. menziesii* (Figures 4A and 3A, respectively). These findings serve to reinforce the central role of stomatal behavior in maintaining hydraulic conductivity (Hetherington and Woodward 2003) as well as the importance of the vulnerability of hydraulic architecture in determining bioclimatic limits (Blackman et al. 2012). Furthermore, these findings suggest that physiological performance of *P. menziesii* maybe be more sensitive to future climate warming at this site than either *P. strobiformis* or *P. ponderosa*.

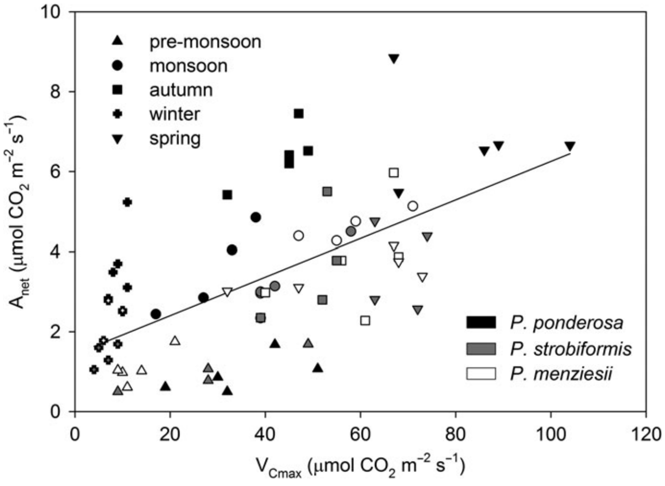

Figure 6. The maximum rate of carboxylation (V_{Cmax}; μmol CO_2 m^{-2} s^{-1}) versus net photosynthesis at ambient [CO_2] (A_{net}; μmol CO_2 m^{-2} s^{-1}) for all measurement dates among three co-occurring tree species in a mixed conifer forest in the Catalina Mountains of southern Arizona. Seasons are indicated by symbols. The regression line indicates a statistically significant best fit line from least squares linear regression.

We observed values of J:V that were generally lower than those reported in a widely cited meta-analysis of C_3 plants by Wullschleger (1993). The low values of J:V that we observed may be explained by the influence of persistent stomatal limitation on investments in J_{max} and V_{Cmax}. Walker et al. (2014) describe a functional trade-off in the relative allocation of resources to electron transport and the Calvin–Benson cycle. Under light limiting conditions, a large investment in J_{max} relative to V_{Cmax} maximizes A_{net}. However, during carboxylation-limited conditions, such as occurs during stomatal closure in response to water limitation, a large investment in J_{max} relative to V_{Cmax} requires dissipating excess electrons to avoid photoinhibition. In the present study, seasonal of patterns of investment in J_{max} relative to V_{Cmax} are consistent with these ideas (Figure 5). During the warmer portions of the growing season, frequent water limitation, high VPD and intense sunlight, a product of our research site's high elevation and, relatively speaking, low latitude, may favor low investment in J_{max} relative to V_{Cmax} as a mechanism to limit photoinhibition. An increase in J:V during cooler winter conditions when VPD declined and sun angles were reduced, may indicate conifers' response to a reduction in carboxylation limitation and the corresponding risk of photoinhibition.

Species' differential ability to extend the seasonal duration of photosynthetic activity may drive interspecific phenological variation in growth rates among co-occurring conifers (Marshall et al. 2001). For example, Nippert et al. (2004) noted that *P. ponderosa* was better able to maintain photosynthesis through the winter than *P. menziesii*, a pattern we observed in the present study (Figure 2). Likewise, the rapid springtime recovery of photosynthetic capacity by *P. ponderosa* relative to *P. menziesii* observed by Marshall et al. (2001) in a common garden experiment is consistent with our April observations (Figure 2). Under future climate scenarios, rising temperatures and reduced snowpack may result in a decline in springtime soil moisture (Garfin et al. 2014). Based on observations of seedlings grown in a common garden, *P. ponderosa* exhibits relatively little population-level variability in photosynthetic traits (Zhang and Marshall 1995). In contrast, regional climate variation in temperature and vapor pressure deficit associated with coastal and interior populations of *P. menziesii* are reflected in their respective photosynthetic traits (Zhang and Marshall 1995). This suggests that the ability of *P. ponderosa* to rapidly respond to ephemeral springtime growing conditions and thereby have an advantage over *P. menziesii* may extend beyond Madrean sky islands mixed conifer communities to other mixed conifer forests in the interior of western North America where climatic warming is predicted to occur.

The possibility that seasonally dynamic, interspecific variation in photosynthetic traits may contribute to a shift in forest community structure and function represents a parameterization challenge for coupled vegetation and climate models and for the interpretation of remotely sensed indices of evergreen phenology (Rogers 2014, Gamon et al. 2016). Our results suggest that models forecasting the effects of future climate on forest productivity might be improved by considering a seasonally dynamic parameterization of conifer physiology. For example, our results indicate that species-specific parameterization of V_{Cmax} by season into coupled vegetation and climate models may reveal seasonally dynamic interactions between CO_2 uptake, carbohydrate synthesis and plant growth and thus provide an additional insight linking plant functional traits with long-term shifts in forest community structure (Sharkey 1985, Wullschleger 1993).

Berndt and Gibbons (1958) noted that on a central Rockies hillslope characterized by thin and rocky soils, *P. ponderosa* possessed a tap root-like root system. At the same site, they also observed that *P. menziesii* developed a shallow, more fibrous root system. In the present study, differences in functional rooting depth among species may have contributed to the seasonal variation in physiological traits we observed. For example, access to deeper, more stable pools of soil moisture could explain *P. ponderosa*'s high rates of photosynthesis during warm, dry autumn conditions (Figure 3A) and consistently low relative stomatal limitation throughout the year (Figure 4A). Likewise, seasonally dynamic responses of relative stomatal limitation in *P. menziesii* might be explained by a well-developed, shallow root system responding to fluctuations in soil moisture (Figure 4A). How soil characteristics and rooting depth interact with intra- and interannual precipitation variability to influence the phenological variability of conifer photosynthetic traits remains uncertain.

Investigations of vegetation elevational range shifts in response to climate change have found upward movement of montane species over decadal time (Kelly and Goulden 2008) and the Madrean sky islands are an important model system in which to explore the effects of rapid climate change on montane ecosystems (Brusca et al. 2013). We built on these ideas and leveraged the region's distinctive hydroclimatology to quantify phenological variation in photosynthetic functional traits among co-occurring tree species in a mixed conifer forest. The results of this novel research cast new light on the well-studied physiological mechanisms that form the foundation for predicting climate- and disturbance-mediated shifts in forest community composition and structure as well as a potential mechanism to explain species coexistence (Nakashizuka 2001, McGill et al. 2006, Coops and Waring 2011). Linking plant functional diversity with ecosystem function has long been an objective of ecology (Chapin et al. 1997, Diaz and Cabido 2001, Hooper et al. 2005). Future research should extend this objective to link plant functional diversity, ecosystem function and interannual climatic variability in marginalized mixed conifer forests, particularly those at species' elevational and range limits.

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Conflict of interest

None declared.

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