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RESEARCH ARTICLE

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Ploidy level predicts differences in minimum leaf conductance in quaking aspen, *Populus tremuloides*

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Abstract

Premise: Leaves lose water in parallel between stomata and the cuticle. Plants regulate stomata to reduce leaf water loss in response to atmospheric demand. However, water may still be lost through leaky stomata and the cuticle, as measured by minimum leaf conductance (g_{min}). We compared g_{min} between co-occurring diploid and triploid individuals of *Populus tremuloides* to determine whether ploidy, leaf traits, or microclimate explain variation in g_{min}. We hypothesized that g_{min} may increase with increasing ploidy because higher ploidy influences traits related to plant water loss, resulting in greater triploid water loss.

Methods: We measured g_{min} in five leaves each of 32 aspen genets in Colorado. We also measured traits associated with plant water-use and structure. We then assessed predictors of differences in g_{min}, including ploidy level.

Results: Minimum leaf conductance differed by ploidy level, with higher g_{min} in triploids than in diploids. Although leaf area and surface-area-to-volume ratio were significant predictors of g_{min}, neither variable accounted for the difference by ploidy level. Slope and aspect explained additional variation in g_{min}, suggesting important microsite effects. However, ploidy level remained a significant predictor of g_{min} across all models, indicating the effect on g_{min} is robust and not due to covariation with structural traits and topography.

Conclusions: Triploids consistently had higher g_{min} than diploids across models; accounting for structural traits and topography. Higher g_{min} in triploids is consistent with hypothesized physical or genetic effects of a larger genome on leaf traits, resulting in differential drought vulnerability across ploidy levels.

KEYWORDS

drought tolerance, gmin, leaf traits, minimum leaf conductance, polyploidy, *Populus tremuloides*, ploidy variation, quaking aspen, Salicaceae, tree ecophysiology

Climate change increases the frequency and intensity of drought and heat waves, with significant impacts on tree physiology and mortality (Breshears et al., 2005; Adams et al., 2010; Allen et al., 2010, 2015; Brodribb et al., 2020; Brodribb, 2020). A key axis of plant response is regulation of water loss through leaf stomatal behavior (Choat et al., 2012; Choat, 2013; Brodribb et al., 2020; Brodribb, 2020). In response to drought, stomata close to minimize water loss and plant water stress (Jarvis, 1976; Farquhar, 1978). However, plants also lose water through the cuticle (g_{cuti}) (Boyer et al., 1997; Riederer and Schreiber, 2001) and through stomatal leaks when stomata are incompletely closed (Schuster et al., 2017). The sum of these two processes is termed minimum leaf conductance (g_{min}) (Kerstiens, 1996a, 1996b). This additional flux after stomatal closure becomes important during extreme heat and drought when uncontrolled water loss can accelerate hydraulic failure (Sinclair and Ludlow, 1986; Barnard and Bauerle, 2013; Sperry et al., 2016; Duursma et al., 2019; Cochard, 2021; Wang et al., 2024).

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Studies have identified some sources of variation in g_{min}. For example, g_{min} varies substantially among species (Brodribb et al., 2014; Schuster et al., 2017; Duursma et al., 2019; Roddy et al., 2023; Wang et al., 2024). In other findings, g_{min} sometimes increases with increasing temperature (Duursma et al., 2019; Wang et al., 2024; Garen and Michaletz, 2025). Additionally, plants acclimated or adapted to drought stress tend to have lower g_{min} to reduce water loss (Bengtson et al., 1978; Kerstiens, 1996a; James et al., 2008). However, high g_{min} may be advantageous during drought conditions because water loss through the cuticle can help plants cool during high temperatures (Slot et al., 2021; Blonder et al., 2023; Garen and Michaletz, 2025). Within species, g_{min} can vary by age (Kane et al., 2020). Genetic factors may also influence g_{min} within species (Kerstiens, 1996a, b; James et al., 2008). For example, low g_{min} has long been a key trait in breeding drought-tolerant crops, with comparisons of g_{min} across genotypes revealing high intraspecific variation (James et al., 2008) and plasticity (Duursma et al., 2019). Although interest in understanding the sources of variation has increased (Duursma et al., 2019; Burlett et al., 2025; Garen and Michaletz, 2025; Liao et al., 2025), the mechanisms underlying variation in g_{min} remain unclear.

In this study, we aimed to understand whether g_{min} differs by ploidy level in quaking aspen (*Populus tremuloides* Michx.; Salicaceae). Aspen is the most widespread tree species in North America, spanning from northern Canada to southern Mexico (DeByle and Winokur, 1985; Mitton and Grant, 1996). Aspen is also a foundation species because it plays a disproportionate role in promoting biodiversity, sequestering carbon, and providing other ecosystem services (Rogers et al., 2020). Aspen forests are experiencing widespread mortality in many areas of their southwestern range due to insects, fungal pathogens, increasing temperature, and drought (Anderegg et al., 2013; Kulakowski et al., 2013; Park Williams et al., 2013; Dudley et al., 2015; Worrall et al., 2013, 2015). There appears to be considerable geographic variation in aspen responses to climate change, which may be driven by phenotypic variation in traits, including g_{min} (Anderegg et al., 2012, 2013; Tai et al., 2017).

Aspen occurs in two ploidy levels: diploid and triploid (Jelinski and Cheliak, 1992). Triploids are common in the southwestern range (Mock et al., 2012; Dixon and DeWald, 2015), while in the northeastern range, diploids almost exclusively occur (Goessen et al., 2022). Diploids and triploids commonly co-occur geographically at scales of <100 m in the Intermountain West of the United States (Mock et al., 2008; Bishop et al., 2019). Triploids are more commonly found on south-facing slopes at lower elevations in warmer, drier microclimates, with earlier snowmelt dates than on north-facing slopes (Blonder et al., 2020, 2021). Diploids are typically located at higher elevations in cooler, wetter environments (Mock et al., 2012).

Several traits in triploid aspen differ from those in diploid aspen (Greer et al., 2018), consistent with hypothesized nucleotypic effects resulting from whole-genome duplication (Bennett et al., 1971). Triploids grow taller and faster, have larger leaves, and higher photosynthetic rates (Greer et al., 2018). Triploids have higher intrinsic water-use efficiency than diploids but have higher stomatal conductance and are less sensitive to increasing vapor pressure deficit. Triploids also have larger stomatal sizes, a trait that may make them less resilient to drought stress than diploids (Greer et al., 2018). These results may help explain the higher mortality rates in triploids in some populations (Blonder et al., 2020, 2021).

This system provides a model for understanding intraspecific g_{min} variation across ploidy levels because it allows us to separate the effects of ploidy level from local topographic variation. Assessing the impact of intraspecific ploidy level on g_{min} will provide insight into this non-stomatal water-loss pathway, especially since g_{min} can be a determinant of drought tolerance, with implications for aspen forest mortality and land management.

Here, we conducted an observational study while statistically controlling for microclimate effects to understand how g_{min} would differ between ploidy levels in similar environments. We also aimed to understand whether any leaf traits are associated with g_{min}. First, we tested whether g_{min} is associated with ploidy and leaf structural traits. Then, we tested whether topographic variables explain additional variation in g_{min} and whether ploidy effects persist after accounting for these microclimatic proxies.

We hypothesized that triploid leaves have higher g_{min} than diploid leaves because polyploidy can increase cell size and alter leaf structural organization via whole-genome duplication. Because g_{min} reflects residual diffusion of water vapor across the epidermis and cuticle after (potentially incomplete) stomatal closure, traits that describe structural allocation per unit area can influence g_{min}. Because leaf mass per unit area (LMA) equals the product of tissue density and thickness ($LMA = \rho T$), thicker triploid leaves at similar LMA imply reduced bulk tissue density. Reduced structural density is expected to lower diffusion resistance across the epidermis and increase residual water loss. We therefore predict that g_{min} will be higher in triploids and will covary negatively with tissue density and positively with leaf thickness, independent of external surface-area-to-volume geometry.

MATERIALS AND METHODS

Study sites and genet selection

For our study, we used a subset of the plots established in the summer of 2018 and studied by Blonder et al. (2021), located across ~400 km^2 of the upper Gunnison River watershed, southwestern Colorado Rocky Mountains, United States (Figure 1A). Blonder et al. (2021) selected the plots to span a range of environments and watersheds, including the Middle East River, Oh-Be-Joyful Creek, Slate River, Washington Gulch, and Coal Creek. At each plot, a living and standing aspen ramet with a minimum diameter of 5 cm at breast height was permanently tagged and assigned an identification number. The ploidy level of a focal ramet was determined by genotyping-by-sequencing in a previous study (Gompert and Mock, 2017).

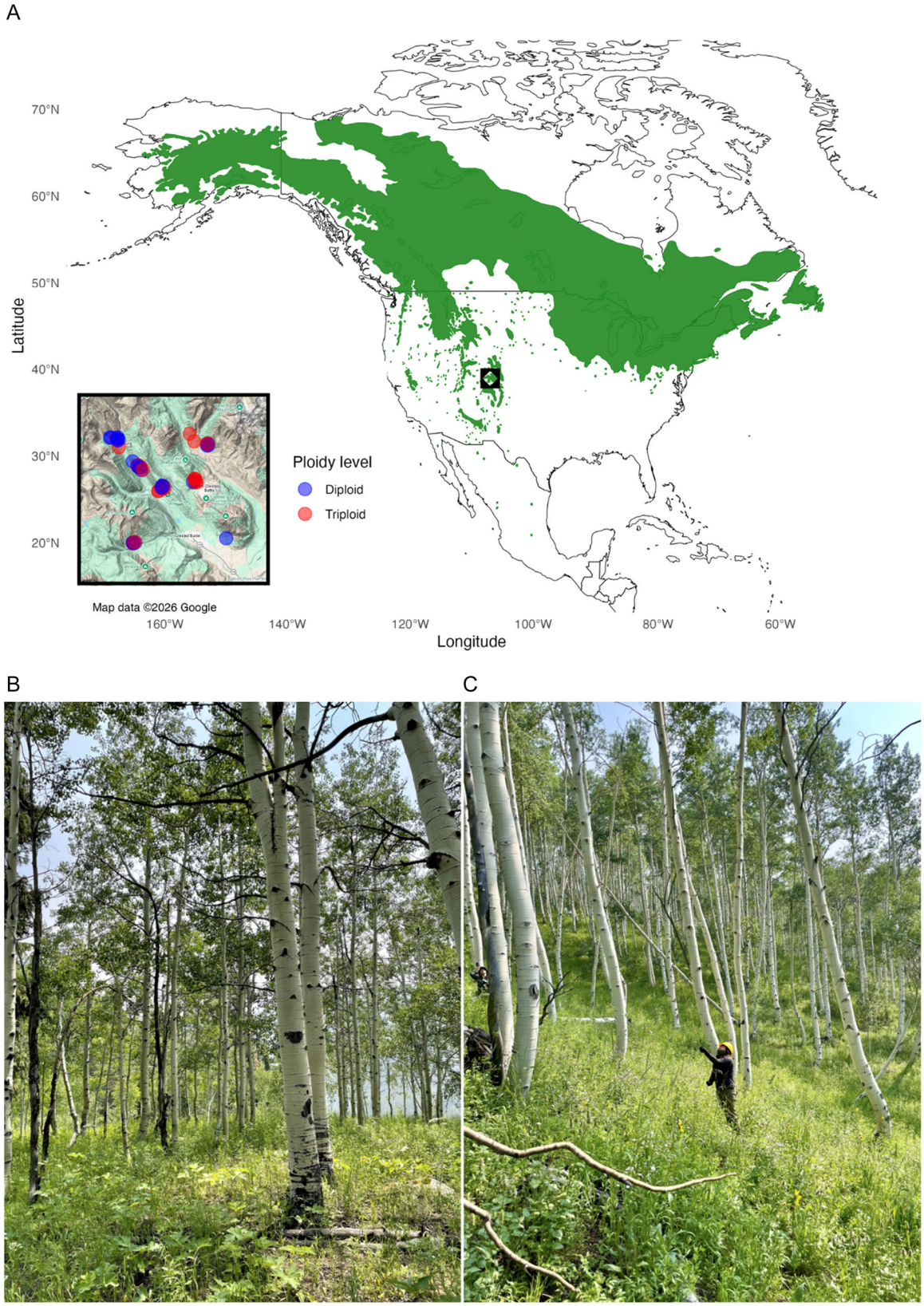

FIGURE 1 (A) Map of the geographic distribution of aspen across its range (green shading). The study area is marked by a black rectangle; inset (bottom left) shows location of focal genets of this study in the Upper Gunnison River watershed in southwestern Colorado. Examples of (B) diploid and (C) triploid aspen genets included in the study.

In July 2021, we selected 32 of the 503 plots for our study (Figure 1A). Plots of similar elevation (16 diploid and 16 triploid) were chosen to minimize environmental variation because our study was observational rather than a common-garden study. Sites were also selected based on accessibility to ensure the safety of the field crew (e.g., closer proximity to the road or trail, to avoid excessive bushwacking) and shorter driving distance from the laboratory (less water loss during transport).

Topographic data were obtained from LiDAR data collected in June 2018, covering the area at 1-m spatial resolution (Chadwick et al., 2020). These data were used to calculate elevation, slope, and cosine aspect (−1 = south-facing; +1 = north-facing) (Goulden et al., 2020). In the genets selected for this experiment, slopes differed significantly by ploidy level (Appendix S1: Table S1, Figure S1). Diploids occurred on slopes of 28° ± 8.67 (mean ± SD), whereas triploids occurred on slopes of 16° ± 6.39 (Appendix S1: Table S2). The cosine aspect (diploids: −0.64 ± 0.38; triploids: −0.70 ± 0.34) and mean elevation (diploids: 2936 ± 67 m; triploids: 2961 ± 20 m) of the genets of each ploidy level were not significantly different (Appendix S1: Table S1, Figure S1). Elevations ranged from 2918 to 2996 m a.s.l. for triploids and from 2853 to 3129 m for diploids.

Branch collection

An aspen ramet in the center of each plot was previously marked by Blonder et al. (2021). In July 2021, one sunlit upper-canopy branch was collected pre-dawn from this ramet using a pole pruner or, when necessary, a slingshot and rope (Figure 1B, C). We ensured each collected branch was at least 1 m long to avoid open vessels (Sperry et al., 1991). Upon collection, each branch was immediately sprayed with distilled water and placed inside a black 100-L plastic bag with moist paper towels to retain moisture. Branches were transported to the laboratory within an hour of collection. Once in the laboratory, each branch was placed in a bucket filled with distilled water, and the proximal end was recut in short sections multiple times under water to prevent loss of hydraulic function. Black plastic bags were placed over the branches to avoid desiccation and exposure to sunlight. The branches were left in water overnight to rehydrate.

Minimum leaf conductance

The morning after branch collection, we assayed g_{min} using the bench-drying protocol (Sack and Scoffoni, 2010) as commonly used in other studies (e.g., Pratt et al., 2005; Scoffoni et al., 2011) and described below.

Leaf selection, preparation, and measurement of area and mass

Five healthy, fully expanded, mature leaves with no visually apparent insect or microbial damage were selected from each collected branch. Leaves were cut from the distal end of the branch where the petiole meets the branch node using a razor blade, lightly cleaned with distilled water, and gently patted dry. We first weighed the fresh leaf to obtain the fresh mass (g). Then we measured the area of the detached leaf and petiole (cm^2) by analyzing a photograph of the leaf using Leafscan (Version 1.3.21, Anderson and Rosas-Anderson, 2017) on a mobile phone (iPhone 12, Apple, Cupertino, CA, USA). We then immediately sealed the petioles with Parafilm (Bemis Co., Neenah, WI, USA) to prevent water loss through the petioles.

Rate of leaf water loss and minimum conductance

We hung leaves on a clothesline, using clothespins attached to the excess Parafilm on the petioles to avoid leaf damage. Two EL-USB-2+ data loggers (Lascar Electronics, Essex, UK) were used to continuously record air temperature and relative humidity in the laboratory throughout the dry-down measurements. These values were then averaged over the dry-down and, along with the local atmospheric pressure (Weather Underground station ID KCOCREST46, Crested Butte, CO, USA), were used to calculate the mole fraction vapor pressure deficit. Fans were placed under the leaves to reduce the boundary layer and dry the leaves until stomatal closure. After 2 h, each leaf was removed from the clothesline every 10–15 min, weighed using a balance (USS-DBS3-2, U.S. Solid Analytical Balance, Cleveland, OH, USA), and then re-pinned until the next measurement to determine the rate of water loss. This process was repeated for at least 2 h.

Leaves were repeatedly weighed as they dried. Hygen (1951) showed that after a leaf is detached and stomata close, a linear decline in mass occurs due to water loss. We estimated g_{min} as the slope of a linear regression on the linear component of the dry-down data (Sinclair and Ludlow, 1986). Because stomata may still be closing during the measurement period, we identified the time range after all stomata closed, during which the rate of water loss was stable (Scoffoni et al., 2011; Schuster et al., 2017; Slot et al., 2021). We first identified leaves with a decline in water-loss rate exceeding 4×10^{-7} g m^{-2} s^{-2}, indicating that stomata had not fully closed when measurements began. We then removed the initial outliers, defined as water loss that exceeded twice the standard deviation of the final four water loss measurements, from the data for these leaves. We calculated g_{min} (mmol m^{-2} s^{-1}) as the rate of water loss per unit leaf area per unit time, divided by the mole fraction of the vapor pressure deficit, within the stable interval.

Leaf traits

Leaf traits were calculated from direct measurements obtained during the g_{min} assay. The traits and the calculations are in Table 1. Leaf area (LA, m^2) was measured

TABLE 1 Leaf traits, abbreviation, units, definitions, and calculations.

Trait	Abbreviation	Units	Definition	Calculation
Minimum leaf conductance	g_{min}	mmol m^{-2} s^{-1}	Minimum water loss of leaf per unit area	Water loss/LA/time
Leaf area	LA	m^2	Area of fresh, expanded leaf	Direct measurement
Leaf density	LD	g m^{-3}	Leaf material per volume	LMA/LT
Leaf mass per area	LMA	g m^{-2}	Leaf dry mass per area	Dry mass/LA
Leaf thickness	LT	μm	Leaf volume per area	Fresh mass/Density of water/LA
Leaf water content	LWC	g g^{-1}	Fraction of leaf mass that is water	(Fresh mass – Dry mass)/Dry mass
Surface-area-to-volume ratio	SA/V	m^{-1}	Total area of leaf per volume	LA × 2 (for each side of the leaf)/Volume

directly and converted to square meter to match the units of g_{min}. Leaf thickness (LT, μm) was estimated from fresh mass per unit area, assuming water density. Although direct micrometer measurements would provide greater precision, this approach provides a standardized proxy for comparative analyses (Vile et al., 2005). Leaves were oven-dried at 65°C for 72 h to constant mass before weighing to obtain dry mass (g) (Pérez-Harguindeguy et al., 2013). Leaf water content (LWC, g g^{-1}) was calculated as water mass (fresh mass minus dry mass) divided by dry mass (Pacey et al., 2020). Leaf mass per area (LMA, g m^{-2}) was derived as dry mass divided by LA, and leaf density (LD, g m^{-3}) was calculated as LMA divided by LT (Witkowski and Lamont, 1991). The leaf surface-area-to-volume ratio (SA/V, m^{-1}) was also calculated. To obtain the surface area, we multiplied the LA by 2 (accounting for the top and bottom leaf surface) for each leaf surface. To obtain volume, we divided dry mass by LD (Shipley and Vu, 2002).

Statistical analyses

All analyses were performed in R version 4.2.3 (R Core Team, 2023). To test for overall differences in trait data (LA, LD, LMA, LT, LWC, and SA/V) between ploidy levels, we performed a multivariate permutational analysis of variance (PERMANOVA) using the function adonis2 in the R package vegan 2.4-6 (Oksanen et al., 2018), accounting for the nonindependence of leaves collected from the same genet using the strata argument. Using the function prcomp, we also performed a principal component analysis (PCA) to visualize the axes of greatest variation in the trait data after scaling and centering.

Collinearity diagnosis

Predictor variables may be collinear, leading to incorrect parameter estimates (Dormann et al., 2013). Before model fitting, we assessed predictor collinearity using variance inflation factors (VIFs) from the R package car 3.1-3 (Fox et al., 2024). Traits LD, LWC, LT, and LMA were excluded from the best model because their VIFs exceeded 5, indicating collinearity among the predictor variables (Zuur et al., 2010). The ratio SA/V is mathematically related to LT (SA/V = 2/LT; Mauseth, 2000) and provided identical model results, but we retained SA/V because it expresses leaf structure as a ratio of evaporative surface to internal water volume, making it a more intuitive descriptor of the structural basis for passive water loss. After excluding these traits, the predictor variables with VIF less than 5 were leaf area (VIF = 1.092), SA/V (VIF = 1.124) and ploidy level (VIF = 1.049).

Linear mixed models

To test for individual trait differences by ploidy level, we used linear mixed-effects models using the R package lme4 1.1-37 (Bates et al., 2024) with ploidy as a fixed effect and genet as a random effect to account for the nonindependence of leaves collected from the same genet. *F*-tests were performed using the R package lmerTest 3.1-3 (Kuznetsova et al., 2017) with a Satterthwaite correction for degrees of freedom. Traits were $\log_{10}$-transformed to reduce the influence of outliers.

To identify the most parsimonious predictors of g_{min}, we compared models with different combinations of the retained traits and topographic variables (elevation, slope, cosine aspect) using the second-order Akaike information criterion (AICc). The AICc prevents overfitting given the small sample size in our data (Anderson and Burnham, 2002). Ploidy level was included and excluded as a fixed effect to assess whether it improved model fit. In all models, genet was included as a random effect. We used the R package MuMIn 1.48.11 (Bartoń, 2025) to calculate marginal R^2 (variance explained by fixed effects only) and conditional R^2 (variance explained by the full model including random effects) for the model with the lowest AICc. Several topographic and leaf traits showed apparent relationships with g_{min} (Figure 2), but these predictors were not retained in the final model based on the AICc. For the list of candidate models, see Table 2.

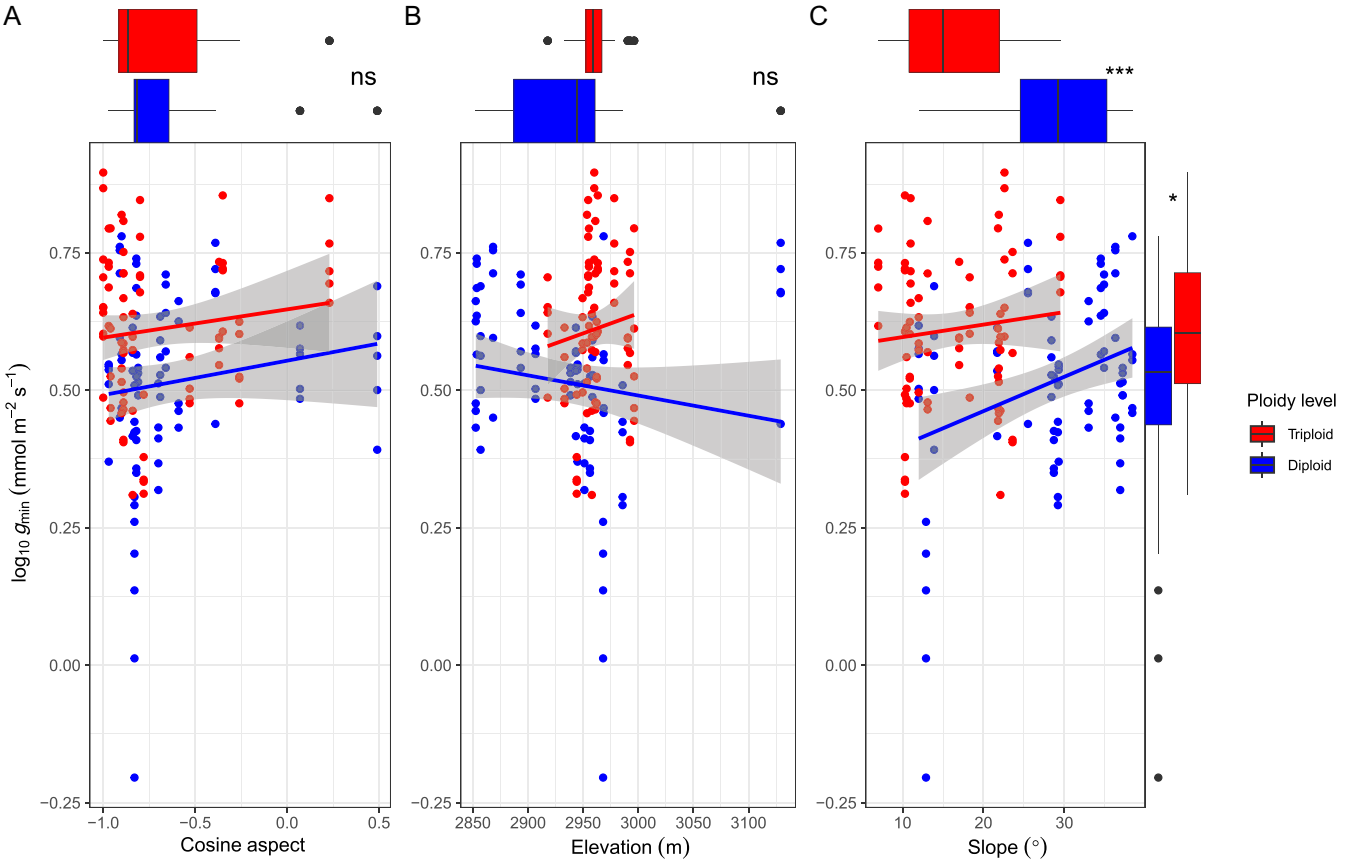

FIGURE 2 Relationship between g_{min} and ploidy level of aspen across values of all topographic predictors (A) cosine aspect, (B) elevation, (C) slope. Scatterplots display the relationship between g_{min} and the variables for triploid (red) and diploid (blue) genets. Each plot includes a regression line and a 95% confidence interval by ploidy level. Points indicate a leaf measurement. Boxplots at the margins show the distribution of each variable by ploidy level. Significant differences in topography and g_{min} by ploidy level are indicated by ***$P < 0.001$ and ns = not significant.

TABLE 2 List of models for predicting g_{min} of *P. tremuloides* leaves with ploidy level, leaf traits, topographic variables, and the combination of the above as predictor variables. Only the AICc and R^2_M/R^2_C of the best model are reported.

Model		Predictor variables	AICc	R^2_M/R^2_C
Ploidy level + Leaf traits	Full model	ploidy level + $\log_{10}$ (LA) + $\log_{10}$ (LD) + $\log_{10}$ (LT) + $\log_{10}$ (LMA) + $\log_{10}$ (LWC) + $\log_{10}$ (SA/V) + (1 | genet)		
	Best model	ploidy level + $\log_{10}$ (LA) + $\log_{10}$ (SA/V) + (1 | genet)	−195.47	13.1/63.4
Ploidy level + Topographic variables	Full model	ploidy level + slope + cosine aspect + elevation + (1 | genet)		
	Best model	ploidy level + slope + cosine aspect + (1 | genet)	−187.65	22.6/61.6
Ploidy level + Leaf traits + Topographic variables	Full model	ploidy level + $\log_{10}$ (LA) + $\log_{10}$ (LD) + $\log_{10}$ (LT) + $\log_{10}$ (LMA) + $\log_{10}$ (LWC) + $\log_{10}$ (SA/V) + slope + cosine aspect + elevation + (1 | genet)		
	Best model	ploidy level + $\log_{10}$ (SA/V) + $\log_{10}$ (LA) + slope + cosine aspect + (1 | genet)	−184.38	24.6/64.5

The R package emmeans 1.11.1 (Lenth, 2025) was used to calculate the estimated marginal means and standard error of g_{min} for each ploidy level, holding the topographic variables at their mean values. Model assumptions were verified by inspecting scaled residuals simulated from the fitted model using the R package DHARMa 0.4.7. (Hartig, 2024), including tests for uniformity, dispersion, and outliers. Predicted relationships from the most parsimonious model were visualized using the R package ggeffects 2.3.1 (Lüdecke, 2018).

RESULTS

Trait differences by ploidy level

Overall, triploid and diploid genets differed in their trait space (PERMANOVA, $F_{1,30} = 3.80$, $R^2 = 0.113$, $P = 0.001$), indicating leaf structural traits associated with water loss differed by ploidy level. The first two axes of the PCA explained 87% of the variance (Figure 4). The first principal component, which accounted for 48% of the variance, had strong positive loadings for LMA, LT, and a strong negative loading for SA/V (Appendix S2). The second principal component, accounting for 39% of the variance, had strong positive loadings for LWC and LA and a strong negative loading for LD (Appendix S2). Additionally, when each trait was considered alone, LT and LWC were greater in triploids than diploids, whereas LD and SA/V were greater in diploids than triploids (Appendix S3). LA and LMA did not differ by ploidy level (Appendix S4).

Effects of ploidy and traits on g_{min}

In the final leaf trait model, LA, SA/V, and ploidy level received high model weights (Appendix S5: Table S5). In this model, LA, SA/V, and ploidy level explained 63% of the variation (conditional $R^2 = 0.634$) in g_{min} when including the random effect (genet) and 13% of the variation (marginal $R^2 = 0.131$) (Table 2). Parameter estimates showed a negative association between LA and g_{min} (estimate = -0.19 ± 0.09 mmol $m^{-2}s^{-1}$, mean ± SE, 95% CI reported in Table 3, Figure 5B). Parameter estimates showed a negative association between SA/V and g_{min} (estimate = -0.53 ± 0.28 mmol $m^{-2}s^{-1}$, mean ± SE, 95% CI reported in Table 3, Figure 5A). In this model, we found g_{min} was 24% greater in triploids (4.00 ± 0.29 mmol $m^{-2}s^{-1}$, mean ± SE) than in diploids (3.24 ± 0.24 mmol $m^{-2}s^{-1}$, mean ± SE).

Effects of ploidy and topography on g_{min}

We then used a linear mixed model to determine whether ploidy level and topography were associated with g_{min}. Slope and cosine aspect explained 62.3% of the variation (conditional $R^2 = 0.623$) in g_{min} when including the random effect (genet) and 22.5% of the variation (marginal $R^2 = 0.225$) in g_{min} (Table 2). In this model, ploidy level, slope, and cosine aspect received high model weights, whereas elevation received little support (Appendix S6: Table S6). Model-averaged estimates indicated that g_{min} increased with slope and with greater south-facing aspect (Figure 5C, D). Triploid leaves maintained higher g_{min} across all supported models. With conditioning on the mean values of the topographic variables in the linear mixed-effects model, model predictions indicated g_{min} was 51% greater in triploids (4.67 ± 0.28 mmol $m^{-2}s^{-1}$, mean ± SE) than in diploids (3.09 ± 0.28 mmol $m^{-2}s^{-1}$, mean ± SE).

Effects of ploidy, traits and topography on g_{min}

Finally, we compared models that combined leaf traits, topographic variables, and ploidy level to determine which parameters explained the most variation in g_{min}. The top-ranked model included ploidy level, leaf area, surface-area-to-volume ratio, slope, and cosine aspect, with ΔAICc <2

TABLE 3 Coefficient estimates from the best model with corresponding standard error (SE), 95% confidence intervals (CI) and *P*-values (Pr(>|*t*|)). Values were $\log_{10}$-transformed.

Model	Predictor	Estimate	SE	95% CI	*P*
Ploidy level + Leaf traits	Intercept	2.841	1.167	0.548–5.097	0.016
	Ploidy level	0.092	0.045	0.004–0.179	0.051
	$\log_{10}$ (SA/V)	−0.528	0.284	−1.078–0.030	0.065
	$\log_{10}$ (LA)	−0.191	0.086	−0.357–0.022	**0.028**
Ploidy level + Topographic variables	Intercept	0.362	0.079	0.212–0.512	<0.001
	Ploidy level	0.214	0.056	0.108–0.319	**0.0006**
	Cosine aspect	0.159	0.065	0.036–0.283	**0.021**
	Slope	0.009	0.003	0.003–0.015	**0.008**
Ploidy level + Leaf traits + Topographic variables	Intercept	2.483	1.177	0.138–4.701	0.037
	Ploidy level	0.209	0.057	0.102–0.315	**0.001**
	$\log_{10}$ (SA/V)	−0.477	0.283	−1.011–0.086	0.094
	$\log_{10}$ (LA)	−0.161	0.085	−0.322–0.009	0.060
	Cosine aspect	0.156	0.067	0.026–0.276	**0.028**
	Slope	0.008	0.003	0.002–0.014	**0.022**

relative to all simpler alternatives (Appendix S6: Table S7). Together, this combination of predictors had the highest cumulative model weight (AICc weight = 0.849) and produced consistent effect directions across the model set. In models including only ploidy level and structural traits, predicted g_{min} was 24% higher in triploids. After we accounted for topography and ploidy level, predicted g_{min} was approximately 51% higher in triploids.

Together, these variables explained 25% (marginal $R^2 = 0.246$) of the variation in g_{min} and 65% (conditional $R^2 = 0.645$) of the variation when including the random effect (genet) (Table 2). The difference between marginal and conditional R^2 indicates that substantial variation in g_{min} occurs among genets and remains unexplained by the measured fixed effects. Model-averaged predictions indicated that triploid (4.51 ± 0.37 mmol $m^{-2}s^{-1}$, mean ± SE, 95% CI in Table 3) leaves maintained approximately 56.4% higher g_{min} than diploid (2.88 ± 0.24 mmol $m^{-2}s^{-1}$, mean ± SE, 95% CI in Table 3) leaves after accounting for trait variation and topography.

DISCUSSION

Our study identified factors associated with variability in minimum leaf conductance (g_{min}) in *P. tremuloides* leaves. Triploid leaves consistently had higher g_{min} across the supported model set, and ploidy level received high model weight in all candidate models. This pattern persisted after accounting for leaf structural traits and after controlling for slope and aspect, indicating that genome duplication is a strong and independent predictor of unavoidable water loss. We also found that g_{min} covaried with leaf area (LA) and surface-area-to-volume ratio (SA/V), consistent with the idea that geometric and anatomical shifts associated with polyploidy influence diffusion pathways and leaf water flux. Topography further modified g_{min}, underscoring the interaction between microclimate and genomic variation in shaping drought-response strategies. These findings support our proposed hypothesis that g_{min} differs by ploidy level and is associated with leaf structural traits.

Implications of potential cell-size differences

Our results demonstrate differences in g_{min} across ploidy levels, with triploid leaves losing more water per unit area than diploid leaves. Polyploids may have adjustments in gene expression that may accommodate increased cell size and metabolic demands, which can affect phenotypic expression and physiology (Stebbins, 1971; Doyle and Coate, 2019). Ploidy level, cell size differences, and related gene expression may thus drive variation in leaf morphological traits. In this species, we found that LMA and LA do not differ by ploidy level, whereas LD, LT, LWC, and SA/V do (Appendix S4). However, other studies have shown that aspen triploids have statistically significantly larger leaves, and so the lack of a detected difference across ploidy levels may be due to our small sample size (Willis et al., 2016; Greer et al., 2018; Barceló-Anguiano et al., 2021).

The SA/V was associated with variation in g_{min}, but it did not explain the higher g_{min} of triploid leaves. Diploid leaves had greater SA/V than triploid leaves, whereas triploid leaves had greater g_{min} (Appendix S3). This mismatch indicates that external leaf geometry alone cannot account for the difference in g_{min} across ploidy levels. Instead, the higher g_{min} of triploids may reflect tissue-level or epidermal properties not captured by bulk SA/V, such as cuticle composition, stomatal leakage, epidermal anatomy, or mesophyll organization.

Although we did not detect a significant effect of ploidy level on LA (Appendix S4), triploid leaves were 12% larger than diploid leaves (Appendix S6). Greer et al. (2018) found that triploids have a greater LA than diploids. LA was negatively associated with g_{min} in both diploid and triploid leaves (Figure 3A), and the relationship was consistent in model estimates (Figure 5B). Leaf area may be associated with variation in g_{min} because, per unit area, smaller leaves lose water faster than larger leaves (Wang et al., 2019). In leaves, the boundary layer provides resistance to water vapor diffusion (Schönherr, 1982; Nobel, 1999), and the thickness of the leaf boundary layer is proportional to the surface area of the leaf (Leigh et al., 2017) relative to the direction of wind (Daudet et al., 1999). Although a larger surface area means more water can be lost, larger leaves may have greater boundary-layer resistance than smaller leaves (Nobel, 1999) because smaller leaves have a thinner boundary layer. In contrast, larger leaves have a thicker boundary layer. However, studies suggest the boundary layer plays a lesser role in water loss in *P. tremuloides* leaves due to fluttering (Roden and Pearcy, 1993), suggesting that LA does not drive resistance to water loss and may not explain the higher g_{min} in triploids compared to diploids.

Although LT was not associated with variation in g_{min}, triploids have greater LT (Appendix S3). Polyploids often have thicker leaves than diploids (Xue et al., 2017) due to anatomical differences, and ploidy-induced leaf anatomical changes may modify diffusion pathways for water vapor (Wilson et al., 2021; Šmarda et al., 2023). Leaves lose water when water flows from the xylem to spongy mesophyll cells through the bundle sheath made by parenchyma cells, where water then evaporates from the surface of the mesophyll (Sack and Holbrook, 2006). Cell types such as epidermal cells and mesophyll parenchyma (Chen et al., 2021) are larger in polyploids, leading to thicker leaves. Polyploids have larger cells (Pacey et al., 2022). Larger cells have a greater water storage capacity and may result in a higher LWC, as seen in the triploid leaves we measured (Appendix S3). Additionally, larger cells may lead to more efficient water transport because they can change shape and be packed more tightly, leaving less airspace in leaves for faster diffusion of water vapor from mesophyll cells (Wilson et al., 2021). Previous studies have found a positive relationship between LT and hydraulic conductance, where water transport is more efficient when there are larger air spaces in the mesophyll cells

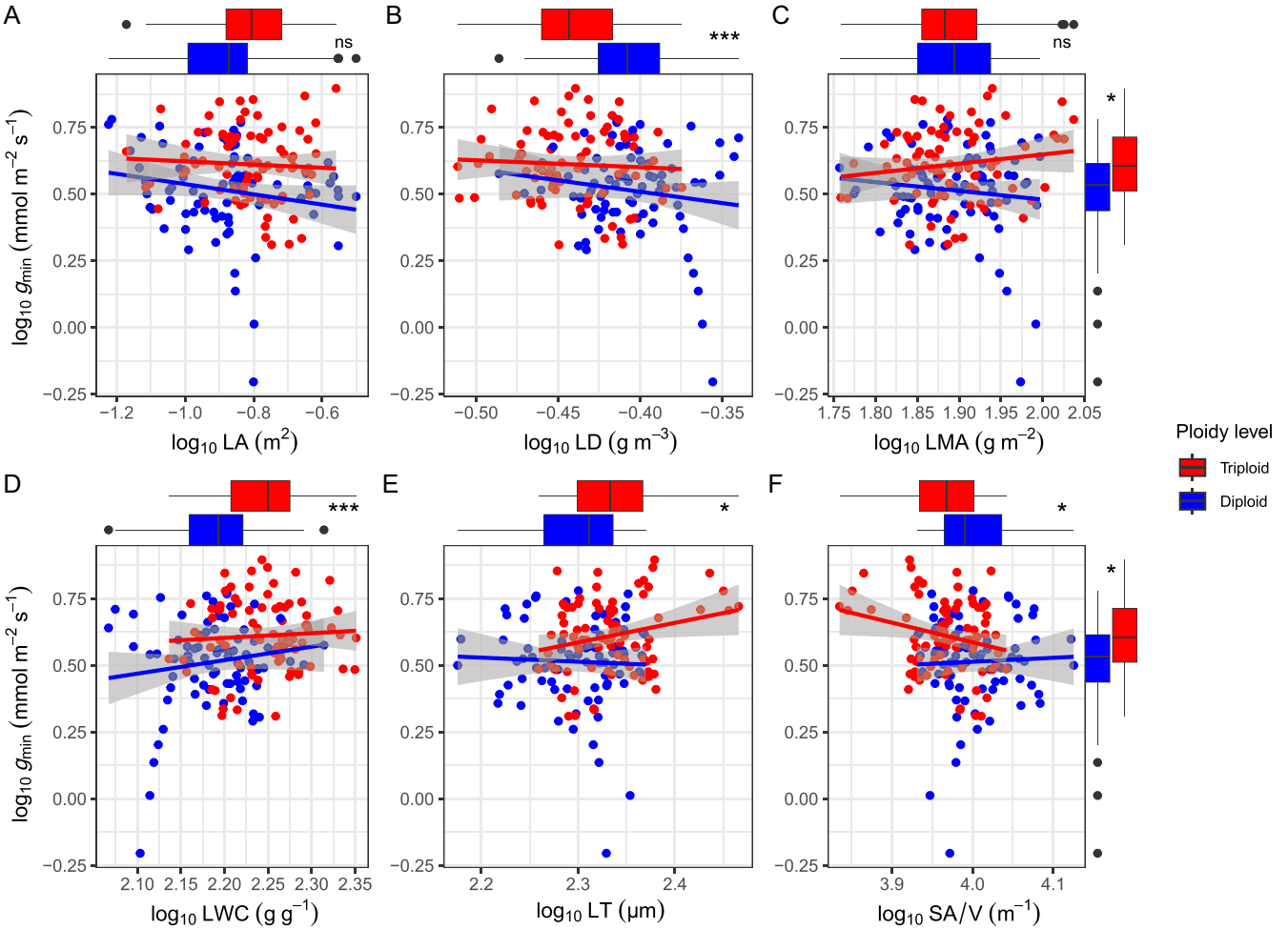

FIGURE 3 Relationship between g_{min} and all trait predictors (A) leaf area (m^2), (B) leaf density (g m^{-3}), (C) leaf mass per area (g m^{-2}), (D) leaf water content (g g^{-1}), (E) leaf thickness (μm), and (F) surface-area-to-volume ratio (m^{-1}) for each ploidy level of aspen. Scatterplots display the relationship between g_{min} and the variables for triploid (red) and diploid (blue) genets. Each plot includes a regression line and a 95% confidence interval by ploidy level. Boxplots are added to the margins to show the marginal distribution of each variable by ploidy level. The boxplots on the *y*-axis correspond to the marginal distribution of g_{min}. Boxplots and points in blue correspond to diploid leaves. Boxplots and points in red correspond to triploid leaves. Significant differences in trait by ploidy level are indicated by $^*P < 0.05$, $^{***}P < 0.001$, and ns = not significant.

close to bundle sheath cells, leading to faster diffusion of water vapor from mesophyll cells through leaky stomata or a permeable cuticle (Oguchi et al., 2018).

Although polyploidy can alter cell size and packing in other systems (Comai, 2005; Beaulieu et al., 2008), we did not directly measure leaf anatomy. Therefore, mechanistic interpretations linking ploidy to cellular organization remain hypotheses requiring anatomical validation. On the other hand, larger cells do not necessarily mean larger surface area because polyploids may have fewer cells per leaf (Robinson et al., 2018). In this case, water transport may not be more efficient, but since other studies have reported that cell number does not differ by ploidy level in *Populus* polyploids (Zhang et al., 2019), greater LT in triploids may make water loss more efficient.

Other studies have found that lower g_{min} is correlated with higher LMA (Reich, 2014; Slot et al., 2021) because LMA is a proxy for leaf construction and cost (Wright et al., 2004). Although triploid aspen leaves have been reported to have a lower LMA (Greer et al., 2018), in our study, we found no association between LMA and g_{min} (Figure 3C) or a significant difference in LMA by ploidy level (Appendix S4). Our findings imply that while g_{min} varies by ploidy level, overall leaf construction costs do not. Leaf mass per area consists of two other components: LD and LT. Similar LMA values can be obtained when LD and LT values are inversely related. In our study, triploid leaves had lower LD and higher LT than diploid leaves (Appendix S3). These findings are consistent with cell-size differences: Whole-genome duplication increases cell volume, resulting in less densely packed cells and reduced LD. However, larger cells increase overall leaf thickness, as we see in triploids (Appendix S4). Per unit area, the cost to build leaves is the same, but the construction can differ by ploidy level. These findings suggest the relationship between LMA and g_{min} may also depend on the other components of LMA.

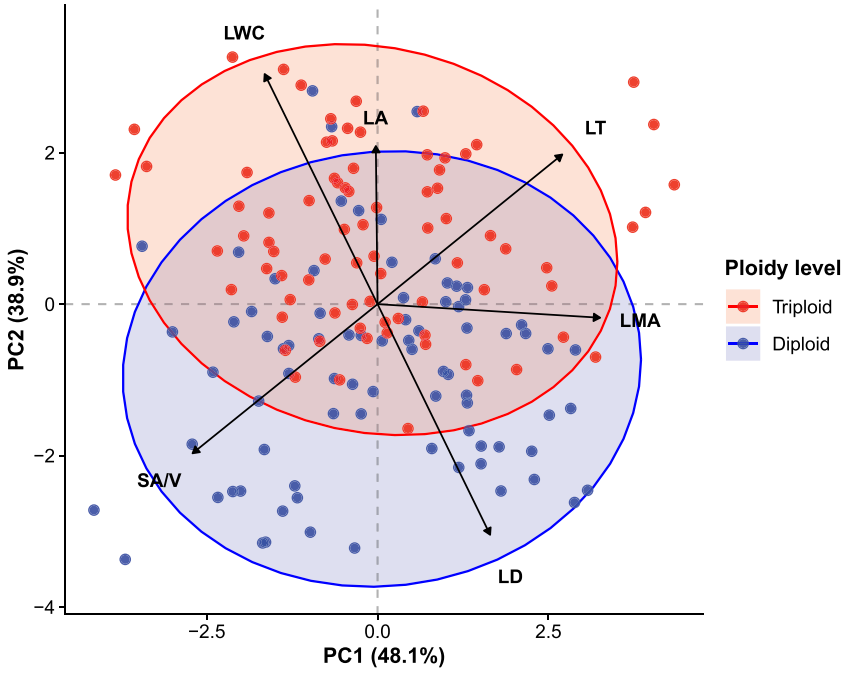

FIGURE 4 PCA of variation in leaf traits in aspen. Diploids and triploids differ in functional trait space occupancy. Principal component analysis (PCA) of variation in leaf traits in *P. tremuloides*. The PCA is based on 160 leaves from 32 genets ($N = 5$ leaves per genet). Traits corresponding to diploid leaves are blue circles, and traits corresponding to triploid leaves are red circles. Vectors indicate the trait loadings on each axis. Each vector is labeled with the abbreviated trait it represents, and its length indicates the correlation with each axis. LA: leaf area (m^2), LD: leaf density (g m^{-3}), LMA: leaf mass per area (g m^{-2}), LT: leaf thickness (μm), LWC: leaf water content (g g^{-1}), and SA/V: surface-area-to-volume ratio (m^{-1}).

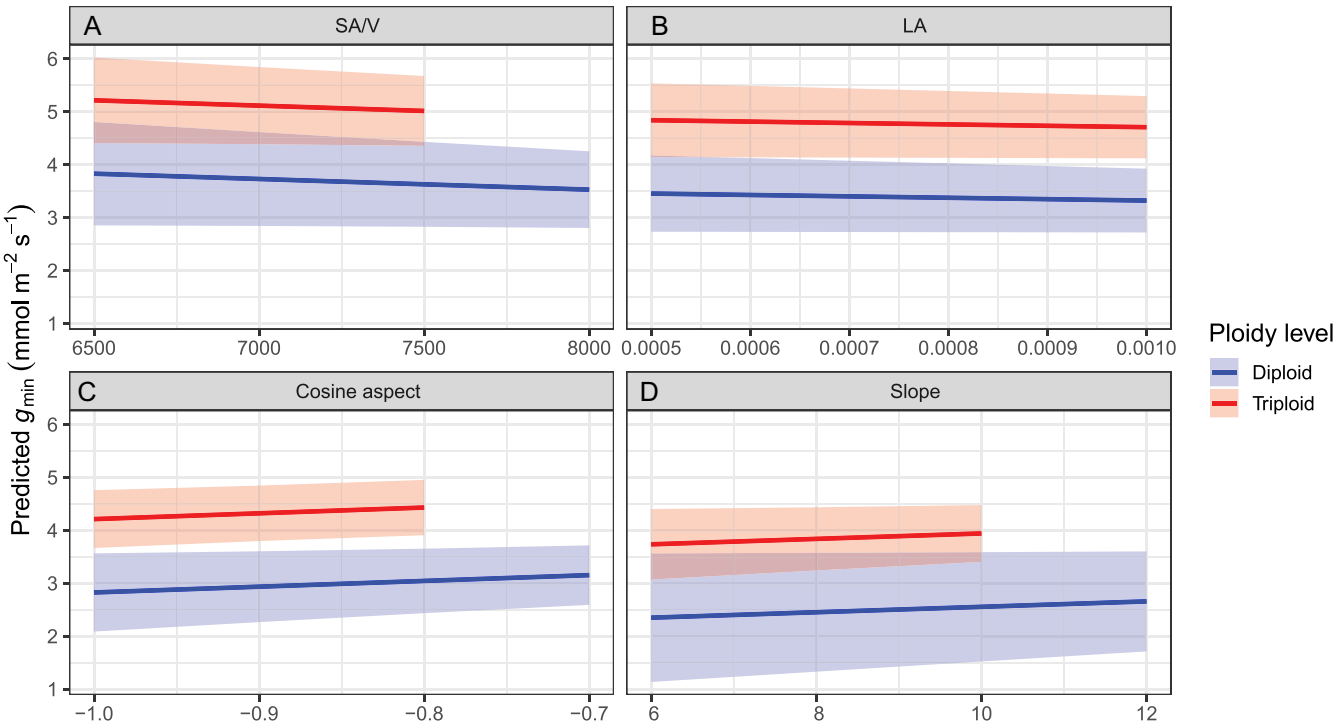

FIGURE 5 Model-predicted g_{min} between (A) surface-area-to-volume ratio (SA/V; m^{-1}) by ploidy level, (B) leaf area (LA; m^2) by ploidy level, (C) cosine aspect by ploidy level, and (D) slope by ploidy level of aspen. Each plot includes a regression line and a 95% confidence interval by ploidy level.

Effects of topography

Differences in environments occupied by each ploidy level can complicate interpretations of trait differences among naturally occurring populations (Trugman et al., 2021) because different environments may select for individuals with different phenotypes. In this plot network, diploids are more common in higher elevations and steeper slopes. Although ploidy level did not differ significantly in cosine aspect or elevation, diploid aspen genets were on slopes 73% steeper than the slopes where triploid aspen genets were found (Appendix S1: Table S2). The angle of a slope, along with its aspect, influences water availability (Badano et al., 2005), especially in headwater valleys where snowmelt is a primary moisture source. The slope steepness also influences solar radiation and soil characteristics (Barbour and Billings, 2000). Although soil moisture was not measured, we infer that water availability may differ between the genets we sampled. We might have sampled diploids in environments with less access to water, causing more drought stress. Plants experiencing greater water limitations may invest in different traits not measured in our study that could minimize g_{min}. The potential mechanisms underlying this relationship are discussed further in the next section.

However, because we controlled for elevation, we may have sampled triploids above their ideal niche in cooler environments and diploids below it in warmer environments. Nonetheless, triploids maintained greater g_{min} than diploids, suggesting the difference between triploid and diploid g_{min} may actually be larger than reported. Still, studying natural populations in situ is key to improving our understanding of plant responses to climate change, especially in keystone species like *P. tremuloides*.

Untested hypotheses

Ploidy level was the strongest predictor of g_{min}, and although several structural traits differed by ploidy level, they explained only a marginal amount of variation in g_{min}. Several untested hypotheses may also contribute to differences in g_{min} across ploidy levels. Below, we discuss other possible contributions of minimum leaf conductance that are yet to be tested.

Cuticle and stomata effects

The cuticle and leaky stomata are the potential sources of water loss in g_{min}, but our study cannot decouple their contributions. However, differences in ploidy may contribute to the different amounts of water loss in triploid and diploid *P. tremuloides* leaves.

For example, the thickness of the cuticle may be negatively correlated with water loss, but experimental evidence supporting this assumption is mixed (Riederer and Schreiber, 2001). Studies that measured cuticle thickness and water loss in other species did not find a relationship (Kamp, 1930; Schönherr, 1982; Lendzian and Kerstiens, 1991). Other findings suggest epicuticular wax deposition rates, crystallization patterns and chemical composition acts as the actual barrier against desiccation (Schönherr, 1982; Schönherr and Riederer, 1989; Parsons et al., 2012; Grünhofer et al., 2024). However, other studies report that differences in amount and chemical composition of wax did not influence g_{min} (Shellakkutti et al., 2022). In another study, autotetraploid leaves retained water better than diploid leaves due to differences in leaf cuticular wax, resulting from differential gene expression by ploidy level (Li et al., 2024). In this study, diploid and autotetraploid leaves contained different wax and crystalloid contents, reducing the cuticle permeability of autotetraploids relative to diploids. However, in our study, polyploids (triploids) had a higher g_{min}, suggesting greater water loss. Without measurements of cuticle thickness and leaf wax composition in triploid and diploid aspen, it is unclear how these traits may differ and influence g_{min}.

Triploid aspen leaves may also have a higher g_{min} than diploid aspen leaves because of different stomatal traits. In aspen triploids, greater guard cell size and length may contribute to higher g_{min} values via "leaky" or incomplete stomatal closure, as seen via foliar water uptake in polyploid coast redwoods, *Sequoia sempervirens* (Noss, 2000; Burgess and Dawson, 2004). Aspen triploid leaves have greater stomatal size and density than diploid leaves (Greer et al., 2018). Even if cuticle traits did not differ by ploidy level, we hypothesized that triploid leaves have a greater g_{min} because their stomatal traits increase the chance of water loss via incomplete stomatal closure, contributing to a greater minimum leaf conductance in triploids than diploids (Kerstiens, 1996a, 1996b).

Leaf age

Leaf age correlates with variation in cuticular conductance. In *Quercus rubra* leaves, younger leaves lose more water through the cuticle than through stomata, while older leaves lose more water through stomata (Kane et al., 2020). Phenological differences by ploidy level have been reported in other studies (Comai, 2005; Van de Peer et al., 2021), including in aspen (Blonder et al., 2023), but the impact of leaf age across species and ploidy level remains unclear. To minimize any differences in leaf age, we collected triploid and diploid leaves simultaneously at the same time of year. We also ensured the measured leaves were fully expanded, mature, and healthy.

Study limitations

We identified an association between ploidy level and g_{min}, but did not identify the full causal mechanism underlying that association. The observational design does not fully account for environmental variation. Specifically, we are unable to

fully rule out differential habitat selection by diploids and triploids, which may occupy distinct microclimates that influence leaf traits. Additionally, our study is limited to one growing season. Leaf traits may be influenced by antecedent climatic conditions (Meier and Leuschner, 2008), and in some years, the effects of topographical differences may be magnified. For instance, water stress in dry years can be exacerbated by topography (Esteban et al., 2021). Therefore, multiyear measurements across wet and dry years are needed to determine whether the ploidy effect on g_{min} is similar across climatic conditions. We suggest future research using multiyear data spanning climatic extremes to better disentangle the relative influence of ploidy level, topography, and structural traits on g_{min}.

Implications for mortality

It is difficult to predict whether polyploids will do better or worse under intensifying drought conditions, although a common hypothesis is that polyploids are better adapted to stressful conditions (Stebbins, 1985; Otto and Whitton, 2000; Brochmann et al., 2004; Edgeloe et al., 2022; Ræbild et al., 2024; Sobhanian et al., 2026). Triploid trees in these plots are less resilient to drought and have higher rates of aboveground mortality than diploids (Blonder et al., 2021). Cochard (2021) proposed a general hypothesis beyond *P. tremuloides*: Minimum leaf conductance may be a critical mechanism underlying tree mortality from heat waves and drought, with high temperatures triggering a cuticle phase transition that causes excessive water loss via $g_{min,}$ leading to uncontrolled cavitation and hydraulic failure. In aspen, the variation in g_{min} observed may therefore influence its mortality risk. Ploidy level affects mortality risk in the species, with triploids having a higher mortality risk than diploids, consistent with this hypothesis (Blonder et al., 2021).

As a continuous source of water loss, g_{min} provides insights into plant vulnerability. Whereas agronomists use g_{min} as a key breeding trait for improving drought tolerance in crops (Sinclair and Ludlow, 1986), g_{min} could be an informative trait for ecologists to evaluate the effects of climate change and drought on forest ecosystems and aspen distribution. Since our results show triploid trees have a greater g_{min} than diploid trees, water loss will not only be underestimated if models do not account for g_{min}, especially in biomes where ambient temperature and vapor pressure deficit increase (Barkhordarian et al., 2019), but the error will be even more significant in forest stands dominated by triploid trees.

CONCLUSIONS

This study highlights polyploidy as an important driver of hydraulic variation in quaking aspen. Polyploidy can influence leaf structure and water flux, and these effects remain detectable even in co-occurring individuals exposed to similar environments. These findings add to the growing understanding of the predictors of g_{min} and point to the value of future mechanistic studies to explore the processes underlying this variation, in this and other species.

AUTHOR CONTRIBUTIONS

J.N. and R.M.C.H. designed the study and collected the data. Karen Mock and Jim Walton provided genetic analyses. J.N. and R.M.C.H. organized the data. J.N., R.M.C.H. and J.M.P. analyzed the data and wrote the first draft. B.W.B. and B.J.E. provided guidance and support throughout all stages of the data collection, analyses, and manuscript writing. All authors contributed to revisions of the manuscript. B.W.B. and R.M.C.H. were supported by the U.S. Department of Agriculture NIFA grant #2022-67019-36366. R.M.C.H. was supported by the National Science Foundation Postdoctoral Research Fellowship #2011030 and the Ford Foundation. B.W.B. was also supported by the Rocky Mountain Biological Laboratory via the Johnson family endowment and the Navjot Sodhi fellowship. J.N. was supported by the National Science Foundation Graduate Research Fellowship #2020300359, the Rocky Mountain Biological Laboratory via the Jean H. Langenheim Graduate Fellowship, and the Rocky Mountain Biological Laboratory Graduate Fellowship.

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DATA AVAILABILITY STATEMENT

The final data sets are available at Open Science Framework (OSF) https://osf.io/g5vs2. The code necessary to access the data, the calculations, and statistical tests are available in an open GitHub repository https://github.com/JocelynNavarro/Aspen_Gmin.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Supplemental Tables 1 and 2 and Figure S1.

Appendix S2: Supplemental Table 3.

Appendix S3: Supplemental Figure S2.

Appendix S4: Supplemental Table 4.

Appendix S5: Supplemental Table 5.

Appendix S6: Supplemental Tables S6–S8.

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