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ORIGINAL RESEARCH

Elevated CO_2 Modifies Within-Species Differences in Relative Drought Resistance With Implications for Recovery in *Populus balsamifera*

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ABSTRACT

Forecasting forest responses to climate change requires mechanistic insight into how rising atmospheric CO_2 concentration ($[CO_2]$) affects within-species drought tolerances. We tested whether elevated $[CO_2]$ alters drought resistance and recovery using cuttings of two ecotypes of *Populus balsamifera* (Peace River, PR; Hay River, HR) grown under factorial combinations of $[CO_2]$ (400 vs. 1000 μmol mol^{-1}) and water regime (well-watered vs. drought followed by re-watering). Under drought, elevated $[CO_2]$ compensated for the water deficit in net photosynthesis (P_n) only in PR, narrowing the ecotypic difference in P_n; this pattern was accompanied by a PR-specific increase in photosynthetic capacity (Vc_{max}), whereas HR showed little CO_2-driven enhancement. Stomatal conductance (g_s) declined with drought across $[CO_2]$ levels, and intrinsic water-use efficiency (WUE_i) increased with both drought and elevated $[CO_2]$. Following re-watering, drought plants showed strong recovery of P_n and photosynthetic capacity (Vc_{max} and J_{max}) with g_s and the ratio of intercellular $[CO_2]$ to ambient $[CO_2]$ becoming comparable to those in well-watered trees, indicating recovery of both diffusional and biochemical limitations. Moreover, whole-plant responses remained ecotype dependent: drought reduced total biomass in both ecotypes, with a greater reduction in PR, while HR expressed lower specific leaf area and leaf area ratio under drought. Ecotype-dependent responses to the interaction between elevated $[CO_2]$ and drought highlight the need to consider intraspecific variation in process-based models to improve projections of population responses under future climates.

1 | Introduction

Predicting forest resilience requires a mechanistic understanding of how trees, particularly the widespread *Populus* spp., respond to dual pressure from rising atmospheric CO_2 concentration ($[CO_2]$) and intensifying drought (Reichstein et al. 2013; Rogers et al. 2023). While drought imposes severe constraints through hydraulic stress and reduced carbon uptake, elevated $[CO_2]$ alters tree physiology by stimulating photosynthetic rates and increasing water use efficiency (Ainsworth and Long 2004; Inoue et al. 2020). Given the ecological importance of *Populus* spp. across temperate and boreal forests, their sensitivity to these shifting conditions is a growing concern amidst predicted water shortages (Schindler and Donahue 2006).

Populus spp. are often described as drought-sensitive because many occupy mesic, water-available environments and maintain high transpirational demand (Street et al. 2006). Yet drought sensitivity is not uniform: drought responses differ among *Populus* species (Tschaplinski et al. 1994) and within

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species, with variation expressed in growth and gas-exchange regulation (Chen et al. 1997; Marron et al. 2002; Monclus et al. 2006; Bonhomme, Monclus, Vincent, et al. 2009; Huang et al. 2009; Lu et al. 2009; Regier et al. 2009; Cocozza et al. 2010; Viger et al. 2013). Within *Populus balsamifera*, intraspecific drought sensitivity is expressed through variation in hydraulics and stomatal regulation, including drought-responsive gene expression (Hacke and Sauter 1996; Almeida-Rodriguez et al. 2010; Hamanishi et al. 2010, 2011). Consistent with these patterns, in *P. balsamifera*, imaging studies reveal intraspecific drought-driven changes in mesophyll porosity and bundle sheath extension abundance that affect internal CO_2 diffusion, water transport, and photosynthesis during stress and recovery (Momayyezi et al. 2025). Accordingly, when intraspecific variation is ignored, inference on drought responses is weakened by averaging contrasting ecotype strategies and obscuring the mechanisms regulating performance under stress (Kemppinen and Niittynen 2022).

Elevated [CO_2] generally increases photosynthetic carbon assimilation and can enhance growth and intrinsic water use efficiency in *Populus*, but acclimation responses also occur, including shifts in photosynthetic capacity parameters (Bernacchi et al. 2003; Ainsworth and Long 2004). Field- and canopy-scale studies show that photosynthetic stimulation under long-term CO_2 enrichment can be sustained across canopy profiles in poplar stands (Bernacchi et al. 2003; Liberloo et al. 2007). Controlled experiments further demonstrate substantial intraspecific variation in the magnitude of CO_2 stimulation, including wide ranges in assimilation enhancement and differences in biomass responses and stomatal sensitivity among *Populus* genotypes (Ceulemans et al. 1996; Kalina and Ceulemans 1997; Wang et al. 2000; Lindroth et al. 2001; Noormets et al. 2001).

Although elevated [CO_2] can mitigate some drought impacts (Ainsworth and Long 2004; Inoue et al. 2020), responses are highly heterogeneous across and within species (Aspinwall et al. 2021), ranging from reduced to negligible or even exacerbated drought effects depending on drought intensity and ecotype (Engel et al. 2004; Perry et al. 2013; Lauriks et al. 2022). Given that *Populus* species employ diverse, ecotype-dependent mechanisms to regulate drought responses, the magnitude of the elevated [CO_2] fertilization effect may hinge on whether an ecotype is primarily limited by internal diffusion or biochemical capacity. For example, in *P. tremuloides*, [CO_2] stimulation of photosynthesis ranged from 14% to 68% among intraspecific variants, and post-drought recovery of stomatal conductance and assimilation also differed, indicating that CO_2 responsiveness is tightly coupled to stomatal control (Wang et al. 2000). Despite evidence that internal physiological differences mediate drought effects, the interaction between elevated [CO_2] and soil water deficit remains poorly studied across *P. balsamifera* variants. Studies in other taxa report divergent outcomes: elevated [CO_2] may lessen drought impacts via improved water use efficiency in wild *Olea europaea* (Aranda et al. 2026), preserve existing tolerance rankings in *Prosopis glandulosa* (Polley et al. 1999), or reorder drought-tolerance hierarchies in *Pinus pinaster* (Sánchez-Gómez et al. 2017). Furthermore, while resistance mechanisms (maintaining function during stress) are well documented, recovery (restoring function after re-watering) processes have only recently been investigated in detail in woody plants, largely outside *Populus* (Warren et al. 2012; Cano et al. 2014; Arend et al. 2016; Hesse et al. 2023). The relationship between these traits is still poorly understood; while some plants excel in both, many tree species exhibit an inverse association, suggesting a physiological trade-off (Gazol et al. 2017; Taleb et al. 2023).

Given that *P. balsamifera*. faces increasing drought risk under climate change (Hogg et al. 2002; Schindler and Donahue 2006; Rosso et al. 2023), characterizing the range of physiological responses within the species is essential for predicting forest resilience. This study investigates the eco-physiological responses of growth and leaf gas exchange in two ecotypes originating from northwestern Canada (the Peace River Parkland in Alberta and the Hay River Lowland in the Northwest Territories) to factorial combinations of [CO_2] and water availability. Although direct comparisons of seedlings versus vegetatively propagated cuttings under elevated [CO_2] or drought are limited in *Populus*, experiments using cuttings show the expected photosynthetic responses under elevated [CO_2] (Taylor et al. 2003). Seedling-based drought studies also report recovery patterns that are qualitatively consistent with those documented in cutting-based *Populus* drought experiments (Lu et al. 2010; Rosso et al. 2023). We hypothesized that elevated [CO_2] would provide a greater relative benefit to the ecotype exhibiting lower inherent drought resistance, thereby narrowing the gaps in growth and gas exchange between the two sources during both the stress and recovery phases.

2 | Materials and Methods

2.1 | Plant Materials

Two *Populus balsamifera* ecotypes were sampled in western Canada: Peace River, Alberta (PR; 56.13° N, 117.26° W; 570.9 m above sea level) and Hay River, Northwest Territories (HR; 60.50° N, 115.46° W; 164.9 m above sea level). PR has a mean annual temperature of 1.2°C, 402 mm of annual precipitation, and 264 mm of May–September rainfall with a mean May–September daily temperature of ~13.0°C; HR has a mean of −2.2°C, 320 mm, and 190 mm, with a mean May–September daily temperature of ~11.5°C, respectively. Frost-free period is 115–125 days at PR and ~85–100 days at HR (Environment and Climate Change Canada 2020). Permafrost near Hay River falls within the southern sporadic zone, where ground ice and high water tables help buffer soil moisture (Heginbottom et al. 1993; Smith and Burgess 2004). Soils at HR are predominantly Organic Cryosols and Gray Luvisols on clayey lacustrine plains with high water tables. In contrast, PR soils are Dark Chernozemic and Luvisolic clays derived from former Lake-Peace sediments, generally well-drained (Agriculture and Agri-Food Canada 2013). Precipitation is more evenly distributed through the year at HR, whereas PR is summer-biased with higher potential evapotranspiration. These ecotypes represent western *P. balsamifera* and are drawn from source regions included in range-wide provenance collections that demonstrate strong climate-of-origin effects on growth, phenology, and ecophysiology (Soolanayakanahally et al. 2009; Keller et al. 2011). These two sources were selected to represent different hydroclimatic settings within the western portion of the species' range. Peace River has a warmer, longer frost-free season and summer-biased precipitation, but relatively high potential evapotranspiration and generally well-drained soils, which can promote episodic growing-season water deficits typical of boreal–parkland transition zones (Hogg et al. 2002; Schindler and Donahue 2006). Hay River is colder with a shorter frost-free season and lower growing-season precipitation, but high water tables and sporadic permafrost can buffer soil moisture (Heginbottom et al. 1993; Smith and Burgess 2004). Neither site is strictly arid; both occur in relatively low-precipitation boreal/parkland environments, with different mechanisms shaping drought exposure (Hogg et al. 2002; Schindler and Donahue 2006).

Cuttings were collected from 1 to 2-year-old branches of 10 spatially separated parent trees in PR and HR. Parent trees were sampled across the site with a minimum separation of 30 m to reduce the likelihood of sampling clones of the same plant and to represent ecotype variation. Parent identity was not tracked beyond collection; cuttings were pooled across the parent plant. Cuttings were chilled at −4°C for > 3 weeks to meet bud-break requirements (Man et al. 2017). Cuttings were standardized to 35 ± 2 cm with a terminal bud and 3–4 axillary buds (mid-stem diameter 4.7–7.7 mm), treated with rooting hormone (Stim-Root #3, Plant Products Co. Ltd.), and planted in styrofoam trays containing a peat: vermiculite mix (7:3, v/v). Trays were maintained under high humidity in a polyethylene tent with continuous misting until the cuttings had developed roots. Greenhouse day/night temperatures were 24/14 (±2)°C, and the photoperiod was extended to 16 h using high-pressure sodium lamps (P.L. Systems). Cuttings were allowed to root in Styrofoam trays before being transplanted into 3.5-L pots on December 11, 2023, which is also when the CO_2 treatment started. The plants remained in the greenhouse throughout the experiment. The rooted cuttings were placed in a non-soil substrate (SunGro Sunshine Mix #4; Sun Gro Horticulture), composed primarily of Canadian sphagnum peat moss and coarse perlite, with smaller amounts of coir, dolomitic limestone, a long-lasting wetting agent, and proprietary RESiLIENCE additive. A soilless substrate was used to provide uniform physical/chemical growing conditions and to minimize variability and contamination risk associated with field soils in a controlled-environment experiment. Pots were irrigated to drip every 2 days and fertilized weekly with Plant-Prod 20–20-20 ($2\,g\,L^{-1}$) with chelated micronutrients (Plant Products Co. Ltd.).

2.2 | Experimental Design

We used a split-split plot design for the greenhouse experiment at Lakehead University (Thunder Bay, Canada). Factors were growth [CO_2] (ambient, $400\,\mu mol\,mol^{-1}$; elevated, $1000\,\mu mol\,mol^{-1}$), watering treatment (well-watered, drought-stressed), and ecotype (Peace River, PR; Hay River, HR). The elevated [CO_2] level approximates end-of-century projections under RCP 8.5 (Pachauri et al. 2014). [CO_2] was the whole-plot (two greenhouses per level), watering treatment was the subplot within each greenhouse, and ecotype was the sub-subplot. Six plants per treatment combination were assigned at random (6 plants × 2 [CO_2] × 2 watering × 2 ecotypes × 2 greenhouse replicates = 96 plants).

2.3 | CO_2 Treatments and Greenhouse Environment Controls

Elevated [CO_2] treatment began immediately after cuttings were transplanted into pots and was maintained continuously throughout the experiment. Each greenhouse was independently controlled with an Argus Titan system (Argus Controls Systems Ltd.). Elevated [CO_2] was supplied by GEN2E gas generators (Custom Automated Products Inc.). Natural daylength was extended to 17.5 h with high-pressure sodium lamps (P.L. Systems). Supplemental lighting provided approximately $600\,\mu mol\,m^{-2}\,s^{-1}$ PAR at canopy level, with less blue light than sunlight but a similar red:far-red ratio. Air temperature and relative humidity were maintained at 25°C ± 3°C (daytime)/14°C ± 3°C (nighttime), respectively, with a relative humidity of 60%.

2.4 | Soil Moisture Treatment

After a 6-week establishment phase under well-watered conditions, plants were randomly assigned to the two watering treatments. Soil volumetric water content (VWC) was measured daily with a Delta-T ML2x probe coupled to an HH2 meter (Delta-T Devices). Well-watered pots were maintained at 35%–50% VWC throughout the experiment. Water-stressed pots were held at 5.5%–10% VWC for 5 weeks (Day 42–77), corresponding to a mean midday leaf water potential (Ψ_L) of −1.2 MPa for *Populus*, consistent with moderate water deficit (Silim et al. 2009). Thereafter, droughted plants were re-watered and maintained at 35%–50% VWC for a 3-week recovery period (Day 77–98). The total experimental duration was 14 weeks (98 days; see Figure 1).

2.5 | Measurements

2.5.1 | Foliar Gas Exchange

Gas exchange was measured with a CIRAS-3 open system and PLC3 Universal Leaf Cuvette (PP Systems). Measurements were made twice: (i) at the end of the 5-week drought (hereafter "drought resistance") and (ii) 3 weeks after re-watering (hereafter "post-drought recovery"). On each occasion, a fully expanded leaf (4th–6th from the apex) was measured on three randomly selected plants per treatment combination. Cuvette conditions were 22°C, VPD≈1.3 kPa (50% RH), and $800\,\mu mol\,m^{-2}\,s^{-1}$ PPFD. For A/C_i curves (net photosynthetic rate versus intercellular CO_2 concentration; Long and Bernacchi 2003), reference [CO_2] was stepped through 400, 300, 200, 150, 100, 50, 400, 500, 600, 750, 850, 1000, 1200, 1500, and $1800\,\mu mol\,mol^{-1}$. From these curves, we extracted net photosynthesis at growth [CO_2] (P_n) ($400\,mol\,mol^{-1}$ CO_2 for the ambient [CO_2] treatment and at $1000\,mol\,mol^{-1}$ CO_2 for the elevated [CO_2] treatment), stomatal conductance (g_s), transpiration (E), the intercellular/ambient [CO_2] ratio (C_i/C_a), and intrinsic water-use efficiency ($WUE_i = P_n/g_s$). Plants used for gas exchange were re-randomised at each campaign to distribute timing effects.

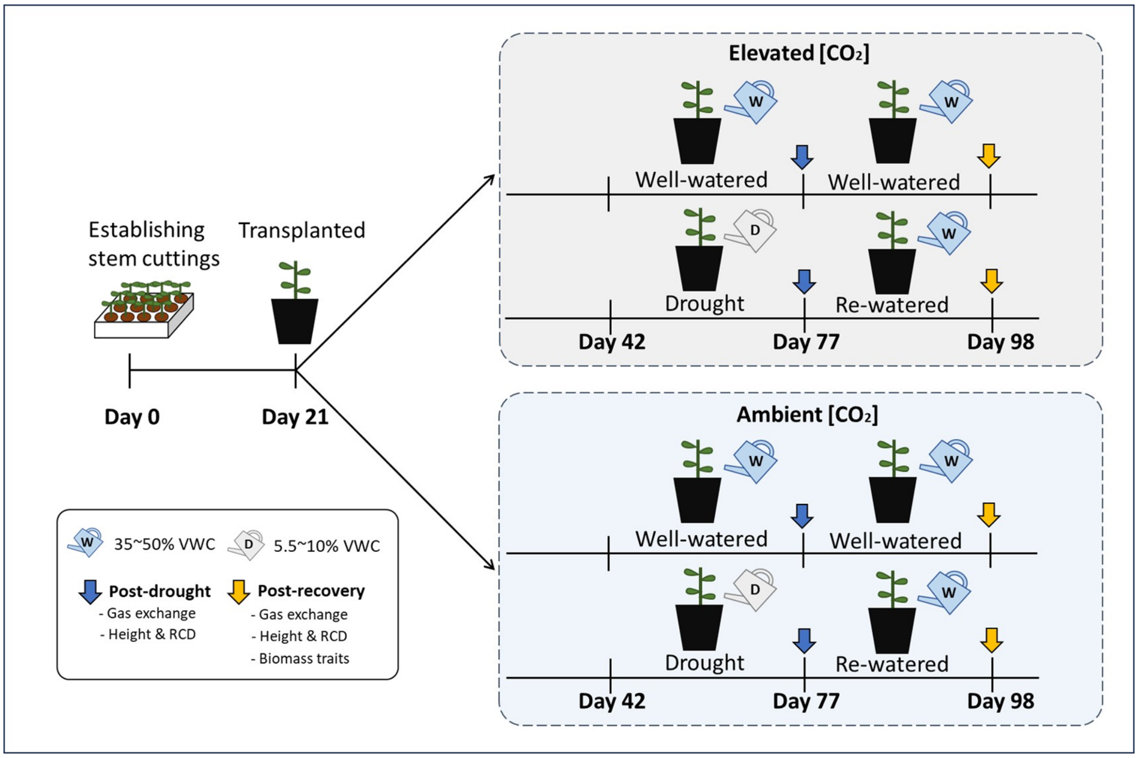

FIGURE 1 | Experimental timeline for *Populus balsamifera* stem segments grown under two CO_2 concentrations (ambient, 400 µmol mol^{-1}; elevated, 1000 µmol mol^{-1}) and subjected to well-watered and drought conditions. Stem segments were established in Styrofoam trays (Day 0–21) and subsequently transplanted to pots (Day 21). CO_2 treatment lasted from Day 21 to Day 98. Watering treatments began on Day 42, with plants maintained either under well-watered conditions or exposed to drought. Droughted plants were re-watered on Day 77 and monitored until Day 98. Physiological and growth measurements (height and root-collar diameter) were conducted at key stages (Day 77–Day 98), while biomass measurements were assessed at the final harvest (Day 98).

A/C_i data were fitted using the function *fitaci* in the R package plantecophys (Duursma 2015) in R v4.1.0 (R Core Team 2023) to estimate the maximum Rubisco carboxylation capacity (Vc_{max}) and the maximum electron-transport capacity (J_{max}) for each seedling.

2.5.2 | Growth and Biomass Allocation

Plant height (Ht) and root collar diameter (RCD) were measured at the end of the drought period (Day 77) and at the end of post-drought recovery (98). Following the recovery measurements, plants were separated into leaves, stems, and roots. Total leaf area and leaf number were determined with WinFolia (Regent Instruments). Organs were dried at 80°C for 48 h and weighed. From these data we calculated specific leaf area (SLA = total leaf area/leaf dry mass), leaf area ratio (LAR = leaf area/total plant dry mass), leaf mass ratio (LMR = leaf mass/total mass), stem mass ratio (SMR), root mass ratio (RMR), and root: shoot ratio (RSR = root mass/(leaf + stem mass)).

2.6 | Data Analysis

All statistical analyses were conducted using R v4.1.0 (R Core Team 2023). Analysis of variance (ANOVA) was used to test for the main effects of growth [CO_2], ecotype, water treatment, and their interactions. Shapiro–Wilk's and Levene's tests were first applied to assess normality and homogeneity of variances, respectively. Variables failing these assumptions were log-transformed prior to analysis. When ANOVA detected a significant ($p \leq 0.05$) interaction, planned simple-effects comparisons were conducted using Fisher's protected least significant difference (LSD) based on the pooled ANOVA error term. These comparisons were restricted to biologically meaningful, pre-specified contrasts used to interpret the interaction (i.e., comparisons among ecotypes within a given [CO_2] × water treatment combination, and comparisons between water treatments within a given ecotype and [CO_2] level); unrestricted all-pairwise comparisons across factor levels were not conducted. Multivariate trait variation was summarised with principal component analysis (PCA). For drought resistance, PCAs were computed separately for PR and HR using gas-exchange and size traits (P_n, g_s, Vc_{max}, J_{max}, WUE_i, Ht, and RCD). For post-drought recovery, the same variables were analysed together with total biomass and allocation traits (SLA, LAR, LMR, RMR, and SMR). Biplots display association vectors and 95% concentration ellipses for the four [CO_2] × watering combinations: (1) ambient [CO_2] with water stress (a[CO_2]–DS), (2) ambient [CO_2] with well-watered conditions (a[CO_2]–WW), (3) elevated [CO_2] with water stress (e[CO_2]–DS), and (4) elevated [CO_2] with well-watered conditions (e[CO_2]–WW).

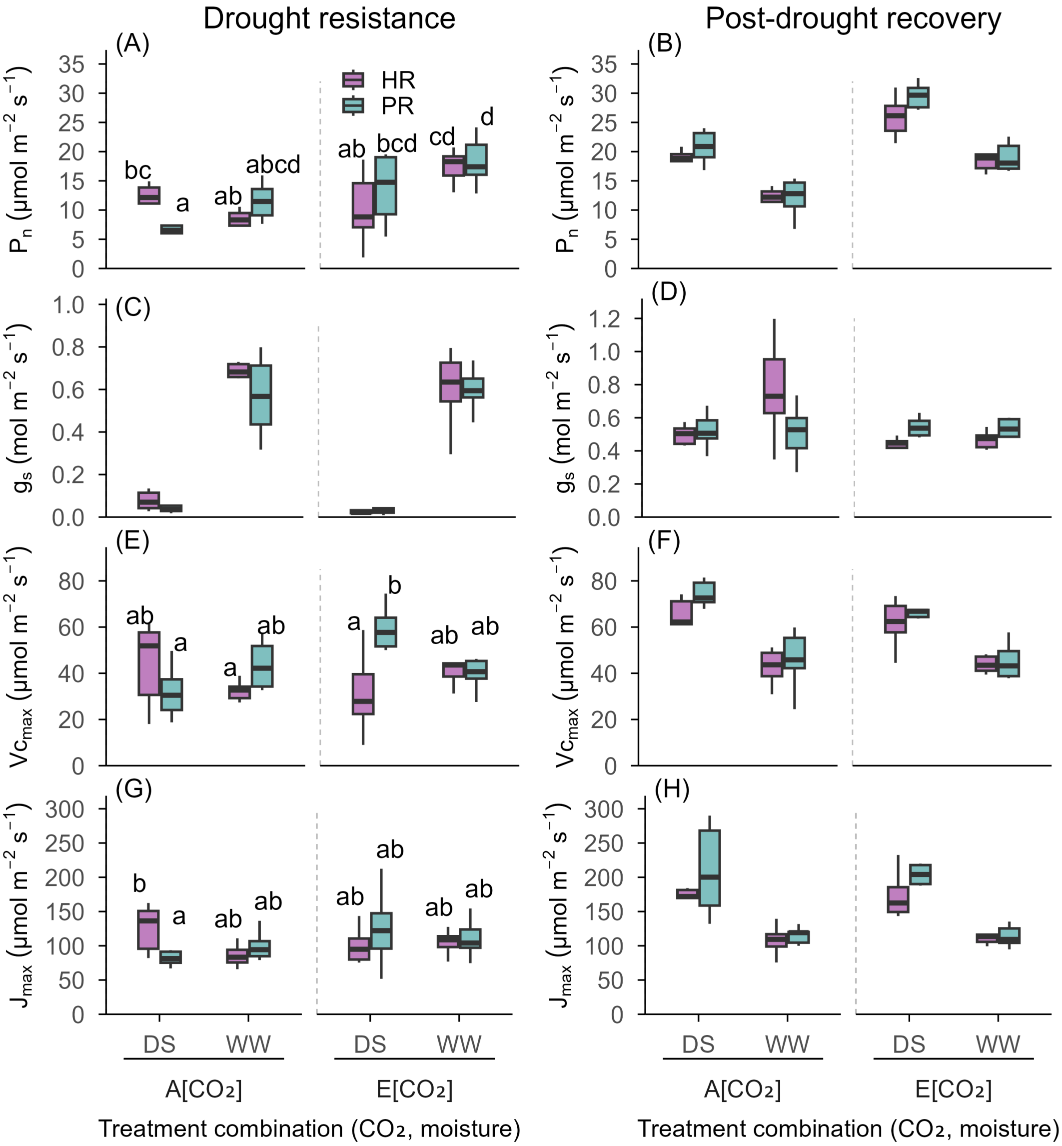

FIGURE 2 | Effects of growth [CO_2] (C), moisture (M), and ecotype (E) on photosynthetic traits of *Populus balsamifera* during the drought-resistance phase (left; end of drought) and post-drought recovery (right; after re-watering). Shown are P_n (400 mol mol^{-1} CO_2 for the ambient [CO_2] treatment and at 1000 mol mol^{-1} CO_2 for the elevated [CO_2] treatment) (A, B) and g_s (C, D), Vc_{max} (E, F), and J_{max} (G, H) for Hay River (HR) and Peace River (PR) origins under drought-stressed (DS) and well-watered (WW) conditions at ambient [CO_2] (A[CO_2]) and elevated [CO_2] (E[CO_2]). Boxes show medians and interquartile ranges with whiskers extending to 1.5× IQR; letters denote LSD groupings within panels ($\alpha = 0.05$). ANOVA significance: (A, E, G) C×M×E; (B) C and M; (C) M; (D) n.s.; (F) M and E; (H) M. Each boxplot is based on $n = 6$ individuals per treatment group.

3 | Results

3.1 | Interplay of Growth [CO_2] and Water Availability on Ecotype Differences in Gas Exchange and Biochemical Capacity

Under ambient [CO_2], HR maintained higher P_n than PR under drought, whereas P_n did not differ between the ecotypes when well-watered (Figure 2A). Under elevated [CO_2], drought suppressed P_n only for HR, and PR showed a larger CO_2-mediated increase than HR under drought; across CO_2 levels, ecotype differences in P_n were therefore contingent on moisture and were indistinct at elevated [CO_2] (Figure 2A). For Vc_{max}, under ambient [CO_2], both ecotypes showed statistically similar values under both moisture regimes (Figure 2C). Under elevated [CO_2], responses diverged with moisture: under drought, PR exceeded HR, whereas under well-watered conditions, ecotype did not differ. Thus, ecotype differences in Vc_{max} emerged only under the combination of elevated [CO_2] and drought, and increasing [CO_2] enhanced Vc_{max} only in PR under drought (Figure 2C). For J_{max}, under ambient [CO_2], HR exceeded PR under drought, while they were similar when well-watered (Figure 2E). Under elevated [CO_2], ecotype differences were not detected under either moisture regime, indicating convergence of J_{max} between HR and PR at elevated [CO_2] (Figure 2E). Stomatal conductance (g_s) declined with water stress across [CO_2] treatments (main effect of moisture, $p = 0.015$; Table 1). Intrinsic water-use efficiency (*WUE*ᵢ) showed a significant [CO_2] × moisture interaction ($p = 0.007$): Under ambient [CO_2], drought modestly increased *WUE*ᵢ relative to well-watered plants, whereas under elevated

TABLE 1 | ANOVA for gas-exchange and size traits measured at the end of the drought phase (drought resistance) in *Populus balsamifera*.

	CO_2 (C)		Moisture (M)		C: M		Ecotype (E)		C: E		M: E		C: M: E	
Variable	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
P_n	8.17	0.104	5.70	0.140	3.41	0.206	0.47	0.499	1.49	0.230	1.67	0.205	6.16	**0.018**
Vc_{max}	0.69	0.495	0.00	0.974	0.01	0.923	1.86	0.182	2.41	0.129	0.00	0.949	8.63	**0.006**
J_{max}	0.28	0.650	0.54	0.539	0.40	0.592	0.17	0.678	3.19	0.083	0.47	0.498	5.26	**0.028**
g_s	1.89	0.303	66.85	**0.015**	1.97	0.296	0.00	0.991	0.17	0.679	0.44	0.509	0.94	0.339
WUE_i	717	**0.001**	633.86	**0.002**	149.17	**0.007**	1.19	0.282	1.05	0.313	1.13	0.295	1.10	0.302
C_i/C_a	1.46	0.350	214.66	**0.005**	2.54	0.252	0.95	0.336	0.61	0.440	0.62	0.437	0.87	0.358
Height	3.61	0.198	14,628	**0.000**	862.11	**0.001**	3.76	0.060	4.80	**0.035**	3.03	0.090	0.01	0.906
RCD	16.73	0.055	0.44	0.575	0.04	0.862	0.30	0.590	2.69	0.110	1.89	0.178	0.27	0.605

Note: Plants were grown in a split–split plot across four greenhouses with growth $[CO_2]$ (C) as the whole-plot factor (ambient 400 vs. elevated 1000 μmol mol^{-1}; two greenhouse replicates per CO_2), moisture (M) as the subplot (well-watered, WW; drought-stressed, DS), and ecotype (E) as the sub-subplot (Hay River, HR; Peace River, PR). Entries are F statistics (F) with associated *p*-values (*p*); significant terms ($\alpha = 0.05$) are bold.
Abbreviations: C_i/C_a, intercellular/ambient CO_2 ratio; g_s, stomatal conductance; Height, shoot height; J_{max}, maximum electron-transport rate; P_n, net photosynthesis evaluated at the growth CO_2; RCD, root-collar diameter; V_{cmax}, maximum Rubisco carboxylation rate; *WUE*i, intrinsic water-use efficiency.

$[CO_2]$, the increase with drought was much larger $[CO_2]$ effects depended on water regime: *WUE*i was higher at elevated than ambient $[CO_2]$ under drought, but $[CO_2]$ had no effect when plants were well-watered.

3.2 | Recovery After Re-Watering Across $[CO_2]$ and Drought Treatments

During recovery, P_n showed main effects of $[CO_2]$ and drought treatment, with higher values at elevated $[CO_2]$ and in plants with a drought treatment, while the ecotype term was not significant (Figure 2B; Table 1). Because P_n differed between ecotypes during drought only under ambient $[CO_2]$ (HR>PR; Figure 2A; Table 1) but converged after re-watering, PR showed a larger absolute increase in P_n from drought to recovery than HR under ambient $[CO_2]$. Recovery Vc_{max} was governed by significant main effects of moisture and ecotype, with values being higher in previously water-stressed plants than in the well-watered control plants, and PR exceeded HR (Figure 2D; Table 1). The recovery of J_{max} showed a significant main effect of moisture, with values being higher in plants that had experienced drought, irrespective of ecotype or $[CO_2]$ (Figure 2F; Table 1). g_s did not differ significantly between CO_2 levels or moisture treatments, and no interactions were detected (all $p > 0.05$; Table 2). By contrast, *WUE*i was significantly higher in plants with a drought exposure (main effect of moisture, $p = 0.026$) and was elevated at the elevated $[CO_2]$ irrespective of moisture treatment ($[CO_2]$ main effect, $p = 0.001$; $[CO_2] \times$ moisture, $p = 0.091$).

3.3 | Ecotype-Dependent Growth and Biomass Allocation

The $[CO_2]$ effects were largely additive responses: stem, leaf, and total biomass increased by 29%, 41%, and 35%, respectively; root-collar diameter increased by 10%; and stem mass ratio decreased by 6% (Table 2). At the end of the drought period, PR was 0.38 cm taller than HR (statistically significant) (Figure 3A). After re-watering, drought treatment plants were, on average, 14.25 cm shorter than continuously well-watered plants, and PR was 6.17 cm taller than HR (Figure 3B). Plants with drought treatment were lower than those that had been continuously well-watered. Post-recovery total biomass also exhibited a moisture × ecotype interaction (Figure 3C). Drought treatment reduced total biomass in both ecotypes, with a greater reduction in PR, whereas under well-watered conditions, PR maintained slightly higher biomass. These biomass patterns were consistent with allocation shifts. Leaf mass ratio (LMR) decreased in plants with drought treatment and was higher in PR than HR (Figure 3D; Table 1). Specific leaf area (SLA) responded differentially by ecotype (Figure 3E). In HR, drought reduced SLA relative to well-watered conditions, and SLA was higher in HR than in PR under well-watered conditions (Table 1). Leaf area ratio (LAR) likewise showed a moisture × ecotype interaction (Figure 3F). Under drought, PR had higher LAR than HR, whereas under well-watered conditions, both ecotypes converged at higher LAR.

3.4 | Multivariate Trait Relationships Differ by Treatment

Principal component analysis (PCA) revealed clear ecotype- and treatment-specific trait associations during drought (Figure 4). In PR, the first two components explained 75.7% of the variance (Figure 4; Top left). PC1 was positively associated with Vc_{max}, WUE_i, and J_{max}, and negatively with g_s, H_t, and P_n; PC2 reflected a gradient from higher structural growth and gas exchange to reduced g_s. Elevated $[CO_2]$ with drought-stress (DS) scored highest on both axes, aligning with greater photosynthetic capacity and WUE_i, whereas ambient $[CO_2]$ with DS was intermediate. Well-watered treatments, particularly elevated $[CO_2]$ with well-watered (WW), were associated with greater g_s and Ht but lower WUE_i. In HR, the first two components accounted for 65.2% of the variance, with PC1 linked to Vc_{max}, J_{max}, and P_n, and PC2 to WUE_i (positive) and g_s and Ht (negative) (Figure 4; Top right). Both DS treatments clustered together with high WUE_i and

TABLE 2 | ANOVA for gas-exchange, growth, and allocation traits measured 2 weeks after re-watering ("post-drought recovery") in *Populus balsamifera*.

	CO_2 (C)		Moisture (M)		C: M		Ecotype (E)		C: E		M: E		C: M: E	
Variable	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
P_n	36.00	**0.027**	35.31	**0.027**	0.10	0.786	2.07	0.158	0.14	0.709	2.10	0.156	0.01	0.907
Vc_{max}	0.62	0.514	33.78	**0.028**	0.93	0.437	5.31	**0.027**	2.34	0.135	2.73	0.107	0.93	0.342
J_{max}	0.08	0.802	33.46	**0.029**	0.06	0.829	2.30	0.138	0.32	0.575	2.64	0.113	0.05	0.831
g_s	11.27	0.078	3.37	0.208	2.44	0.259	0.03	0.857	4.00	0.053	2.69	0.110	2.89	0.098
WUEi	1115.08	**0.001**	36.34	**0.026**	9.54	0.091	0.66	0.422	3.30	0.077	0.90	0.350	1.89	0.178
Ci/Ca	72.70	**0.013**	18.26	0.051	3.28	0.212	0.53	0.473	1.45	0.236	0.31	0.582	1.00	0.323
Height	3.57	0.199	85.19	**0.012**	3.57	0.199	7.54	**0.009**	1.79	0.190	4.96	**0.032**	0.00	1.000
RCD	20.36	**0.046**	6.00	0.134	0.84	0.457	1.31	0.259	1.39	0.246	1.26	0.269	0.96	0.333
Root	14.57	0.062	5.96	0.135	0.15	0.736	1.37	0.250	1.89	0.177	0.11	0.744	0.10	0.750
Stem	69.30	**0.014**	13.51	0.067	0.80	0.465	0.05	0.830	0.94	0.340	6.03	**0.019**	0.71	0.406
Leaf	59.17	**0.016**	275.21	**0.004**	1.55	0.339	3.01	0.091	1.70	0.201	1.39	0.245	0.01	0.917
LA	8.95	0.096	143.84	**0.007**	2.37	0.264	3.16	0.084	0.55	0.463	0.30	0.587	0.01	0.907
Total	60.34	**0.016**	28.38	**0.033**	1.15	0.395	0.39	0.536	1.28	0.266	4.22	**0.047**	0.14	0.710
SLA	5.83	0.137	0.59	0.523	0.04	0.868	0.61	0.440	3.79	0.059	7.93	**0.008**	0.09	0.761
LAR	0.09	0.789	153.19	**0.006**	0.10	0.783	7.08	**0.012**	0.18	0.671	5.53	**0.024**	0.04	0.836
LMR	6.06	0.133	188.90	**0.005**	0.00	0.992	11.16	**0.002**	0.78	0.384	0.02	0.881	0.26	0.613
RMR	1.63	0.329	6.03	0.133	0.38	0.601	1.88	0.179	0.49	0.489	1.06	0.310	0.00	0.958
SMR	25.72	**0.037**	106.77	**0.009**	0.55	0.536	6.15	**0.018**	1.85	0.182	0.70	0.407	0.23	0.632
RSR	1.49	0.346	5.29	0.148	0.53	0.544	1.66	0.205	0.50	0.483	1.05	0.312	0.00	0.999

Note: Plants were grown in a split–split plot across four greenhouses with growth [CO_2] (C) as the whole-plot factor (ambient 400 vs. elevated 1000 μmol mol^{-1}; two greenhouse replicates per [CO_2]), moisture (M) treatment as the subplot (well-watered, WW; drought-stressed, DS), and ecotype (E) as the sub-subplot (Hay River, HR; Peace River, PR). Entries are F statistics (F) with associated *p*-values (*p*); significant terms ($\alpha = 0.05$) are in bold.

Abbreviations: C_i/C_a, intercellular/ambient CO_2 ratio; g_s, stomatal conductance; J_{max}, maximum electron-transport rate; LA, leaf area; LAR, leaf-area ratio; LMR/SMR/RMR, leaf/stem/root mass ratios; P_n, net photosynthesis at the growth [CO_2]; RCD, root collar diameter; RSR root, shoot ratio; SLA, specific leaf area; Vc_{max}, maximum Rubisco carboxylation rate; *WUEi*, intrinsic water-use efficiency.

moderate to high J_{max}. Well-watered plants occupied the lower half of the plot, while elevated [CO_2] with WW was separated from ambient [CO_2] with WW along PC1 due to the [CO_2]-driven enhancement of photosynthetic capacity.

Following re-watering, the first two components explained 68.43% of the variance in PR and 69.87% in HR (Figure 4). In PR, PC1 was positively associated with LMR, LAR, RMR, H_t, and RCD, and negatively with SLA, SMR, g_s, and photosynthetic traits, while PC2 had higher WUE_i and photosynthetic capacity with greater leaf structural investment (Figure 4; Top left). Elevated [CO_2] with DS was positioned on the negative side of PC1 and the high side of PC2, indicating greater physiological efficiency under stress, whereas WW treatments scored positively on PC1, reflecting increased morphological growth. In HR, PC1 was positively associated with biomass, RCD, H_t, LMR, and LAR, and negatively with WUE_i and photosynthetic capacity; PC2 contrasted biomass allocation traits with WUE_i and photosynthetic capacity (Figure 4; Top right). Elevated [CO_2] with WW scored highest on PC1, reflecting maximum morphological growth, while elevated [CO_2] with DS scored lower on PC1 but higher in physiological efficiency traits. Ambient [CO_2] with WW and DS were intermediate, with partial overlap between stress and well-watered conditions.

4 | Discussion

4.1 | Intraspecific Responses of Gas Exchange to [CO_2] × Drought

Long-term CO_2 enrichment can alter leaf gas-exchange traits in *Populus*, including sustained stimulation of photosynthesis and associated shifts in stomatal conductance and photosynthetic capacity across the canopy and through time (Bernacchi et al. 2003; Calfapietra et al. 2005; Liberloo et al. 2007). This acclimation context is important for interpreting CO_2 × moisture interactions because baseline CO_2 responses can change the degree to which drought constrains carbon assimilation. Our study examined the combined effects of elevated [CO_2] and drought stress on leaf gas exchange, growth, and key biomass traits in *Populus balsamifera* stem cuttings. In line with our prediction, elevated [CO_2] reduced

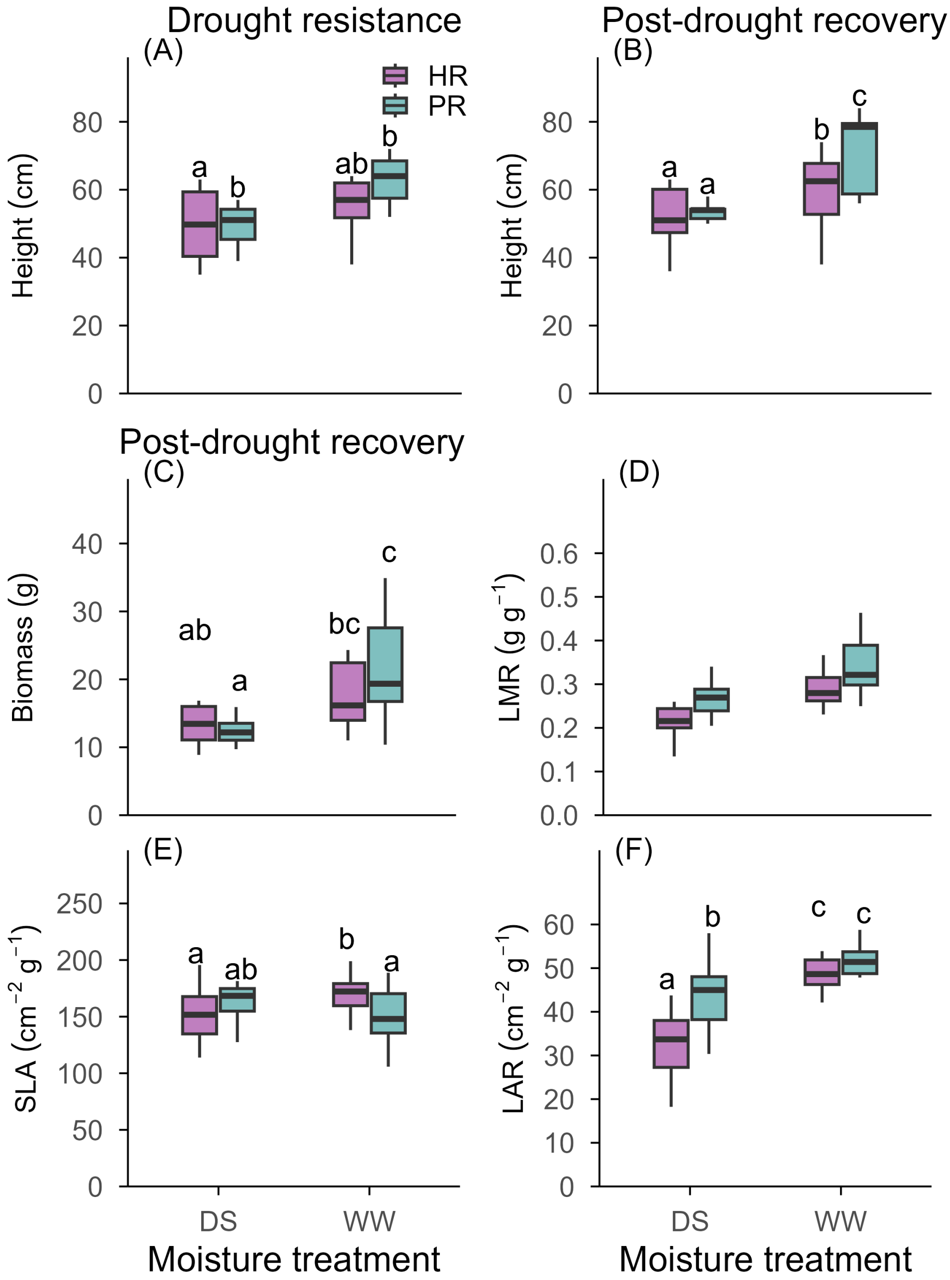

FIGURE 3 | Growth, biomass, and leaf-allocation responses to moisture (M) in two *Populus balsamifera* ecotypes (E). Boxplots show (A) height at the end of drought, (B) height after re-watering, (C) total biomass at harvest, (D) leaf mass ratio (LMR), (E) specific leaf area (SLA), and (F) leaf area ratio (LAR). Boxes show medians and interquartile ranges with whiskers to 1.5 × IQR; letters denote LSD groupings ($\alpha = 0.05$). ANOVA significance: (A–C, E–F) M × E; (D) main effects of M and E. Each boxplot is based on $n = 12$ individuals per moisture × ecotype group (M × E), pooled across [CO_2] treatments.

the ecotype-based variation in net photosynthesis under drought by providing a proportionally greater benefit to the initially weaker PR ecotype, thereby narrowing the drought-performance gap.

At ambient [CO_2], the HR ecotype maintained higher P_n than PR under drought conditions, despite both ecotypes exhibiting similar stomatal limitations. This divergence is consistent with previous findings where *P. deltoides* × *P. nigra* from different maternal environments exhibit unique photosynthetic behavior (Monclus et al. 2006) and likely reflects inherent variations in mesophyll CO_2 conductance reported in other species (Théroux Rancourt et al. 2015) or distinctive drought-management strategies evolved within *P. balsamifera* (Hamanishi et al. 2010). Such findings also align with broader evidence of clonal variation in drought response across the *Populus* genus (Marron et al. 2002; Zhang et al. 2004). However, under elevated [CO_2] and drought, these ecotypic differences were no longer evident. Our results demonstrate that the benefit of elevated [CO_2] was ecotype-dependent: while elevated [CO_2] enhanced carbon gain in PR, it had little effect in HR. This increased stimulation was supported by an increase in Vc_{max} under drought only in the PR ecotype, indicating that its [CO_2]-driven gain was attributable to changes in photosynthetic carboxylation capacity (Vc_{max}) rather than to diffusional limitations via stomatal conductance (g_s). Indeed, the lack of a significant CO_2 × moisture

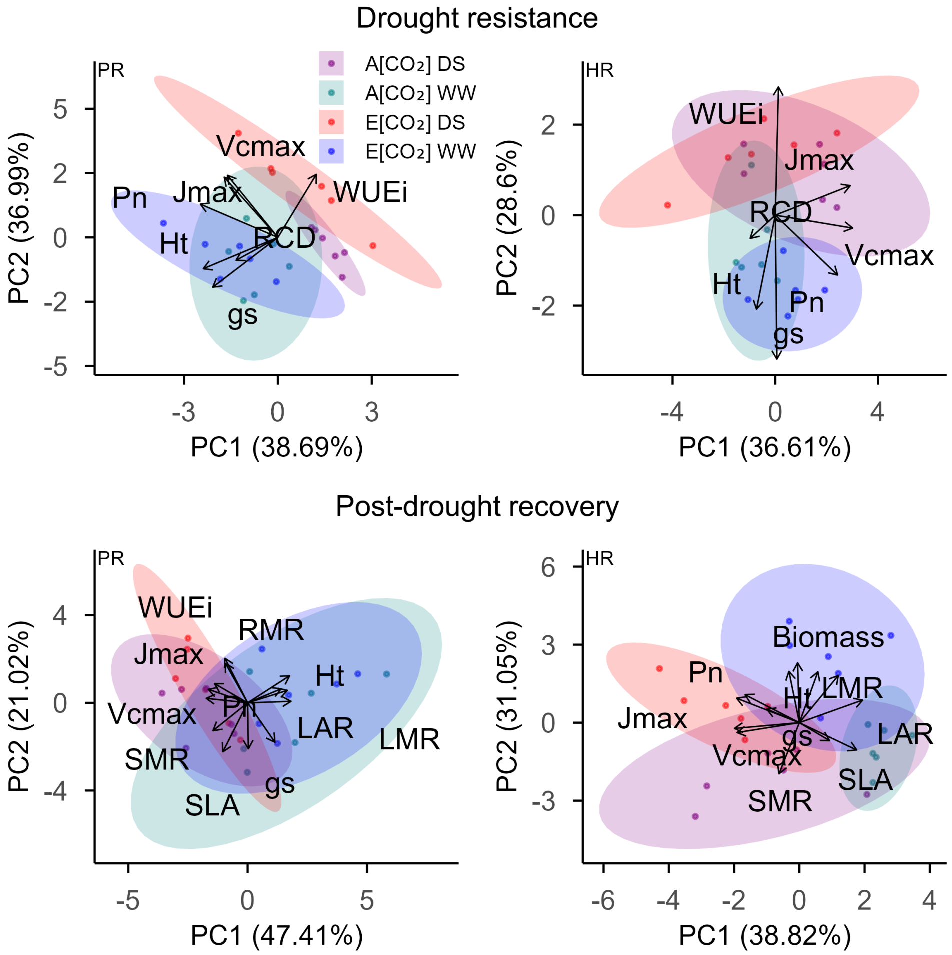

FIGURE 4 | Principal component analyses (PCA) for Peace River (PR; left) and Hay River (HR; right) ecotypes during the drought-resistance phase (top) and post-drought recovery (bottom). Drought PCAs are based on gas-exchange and size traits: Net photosynthesis (P_n), stomatal conductance (g_s), maximum carboxylation rate (V_{cmax}), maximum electron transport rate (J_{max}), intrinsic water-use efficiency (*WUE*ᵢ), height (Ht), and root-collar diameter (RCD). Recovery PCAs included these variables, along with total biomass and allocation traits: Specific leaf area (SLA), leaf area ratio (LAR), leaf mass ratio (LMR), root mass ratio (RMR), and stem mass ratio (SMR). Colored ellipses (95% confidence) represent factorial combinations of [CO_2] and moisture: Ambient [CO_2] (A[CO_2]) × drought stress (DS), A[CO_2] × well-watered (WW), elevated [CO_2] (E[CO_2]) × DS, and E[CO_2] × WW. PC scores and ellipses are based on $n=6$ individuals per treatment group within each ecotype and phase.

interaction for g_s confirms that drought-driven stomatal limitation was expressed similarly across treatments, reflecting the conservative water-use response reported in *Populus* (Giovannelli et al. 2007). Similar within-species variation has been reported in other woody taxa: in wild olive, CO_2 enrichment increased WUE_i and reduced water consumption, but the sensitivity to soil water availability differed among genotypes (Aranda et al. 2026). This suggests that the increased partial pressure of CO_2 provides a metabolic buffer that overrides the structural and biochemical limitations differentiating these ecotypes at ambient levels and drought. This suggests that within-species responses to environmental stress are not static; rather, they can shift under changing [CO_2] and water regimes.

4.2 | Physiological and Biochemical Drivers of Post-Drought Recovery

Trees often resume metabolic activity rapidly after drought release, but full photosynthetic recovery can lag for days to weeks, depending on the stress severity and duration, with differences evident even among closely related taxa (Gallé and Feller 2007; Warren et al. 2012; Arend et al. 2016). In *P. balsamifera*, post-drought recovery can be genotype-dependent, with drought-induced structural changes fully reversible in some genotypes but not others (Momayyezi et al. 2025) and with contrasting recovery among clones and hybrids after an imposed drought followed by re-watering (Larchevêque et al. 2011). Consistent with this pattern, PR showed higher Vc_{max} than HR during the recovery period in our study. In our study, drought-stressed trees showed marked post-recovery after 15 days of re-watering: P_n, Vc_{max}, J_{max}, and WUE_i increased, recovering much of the carbon lost during stress. Across *Populus*, findings range from rapid recovery after severe drought in *P. tremuloides* (Lu et al. 2010) to being unable to recover after prolonged drought in *P. balsamifera* (Galvez et al. 2013), and delayed recovery has been observed even under less extreme conditions, with *P. nigra* returning to pre-stress photosynthesis only after ~15 days of re-watering (Fortunati et al. 2008). Evidence further indicates that recovery can be asynchronous among limitations, including delayed recovery of stomatal versus internal conductance components during drought–recovery cycles in *P. balsamifera*-derived material (Théroux Rancourt et al. 2015). The mechanisms underlying post-drought recovery remain incomplete, but increased stomatal sensitivity to abscisic acid after drought relief (Loewenstein and Pallardy 2002), drought-period limitation that may extend leaf lifespan, have been implicated (Arend et al. 2016). Similar overcompensation of g_s and P_n after re-watering has been reported in other woody species, with g_s fully restored or exceeding non-stressed controls (Gallé et al. 2007; Izanloo et al. 2008; Xu et al. 2010).

Drought had much less effect on photosynthetic capacity than on g_s and C_i/C_a, likely due to protective mechanisms (enzyme stabilization, antioxidant activity) that preserve photosynthetic integrity (Flexas et al. 2006; Lawlor and Tezara 2009), enabling rapid recovery and higher post-drought rates than controls. After re-watering, similar g_s and C_i/C_a between treatments indicate that enhanced photosynthesis arose from intrinsic biochemical recovery rather than improved CO_2 diffusion. Delayed g_s recovery elsewhere has been linked to residual stomatal limitations, mesophyll alterations, or stomatal morphological changes (Flexas et al. 2004; England and Attiwill 2006; Gallé et al. 2007; Cano et al. 2014). Typically, faster recovery of photosynthesis than g_s elevates WUE_i (Miyashita et al. 2005), consistent with our observations. Sustained post-drought stimulation without altered g_s (Pflug et al. 2018) further suggests that some woody species, including *Populus*, can maintain elevated WUE_i even after g_s fully recovers.

4.3 | Variation in Biomass Allocation and Growth Strategies

After the stress period, PR showed slightly greater height growth than HR. Following re-watering, however, both previously drought-stressed ecotypes remained shorter than continuously watered controls, and PR's advantage during the stress period diminished, indicating that superior drought tolerance did not translate into faster recovery. In the absence of drought, PR was taller than HR. Common-garden studies likewise show PR-like, warm-origin genotypes often surpass northern sources such as HR for height when moisture is adequate (Keller et al. 2011), suggesting inherited allocation patterns favoring rapid growth when resources permit. During recovery, drought reduced total biomass, whereas elevated $[CO_2]$ generally increased it. Both ecotypes previously exposed to drought had lower biomass than continuously watered controls; the reduction was greater in PR, while HR maintained biomass similar to controls. This accords with conservative growth and reduced sensitivity to drought-induced biomass loss in northern populations with shorter seasons and colder, wetter soils (Olson et al. 2013).

SLA responses further indicate divergent strategies: HR reduced SLA, retaining thicker, stress-adapted leaves that aid water conservation and resilience (Monclus et al. 2006; Poorter et al. 2012). Although PR leaves were thicker than HR under well-watered conditions, previously stressed and recovered PR showed no SLA adjustment, suggesting limited structural plasticity despite fast growth under ample water. The drought effects on LAR are consistent with resource-conservative strategies to limit water loss (Chaves et al. 2009). PR's higher LAR than HR following drought indicates greater allocation to leaf area during recovery, potentially enhancing carbon gain and conferring an advantage in fluctuating environments, while also reflecting greater sensitivity to drought-related productivity loss (Poorter et al. 2012). Conversely, HR's lower LAR emphasizes structural maintenance over rapid regrowth, typical under water limitation (Poorter and Nagel 2000), and adaptive in drought-prone settings where reducing evaporative surface area supports hydraulic and carbon balance during prolonged or recurrent stress (Chaves et al. 2003). These results provide a controlled mechanistic baseline for understanding how $[CO_2]$ and drought interact; however, their expression in the field may be influenced by additional environmental feedback (Ainsworth and Long 2004; Leakey et al. 2009; Poorter et al. 2012). Because the rooting environment can influence sink strength and the degree of photosynthetic acclimation under elevated $[CO_2]$ (Arp 1991), FACE studies provide complementary evidence showing that drought × $[CO_2]$ responses depend on whole-plant sink development (Jiang et al. 2021).

5 | Conclusion

While intraspecific variation in *Populus* responses to drought and elevated $[CO_2]$ is documented, few studies have simultaneously evaluated leaf-level physiology and whole-plant growth through both drought and recovery phases in *P. balsamifera*. This study demonstrates that elevated $[CO_2]$ narrows ecotypic gaps in drought-phase photosynthesis. This shift occurred primarily because the PR ecotype exhibited a larger $[CO_2]$-associated gain and increased photosynthetic capacity (Vc_{max}), even as stomatal conductance declined across all treatments. Following re-watering, both ecotypes showed robust recovery of P_n, Vc_{max}, J_{max}, and WUE_i, indicating substantial biochemical resilience. However, this leaf-level convergence did not extend to the whole-plant level; HR maintained biomass closer to well-watered controls via a conservative trait suite (lower SLA and LAR), while PR suffered greater growth reductions. Ultimately, elevated $[CO_2]$ can reduce ecotypic differences in drought-phase carbon gain without removing ecotype-dependent differences in growth and post-stress performance, emphasizing the importance of incorporating intraspecific variation when predicting population responses to future $[CO_2]$ and drought regimes.

Author Contributions

Sahari Inoue: conceptualization, funding acquisition, methodology, investigation, writing. Binyam Tedla: formal analysis, investigation – cutting collection, writing. Qing-Lai Dang: resources, writing – review and editing. Yuzou Sano: resources, funding acquisition, writing – review and editing.

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Data Availability Statement

The datasets generated for this study may be provided upon request.

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