

ORIGINAL RESEARCH

Water use efficiency of poplars grown for biomass production in the Midwestern United States

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Abstract

Water availability and other site conditions influence poplar biomass productivity and affect clonal performance due to genotype × environment interactions. It is important to select genotypes with high water use efficiency (WUE) that maximizes yield with available amounts of water at sites, while avoiding drought stress and growth impacts to the trees. During drought, stomatal closure induces increased accumulation of $\delta^{13}\text{C}$ carbon isotope in tree tissues, which is strongly correlated with WUE of trees and usually expressed through carbon isotope discrimination (Δ). Our primary objectives were to evaluate differences in WUE among poplar genotypes grown in the Midwestern United States, and to identify genotypes with high WUE for future deployment on water-limited sites in the region. Sites included 10-year-old biomass plantations in Escanaba, Michigan; Waseca, Minnesota; and Ames, Iowa established from 2000 to 2001 with seven poplar genotypes' three genomic groups. Following harvest, height, diameter, and biomass were determined. Wood samples were collected from individual growth rings to assess annual ring width and WUE through $\delta^{13}\text{C}$ and Δ . Aboveground dry biomass varied among sites ($p = .0007$), clones ($p < .0001$), and their interactions ($p = .0134$), ranging from 3.1 to 14.0 Mg ha⁻¹ year⁻¹. Δ varied among sites ($\Delta = 18.9\text{--}19.7\text{‰}$; $p < .0001$) and clones ($\Delta = 18.6\text{--}19.9\text{‰}$; $p < .0001$), indicating effects of site conditions on WUE of tested genotypes. Clones varied in their water-conserving strategies. Some clones were characterized as water consumers with high growth and high WUE ("C916000"; "C916400"), while other genotypes were water conservers using lower amounts of water with moderate biomass production ("NC13624"; "NC13649"; "NM2" to a certain extent). Although $\delta^{13}\text{C}$ carbon isotope accumulation correlates with WUE, Δ should only be used for selection when it is integrated with other parameters such as productivity, soils information, and climate data.

KEYWORDS

biomass, carbon isotope ($\delta^{13}\text{C}$), carbon isotope discrimination (Δ), clones, genotype × environment interactions, mean annual increment, *Populus*

1 | INTRODUCTION

Populus species and their hybrids (hereafter referred to as poplars) are among the most widespread plantation forests in the world, with poplar biomass systems encompassing upward of 31 million hectares in over 30 countries worldwide (Carle, 2016). As with other short rotation woody crops (SRWCs), plantation type and silvicultural prescriptions such as spacing (i.e., plantation density), rotation length (i.e., harvest age), and establishment technologies (e.g., planting stock type, site preparation, etc.) are determined by the end use of poplar products (Zalesny et al., 2021). Besides traditional poplar culture (e.g., timber, fiber, and pulp production), recent trends emphasize increased use of short rotation biomass plantations as energy feedstocks that contribute to climate change mitigation and more efficient usage of renewable energy sources (Zalesny et al., 2019), thus avoiding additional emissions of carbon dioxide (CO₂; Rončević et al., 2013).

In regions such as the Midwestern United States, poplars are strategically grown to increase biomass production, carbon sequestration, and additional ecosystem services (Lazarus et al., 2015; Zalesny et al., 2012; Zalesny, Stanturf, Gardiner, Perdue, et al., 2016). From an ecological sustainability standpoint, poplar feedstocks contribute positively to several energetic, economic, and environmental benefits: (i) reducing the use of nonrenewable energy sources, (ii) providing cheaper alternatives to nonrenewable energy sources, and (iii) maintaining year-round accessibility of energy feedstocks with elevated energy output:input ratios (Volk et al., 2004; Zalesny et al., 2009). Along with these benefits and uses, poplar productivity levels are substantially greater than other temperate-grown deciduous trees, making them ideal for phytoremediation, mine reclamation, brownfields restoration, and associated phyto-technologies (Nelson et al., 2018; Riemenschneider et al., 2001; Zalesny, Stanturf, Gardiner, Bañuelos, et al., 2016).

Photosynthesis and transpiration of terrestrial ecosystems, including poplar biomass production systems, greatly influence global carbon and hydrologic cycles through extensive exchange of water and carbon between land and atmosphere (Schimel, 1995). The intensity and frequency of climate extremes (e.g., temperature, precipitation, drought) are predicted to increase with elevated CO₂ and other greenhouse gases in the atmosphere (IPCC, 2001). For example, in the Midwestern United States, winters and duration of ice cover on lakes are predicted to be shorter, annual average temperatures warmer, and heavy rainstorms more frequent (Handler et al., 2014; Kling et al., 2003). Some poplar genotypes are sensitive to such climatic extremes. Environmental stresses such as heat, drought, or pests can strongly impact poplar biomass production and carbon mitigation potential (Migliavacca

et al., 2009). In general, climate change effects are predicted to be much more pronounced for many SRWCs relative to other, less intensively managed forest types that have lower productivities over greater rotation lengths (Loustau et al., 2005). However, some lignocellulosic crops have exhibited improved physiological responses (e.g., drought tolerance) and biomass productivity under elevated atmospheric CO₂ (Oliver et al., 2009). For example, certain poplars have the capability to use and transpire large volumes of water while others are adapted to drought conditions (Newman et al., 1997; Pilipović et al., 2015), thus optimizing the overall trade-offs between water usage and carbon mitigation (King et al., 2013). Given the broad genetic variability among poplar species and hybrids (Eckenwalder, 1984; Orlović et al., 2009), identifying genotypes that exhibit satisfactory growth and higher water use efficiency (WUE) may be achievable (Toillon et al., 2013). For example, through breeding and selection of poplar genotypes with improved drought tolerance and WUE, it is possible to extend poplar productivity zones from flood plains and bottomlands to uplands with less soil moisture availability and more frequent seasonal water shortages (Monclus et al., 2006).

In plant sciences, WUE is defined based on particular growth and physiological processes involved. For example, from the perspective of plant growth, WUE is calculated as aboveground biomass production per unit area per unit water that is evapotranspired, while from the leaf physiology perspective, WUE is the ratio of the rate of assimilated CO₂ through photosynthesis to the rate of water transpired (Kirkham, 2005). Since water use at the leaf level is mostly regulated by stomatal conductance (g_s ; Hatfield & Dold, 2019), intrinsic water use efficiency (WUE_i; i.e., the ratio of net photosynthesis to stomatal conductance) is a more reliable parameter related to drought tolerance in plants because WUE_i is influenced by climate (Schulze et al., 2004). Under drought conditions, stomatal behavior not only influences water loss but also the amount of CO₂ uptake needed for photosynthetic processes, resulting in the change of the ratio of internal and atmospheric CO₂ concentration (C_i/C_a ; Gebrekirstos et al., 2011).

Disturbed C_i/C_a changes the ratios of assimilated carbon isotope $\delta^{13}\text{C}$ (Farquhar et al., 1982), resulting in strong correlations between WUE and $\delta^{13}\text{C}$ (Farquhar et al., 1989; Farquhar & Richards, 1984). $\delta^{13}\text{C}$ ratios differ based on photosynthetic pathway processes involved. Values of $\delta^{13}\text{C}$ differ among tree genera and species; according to Wang and Feng (2012), conifer trees have lower $\delta^{13}\text{C}$ rates, while broadleaved tree species show greater sensitivities in the response of C_i to C_a . For C₃ plants, such as poplars, $\delta^{13}\text{C}$ values range from -20 to -32‰ (Ehleringer, 1993). Although correlations among WUE, $\delta^{13}\text{C}$, and carbon isotope discrimination (Δ) exist in plants, direct linkages with

productivity are still uncertain. For example, positive correlations between $\delta^{13}\text{C}$ and growth were found in *Acacia* species and *Balanties aegyptica* (L.) Delile (Gebrekirstos et al., 2011), *Populus catahayana* Rehder and *Populus przewalskii* Maxim. (Yin et al., 2005), and Euramerican (*Populus deltoides* Bart. Ex Marsh. $\times$ *P. nigra* L.) poplars (Voltas et al., 2006), while other studies showed the lack of or weak correlations between growth and Δ (Dillen et al., 2008; Monclus et al., 2005, 2006). Nevertheless, there was significant genotypic variation in WUE, $\delta^{13}\text{C}$, and Δ for poplars, indicating the potential for gains from future breeding and selection (Dillen et al., 2008; Monclus et al., 2005, 2006; Pointeau & Guy, 2014; Voltas et al., 2006).

Furthermore, successful deployment of poplars for bioenergy requires that genotypes be properly matched to site conditions (Zalesny et al., 2009). For example, poplars with high WUE have been observed to be less susceptible to water stress than genotypes with low WUE (Yin et al., 2005). Thus, poplars with high WUE can be identified and selected for the purpose of ensuring high biomass productivity on water-limited sites (Monclus et al., 2005). Chemical analysis of $\delta^{13}\text{C}$ in growth rings has proven useful in evaluating WUE in trees (Schulze et al., 2004). Specifically, genotypes with higher WUE exhibit greater stomatal closure, which in turn affects $\delta^{13}\text{C}$ as heavier isotopes are absorbed at a higher rate when stomata are closed (Leffler & Evans, 1999). Thus, differences in WUE can be identified by differences in $\delta^{13}\text{C}$ in the trees, as previous research has demonstrated for various species, including poplars and their hybrids (Dillen et al., 2008; Ripullone et al., 2004; Monclus et al., 2006). While these studies have examined WUE and correlations among stomatal characteristics, growth parameters, and Δ for poplars in Europe (Dillen et al., 2008; Monclus et al., 2005, 2006), North America (Leffler & Evans, 1999; Pointeau & Guy, 2014), and Asia (Yin et al., 2005), similar information on hybrid poplars in the United States is lacking. In particular, in the Midwestern United States, the significance of poplar improvement for fiber and biomass production was thoroughly investigated from the standpoint of productivity potential (Headlee, Zalesny, Donner, et al., 2013; Lazarus et al., 2015; Nelson et al., 2018; Riemenschneider et al., 2001; Zalesny, Stanturf, Gardiner, Bañuelos, et al., 2016), available land base (Zalesny et al., 2012), and climate change (Zalesny & Headlee, 2015), yet physiological parameters such as WUE, $\delta^{13}\text{C}$, and Δ were not studied.

To address this knowledge gap, our primary objectives were to evaluate differences in WUE among genotypes grown in the Midwestern United States, and to identify genotypes with high WUE for future deployment on water-limited sites in the region. We hypothesized such differences in WUE would exist, as differences in susceptibility to water stress for these genotypes have already

been observed in connection with specific gravity of their wood (Headlee, Zalesny, Hall, et al., 2013). Our specific objectives were to: (1) evaluate $\delta^{13}\text{C}$ and Δ within the growth rings of seven poplar genotypes representing three genomic groups harvested from three contrasting sites in the region, (2) compare these genomic groups to evaluate the extent to which different parent species contributed to WUE, and (3) correlate WUE, environmental conditions (e.g., climate and soils), and biomass productivity to further improve our understanding of how genotype-specific WUE interacts with site conditions to impact biomass yields. The results of this research are expected to be useful for selecting appropriate genotypes for water-limited sites and/or future climate scenarios with reduced water availability, thereby enhancing feedstock production for bioenergy applications.

2 | MATERIALS AND METHODS

2.1 | Site and clone selection

A regional network of hybrid poplar clone and yield trials was established in the Midwestern United States during 2000–2001, as previously described by Headlee, Zalesny, Hall, et al. (2013), Zalesny et al. (2009), and Riemenschneider et al. (2001). For the current study, three sites were selected from these Midwestern trials [Escanaba, Michigan, 45.7708°N, –87.1978°W; Waseca, Minnesota, 44.0621°N, –93.5438°W; Ames, Iowa, 42.0465°N, –93.6585°W], representing a latitudinal gradient of poplar biomass productivity supply sheds in the region. A planting density of 1075 trees ha^{–1} was used at all sites. Table 1 highlights site- and climate-related properties for each site, including information specific to tree establishment and stand harvest metrics (i.e., year planted, stocking, harvest age, height, diameter). In addition, monthly averages for climate and drought data were determined across each growing season (April–October) of the 7 years reported in the study (Waseca, Ames: 2002–2008; Escanaba: 2003–2009; when the trees were 3–9 years old) and then summed/averaged to obtain annual values for analyses. In particular, precipitation (P, mm) along with average (T_{avg} , °C), maximum (T_{max} , °C), and minimum (T_{min} , °C) air temperatures were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climate Data Center (<https://www.ncdc.noaa.gov/cdo-web/>) according to the nearest weather station (Escanaba: USC00201802; Waseca: USC00218692; Ames: USW00094989), and the difference between maximum and minimum temperatures (i.e., $T_{\text{diff}} = T_{\text{max}} - T_{\text{min}}$, °C) was calculated for each site. T_{max} and T_{avg} for individual growing seasons at all three sites

TABLE 1 Site- and climate-related properties at three sites in the Midwestern United States where water use efficiency of seven hybrid poplar clones was tested

	Escanaba, Michigan	Waseca, Minnesota	Ames, Iowa
Latitude, Longitude	45.7708°N, −87.1978°W	44.0621°N, −93.5438°W	42.0465°N, −93.6585°W
County	Delta	Waseca	Story
Year Planted	2001	2000	2000
Stocking (trees ha ^{−1})	1075	1075	1075
Tree age at harvest (year)	9	12	11
Height at harvest (m; mean ± 1 SE)	12.0 ± 0.2	16.2 ± 0.5	14.8 ± 0.5
Diameter at harvest (cm; mean ± 1 SE)	15.3 ± 0.7	21.5 ± 0.8	16.6 ± 0.6
Growing season precipitation (P; mm)	511 ± 11	698 ± 23	725 ± 23
Average temperature (T_{avg} ; °C)	13.5 ± 0.1	16.0 ± 0.1	17.1 ± 0.1
Maximum temperature (T_{max} ; °C)	20.0 ± 0.1	21.9 ± 0.1	23.5 ± 0.1
Minimum temperature (T_{min} ; °C)	6.9 ± 0.1	10.0 ± 0.1	10.7 ± 0.1
Maximum–minimum temperature (T_{diff} ; °C)	13.1 ± 0.0	11.9 ± 0.1	12.8 ± 0.1
Drought index (abnormally dry; D0; %)	53.5 ± 4.8	22.8 ± 2.9	23.2 ± 2.9
Drought index (moderate drought; D1; %)	22.1 ± 3.2	15.4 ± 2.2	10.8 ± 2.5
Drought index (severe drought; D2; %)	9.3 ± 2.0	5.2 ± 1.3	4.1 ± 1.6
Drought index (extreme drought; D3; %)	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

Note: Climate and drought data values are means ± 1 SE across each growing season (April–October) of the 7 years reported in the study (Waseca, Ames: 2002–2008; Escanaba: 2003–2009) when the trees were 3–9 years old.

Climate data source: National Oceanic and Atmospheric Administration (NOAA) National Climate Data Center (<https://www.ncdc.noaa.gov/cdo-web/>).

Drought index source: United States Drought Monitor (<https://droughtmonitor.unl.edu/>); percent of area within the county in each category (D0–D3).

are shown in Table 2. Drought index scores were obtained from the United States Drought Monitor (<https://droughtmonitor.unl.edu/>) according to percent area within each county belonging to the following drought index categories: D0 (abnormally dry), D1 (moderate drought), D2 (severe drought), D3 (extreme drought), and D4 (exceptional drought). Category D4 was negligible throughout the study period and, therefore, was not included in the final analyses.

Physical and chemical soil properties were obtained from the USDA Natural Resources Conservation Service (NRCS) Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov/>) and are listed in Table 3. Soils of the Escanaba site are Onaway-Ossineke fine sandy loams with 1%–6% slopes classified as fine-loamy, mixed, active, frigid Inceptic Hapludalfs. Waseca soils are Webster clay loam with 0%–2% slopes classified as fine-loamy, mixed, superactive, mesic Typic Endaquolls. Ames soils are Hanlon fine sandy loam with 0%–2% slopes classified as coarse-loamy, mixed, mesic Cumulic Hapludolls.

Seven hybrid poplar clones belonging to three genomic groups were tested (Table 4). The *Populus deltoides* clones (“C916000,” “C916400,” “C918001”) were selected given their varying responses to water stress (Headlee, Zalesny, Hall, et al., 2013), while clones with (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* parentage (“NC13563,”

“NC13624,” “NC13649”) were selected given their extensive genetic and phenotypic variability in previous studies (Riemenschneider et al., 2001; Zalesny et al., 2009). Clone “NM2” (*P. nigra* × *P. maximowiczii* A. Henry) was selected as the commercial control.

2.2 | Field sampling

In the field, diameter at breast height (i.e., DBH at 1.37 m) was determined to the nearest 0.1 cm and trees were marked at DBH on two to four dominant trees per clone (Table 4). A variable number of trees were sampled given mortality of some clones at the sites. Selection of harvest trees (i.e., experimental units) for the current study was also limited to those that had full competition on all sides, thus mitigating potential biases from different canopy sizes and time periods to canopy closure. The trees were felled, and total tree height was determined to the nearest 0.1 m. Total leafless aboveground fresh biomass per tree was weighed to the nearest 0.1 kg. A subsample of branches was collected and its fresh biomass weighed to the nearest 0.1 kg. Cross-sectional disks were harvested from each bole at DBH, and fresh mass to the nearest 0.1 g was determined for each disk. Branch subsamples and disks were transported to the analytical laboratory at

TABLE 2 Maximum ($T_{\max}$) and average (T_{avg}) temperature ($^{\circ}\text{C}$) from 3 to 9 years after planting for three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa] where water use efficiency of seven hybrid poplar clones was tested

Site (age, years)	$T_{\max}$	Site (age, years)	T_{avg}
Ames (6)	24.49	Ames (8)	18.24
Ames (8)	24.39	Ames (6)	17.82
Ames (7)	23.67	Ames (7)	17.35
Ames (4)	23.60	Waseca (6)	17.01
Ames (3)	23.23	Ames (3)	16.84
Ames (5)	22.94	Ames (4)	16.79
Waseca (8)	22.94	Waseca (8)	16.73
Waseca (6)	22.71	Ames (5)	16.56
Ames (9)	22.54	Ames (9)	16.40
Waseca (4)	22.04	Waseca (7)	16.21
Waseca (7)	22.01	Waseca (4)	15.66
Escanaba (5)	21.51	Waseca (5)	15.53
Waseca (5)	21.44	Waseca (3)	15.41
Waseca (9)	21.26	Waseca (9)	15.14
Waseca (3)	21.08	Escanaba (5)	14.73
Escanaba (7)	20.99	Escanaba (7)	14.33
Escanaba (6)	20.32	Escanaba (6)	13.77
Escanaba (8)	19.80	Escanaba (3)	13.15
Escanaba (3)	19.71	Escanaba (8)	13.14
Escanaba (4)	19.06	Escanaba (4)	12.74
Escanaba (9)	18.81	Escanaba (9)	12.40

the USDA Forest Service, Institute for Applied Ecosystem Studies in Rhinelander, WI, United States. The branches and disks were oven-dried at 55°C until constant mass, which was recorded with the same precision as fresh mass. After determining moisture content of the branch and bole wood, total leafless aboveground dry biomass per tree was determined. Tree height, diameter, and biomass were divided by harvest age to determine mean annual increment (MAI) of each trait (i.e., $\text{HEIGHT}_{\text{MAI}}$, DBH_{MAI} , and $\text{BIOMASS}_{\text{MAI}}$), which were used for data analyses.

2.3 | Ring width measurements and laboratory sampling

One cross-sectional area of each disk was sanded, wetted, and imaged for ongoing studies. The sanded disks were then cut in half along a plane extending through the pith, and the cut face of each half-disk was sanded. A wafer, free of bark and defects, was cut from each half-disk. From each wafer, annual ring width measurements

were determined to the nearest 0.1 cm using a Wild E5-86550 Stereomicroscope with $10\times$ power eyepieces and a $6\times$ power setting of the objective (Wild Company, Heerbrugg). Diameter increments (i.e., $\text{DIAMETER}_{\text{INC}}$) were standardized by dividing each increment by the overall average increment for the corresponding tree age, in order to avoid confounding age-related trends in diameter increment (e.g., slower growth in later years) with climate-related trends (Briffa & Melvin, 2011). These $\text{DIAMETER}_{\text{INC}}$ values were used in all analyses. After ring width was determined, wood samples ranging from 1 to 2 mg dry mass were extracted from each annual ring for carbon isotope ($\delta^{13}\text{C}$) analyses.

2.4 | Carbon isotope ($\Delta^{13}\text{C}$) analyses and discrimination (Δ) calculations

Stable carbon isotope ($\delta^{13}\text{C}$) values were measured using a FinniganTM MAT DELTA^{plus}XL mass spectrometer (Thermo Fisher Scientific) in continuous flow mode connected to a Costech 4010 Elemental Combustion System Elemental Analyzer (Costech Analytical Technologies, Inc.). Reference standards [caffeine (IAEA-600), cellulose (IAEA-CH-3), and acetanilide (laboratory standard)] were run every 10 samples and used for isotopic corrections and to assign the data to the appropriate isotopic scale. Regressions were used for data corrections, with combined uncertainty (analytical uncertainty and average correction factor) for $\delta^{13}\text{C}$ of $\pm 0.05\text{--}0.09\text{‰}$ (VPDB).

Carbon isotope discrimination (Δ), the factor of isotope fractionation by the photosynthetic process in leaves relative to the source carbon, was calculated according to Farquhar et al. (1989):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p},$$

where δ_a equals the isotopic composition of air that is assumed to be -8‰ , and δ_p equals the isotopic composition ($\delta^{13}\text{C}$) of the wood sample analyzed in the current study. These Δ values were used in all analyses.

2.5 | Experimental design and data analysis

$\text{HEIGHT}_{\text{MAI}}$, DBH_{MAI} , and $\text{BIOMASS}_{\text{MAI}}$ data were subjected to analyses of variance (ANOVA) and analyses of means (ANOM) using SAS[®] (PROC GLM; PROC ANOM; SAS INSTITUTE, INC.) assuming a two-way factorial design including three sites, seven clones, and their interactions. Multiple comparison analyses with Tukey's

TABLE 3 Soil properties at three sites in the Midwestern United States where water use efficiency of seven hybrid poplar clones was tested

	Escanaba, Michigan	Waseca, Minnesota	Ames, Iowa
Soil series	Onaway-Ossineke	Webster	Hanlon
Drainage class	Well drained	Poorly drained	Moderately well drained
Slope (%)	1–6	0–2	0–2
K Factor (erodibility, 0.02–0.69 scale)	0.32	0.28	0.32
Texture	Fine sandy loam	Clay loam	Fine sandy loam
Sand (%)	55.3	36.6	65.3
Silt (%)	32.1	37.3	22.3
Clay (%)	12.6	26.1	12.4
Organic matter (%)	0.5	2.1	1.7
Soil organic carbon (%)	0.27	1.19	0.96
pH	7.5	7.5	6.6
Bulk density (g cm ⁻³)	1.73	1.44	1.55
Cation exchange capacity (meq 100 g ⁻¹)	6.7	20.5	11.6
Saturated hydraulic conductivity (Ksat; $\mu\text{m s}^{-1}$)	8.3	5.8	28.0
Frost free days (#)	120	155	183
Depth to water table (cm)	>200	30	122
Available water capacity (cm cm ⁻¹)	0.09	0.18	0.15
Available water storage (cm)	19.03	35.64	23.76
Water content (15 Bar; %)	9.0	16.6	10.9
Water content (1/3 Bar; %)	18.0	29.8	20.3

Note: Soil data source: USDA Natural Resources Conservation Service (NRCS) Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov/>).

United States

Genomic group ^a	Clone	Escanaba, Michigan	Waseca, Minnesota	Ames, Iowa
<i>P. deltoides</i>	C916000	4	3	3
	C916400	3	4	4
	C918001	4	4	3
<i>(P. trichocarpa × P. deltoides) × P. deltoides</i>	NC13563	4	3	3
	NC13624	4	4	4
	NC13649	4	2	3
<i>P. nigra × P. maximowiczii</i>	NM2	4	3	2
	Total	27	23	22

^aAuthorities for the aforementioned species are: *P. deltoides* Bartr. Ex Marsh; *P. trichocarpa* Torr. & Gray; *P. nigra* L.; *P. maximowiczii* A. Henry.

TABLE 4 Number of trees tested for each combination of site and clone in a study testing the water use efficiency of poplars at three sites in the Midwestern

adjustment were used to identify significant differences among least-squares means for main effects and interactions at $p < .05$.

DIAMETER_{INC} and Δ data were subjected to analyses of variance (ANOVA) and analyses of means (ANOM) using SAS[®] (PROC MIXED; PROC ANOM; SAS INSTITUTE, INC.) assuming a three-way, repeated measures factorial design with fixed effects including three sites, seven clones, seven ages, and their interactions. The

seven ages (representing tree growth from years 3 to 9 of plantation development) were analyzed as the repeated measure. To account for pseudo-replication associated with taking multiple measurements over time from the same trees, six different covariance structures (i.e., vc, cs, ar(1), toep, ante(1), un) were tested in PROC MIXED to determine which one provided the best model fit based on the lowest BIC scores. The ar(1) and cs covariance structures exhibited the lowest BIC scores and were used

for $\text{DIAMETER}_{\text{INC}}$ and Δ , respectively. Using these covariance structures, ANOVA were conducted in PROC MIXED for both traits, and multiple comparisons analyses were conducted as with $\text{HEIGHT}_{\text{MAI}}$, DBH_{MAI} , and $\text{BIOMASS}_{\text{MAI}}$ data.

In addition, the relationships between climate variables (Table 1) and the response variables of $\text{DIAMETER}_{\text{INC}}$ and Δ , respectively, were subjected to regression analyses using PROC NLIN in SAS[®]. Initially, a linear model with a forward selection process was used to identify which of the climate variables had statistically significant ($p < .05$) relationships with $\text{DIAMETER}_{\text{INC}}$ (which was averaged across trees within each clone for each site and year to match the resolution of the climate data; $n = 147$). The forward selection process for $\text{DIAMETER}_{\text{INC}}$ resulted in a single significant predictor variable, T_{max} ($p = .0193$). The nonlinear nature of tree growth with respect to temperature, and the concept of an optimal temperature for growth, is prominent in process-based models (e.g., Sands & Landsberg, 2002) and was supported in this study by comparisons of the linear model to the following nonlinear model which had a stronger fit (i.e., lower mean-squared error):

$$\text{DIAMETER}_{\text{INC}} = \frac{a_{\text{Dmax}}}{1 + a_{\text{Tsens}} (T_{\text{max}} - a_{\text{Topt}})^2}.$$

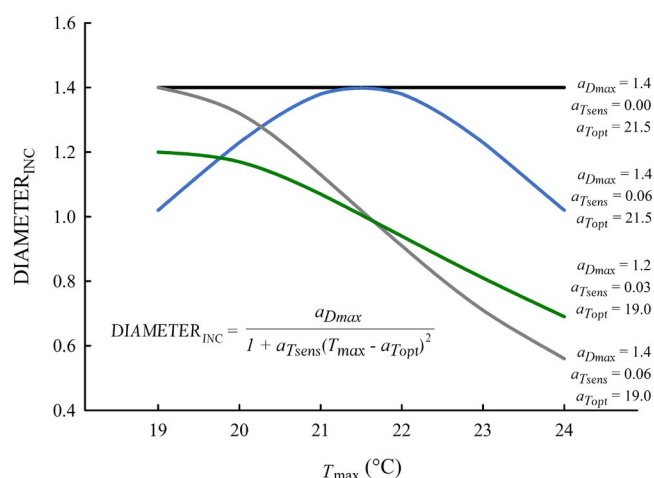


FIGURE 1 Nonlinear model for proportional difference in the response of the annual diameter increment relative to the overall mean ($\text{DIAMETER}_{\text{INC}}$) to the maximum temperature (T_{max}), using the approximate range of T_{max} from the current study. The response curve is flat when a_{Tsens} equals zero, while values greater than zero produce a curve with a peak having (x, y) coordinates equal to (a_{Topt} , a_{Dmax}). a_{Dmax} , predicted maximum value of $\text{DIAMETER}_{\text{INC}}$; a_{Topt} , temperature that maximizes $\text{DIAMETER}_{\text{INC}}$; a_{Tsens} , sensitivity of $\text{DIAMETER}_{\text{INC}}$ to deviations from a_{Topt}

In the above equation, T_{max} represents the observed maximum temperature (as previously described), and the remaining parameters are estimated based on best fit. The parameter a_{Dmax} is the predicted maximum value of $\text{DIAMETER}_{\text{INC}}$, parameter a_{Topt} is the temperature that maximizes $\text{DIAMETER}_{\text{INC}}$, and parameter a_{Tsens} represents the sensitivity of $\text{DIAMETER}_{\text{INC}}$ to deviations from a_{Topt} . As illustrated in Figure 1, the response curve is flat when a_{Tsens} equals zero, while values greater than zero produce a curve with a peak having (x, y) coordinates equal to (a_{Topt} , a_{Dmax}). Starting values for estimating a_{Dmax} and a_{Topt} were set equal to the mean of the observed maximum clonal values of $\text{DIAMETER}_{\text{INC}}$ and the temperatures at which the increments were observed, respectively, while the starting value for a_{Tsens} was zero (corresponding to a null hypothesis of no significant response to T_{max}). The best-fit parameter estimates (and associated 95% confidence intervals) for the global model were recorded, and then, clone-specific parameters were also generated to facilitate comparisons to the global model.

For Δ , the initial linear model with forward selection identified a single significant predictor variable, T_{avg} ($p < .0001$). The linear model was compared to a nonlinear model of the form shown above, which indicated that the linear model below fits the data better (i.e., lower mean-squared error):

$$\Delta = b_{\text{int}} + b_{\text{slope}} T_{\text{avg}}.$$

In the above equation, T_{avg} represents the observed average temperature (as previously described), and the remaining parameters are estimated based on best fit. The parameters b_{int} and b_{slope} are the intercept and slope, respectively, for the linear relationship between T_{avg} and Δ . Again, the best-fit parameter estimates (and associated 95% confidence intervals) for the global model were recorded, and then clone-specific parameters were generated to facilitate comparisons to the global model.

3 | RESULTS

3.1 | Height, diameter, and biomass at harvest

The site $\times$ clone interaction was significant for $\text{HEIGHT}_{\text{MAI}}$ ($p = .0061$; Table 5). $\text{HEIGHT}_{\text{MAI}}$ ranged from 0.94 ± 0.15 (Waseca, “NC13624”) to 1.62 ± 0.15 (Ames, “C916400”) m year^{-1} , with an overall mean of $1.34 \pm 0.02 \text{ m year}^{-1}$ (Figure 2a). Differences in $\text{HEIGHT}_{\text{MAI}}$ among sites were less than 1%, leading to a non-significant site main effect ($p = .7928$). In contrast, trees of “C916400” were 43% taller than those of “NC13624” ($p < .0001$), which was the least

TABLE 5 Probability values from analyses of variance for mean annual increment (MAI) of tree height (HEIGHT_{MAI}), diameter at breast height (DBH_{MAI}), and aboveground leafless dry biomass (BIOMASS_{MAI}), as well as proportional difference in annual diameter increment relative to the overall mean (DIAMETER_{INC}) and carbon isotope discrimination (DISCRIMINATION_Δ) for seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]

	HEIGHT _{MAI}	DBH _{MAI}	BIOMASS _{MAI}	DIAMETER _{INC}	DISCRIMINATION _Δ
Site	0.7928	0.0002	0.0006	0.0004	<0.0001
Clone	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site × Clone	0.0061	0.0054	0.0137	0.0209	0.0899
Age	na ^a	na	na	0.9989	<0.0001
Site × Age	na	na	na	0.0020	<0.0001
Clone × Age	na	na	na	0.0026	0.0009
Site × Clone × Age	na	na	na	0.0743	0.0001

Note: Annual tree ages from 3 to 9 years were included in the analyses for DIAMETER_{INC} and DISCRIMINATION_Δ. Significant values are listed in bold.

^aNot applicable; age effects were not included in the ANOVA model for HEIGHT_{MAI}, DBH_{MAI}, nor BIOMASS_{MAI}.

productive clone across sites. In general, F₁ hybrid D × D clones exhibited 14% greater HEIGHT_{MAI} than their BC₁ backcross hybrid TD × D counterparts and 3% taller trees than “NM2.” Despite a lack of significant differences among sites within clones, site × clone interactions for HEIGHT_{MAI} exhibited trends based on genomic groups: (1) HEIGHT_{MAI} consistently increased for D × D clones with decreasing latitudes, (2) HEIGHT_{MAI} was highly variable for TD × D clones, with significant changes in both rank and magnitude for particular site × clone interactions, and (3) HEIGHT_{MAI} was greater in northern latitudes for “NM2” (Figure 2a).

The site × clone interaction was significant for DBH_{MAI} ($p = .0054$; Table 5). DBH_{MAI} ranged from 1.2 ± 0.1 (Escanaba, “C918001”) to 2.4 ± 0.2 (Escanaba, “NM2”) cm year⁻¹, with an overall mean of 1.7 ± 0.0 cm year⁻¹ (Figure 2b). Trees growing at Waseca had the greatest DBH_{MAI} ($p = .0002$), which was 5% and 19% greater than Escanaba and Ames, respectively. Trees of “C916000” had 47% more DBH_{MAI} than those of “C918001” ($p < .0001$), which was the least productive clone across sites. “NM2” had the largest DBH_{MAI}, which was 25% and 30% greater than the D × D and TD × D genotypes, respectively. Genomic group trends for site differences within clones were similar to HEIGHT_{MAI} for the D × D clones, yet DBH_{MAI} rankings for sites within the TD × D clones were much more stable than for HEIGHT_{MAI} (i.e., with the exception of Waseca being the best for “NC13563,” DBH_{MAI} decreased with lower latitudes). For “NM2,” DBH_{MAI} was 49% significantly greater at Escanaba than Ames (Figure 2b).

The site × clone interaction was significant for BIOMASS_{MAI} ($p = .0137$; Table 5). BIOMASS_{MAI} ranged from 3.13 ± 1.55 (Waseca, “NC13624”) to 14.01 ± 3.50 (Waseca, “NM2”) Mg ha⁻¹ year⁻¹, with an overall mean of

5.96 ± 0.46 Mg ha⁻¹ year⁻¹ (Figure 2c). Trees growing at Waseca had the greatest BIOMASS_{MAI} ($p = .0006$), which was 31% and 55% greater than Escanaba and Ames, respectively. Trees of “NM2” had 159% more BIOMASS_{MAI} than those of “NC13624” ($p < .0001$), which was the least productive clone across sites. Site × clone interactions for BIOMASS_{MAI} exhibited trends based on genomic groups that were different than those for HEIGHT_{MAI} and DBH_{MAI} for the D × D genotypes yet similar for the other groups: (1) BIOMASS_{MAI} was consistently greatest at Waseca for D × D clones, with BIOMASS_{MAI} being 98% significantly greater at Waseca than Escanaba for “C916400”; (2) BIOMASS_{MAI} was highly variable for TD × D clones, with significant changes in both rank and magnitude for particular site × clone interactions; and (3) BIOMASS_{MAI} was greater in northern latitudes for “NM2” (Figure 2c).

3.2 | Diameter increment and carbon isotope discrimination throughout plantation development

While the site and clone main effects were significant for DIAMETER_{INC} ($p = .0004$, $p < .0001$, respectively), their interaction was most important for this trait ($p = .0209$; Table 5). DIAMETER_{INC} which was governed by site differences for “C916000” ($p = .0027$) and “NM2” ($p = .0014$; Table 6). For “C916000,” DIAMETER_{INC} was greatest for Waseca which was 42% and 51% significantly greater than Escanaba and Ames, respectively, which were not different from one another (Figure 3a). For “NM2,” Waseca and Escanaba exhibited the greatest DIAMETER_{INC} that was not different from one another but was 61% and 69% significantly greater than Ames, respectively (Figure 3b). DIAMETER_{INC} was similar at Waseca and Escanaba,

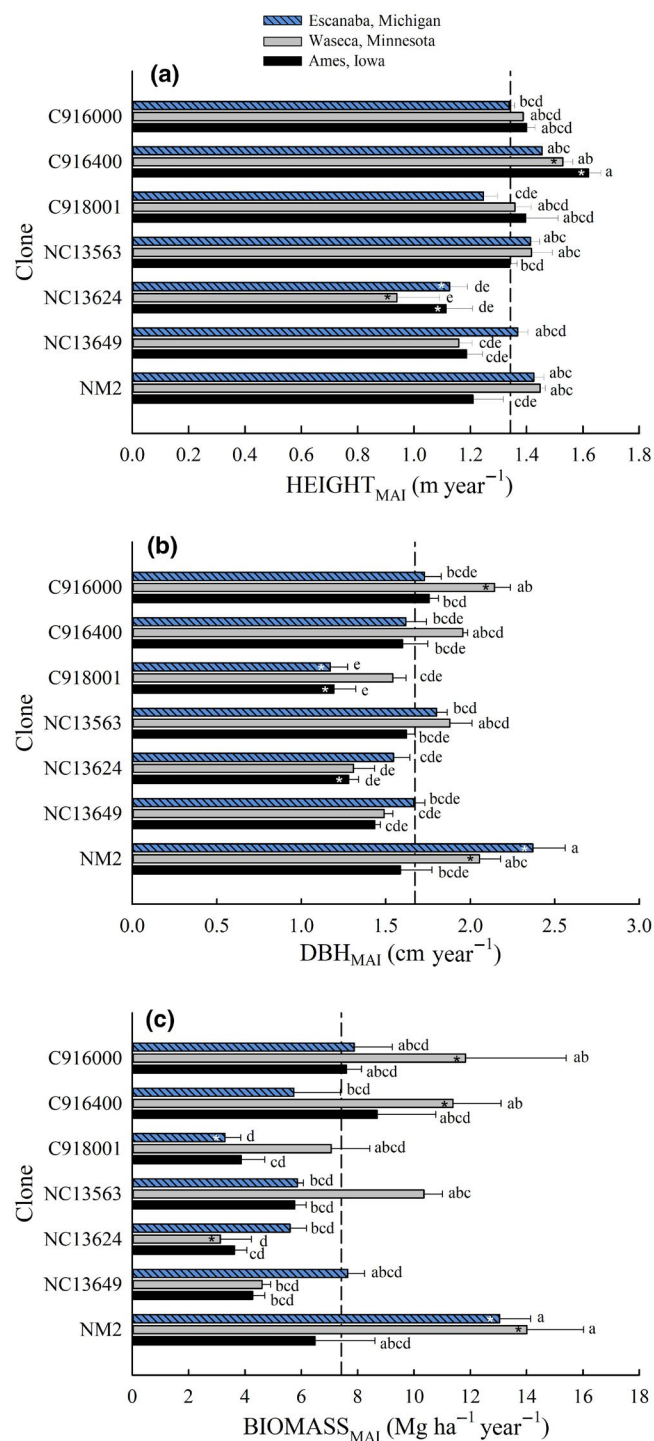


FIGURE 2 Mean annual increment (MAI) of tree height (a; m year⁻¹), diameter at breast height (DBH) (b; cm year⁻¹), and aboveground leafless dry biomass (c; Mg ha⁻¹ year⁻¹) of seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] harvested at 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]. The dashed line is the overall mean; means differing from the overall mean at $p < .05$ are indicated with asterisks. Bars with the same letters are not different at $p < .05$

which were 29% and 17% significantly larger than Ames (Figure 4a). For clones, DIAMETER_{INC} ranged from 0.78 ± 0.07 (“NC13624”) to 1.19 ± 0.06 (“C916000”), with an overall mean of 0.99 ± 0.05 (Figure 4b). Within genomic groups, “C916000” had 41% significantly greater DIAMETER_{INC} than “C918001” for the D × D clones, while “NC13563” was 41% higher than “NC13624” for the TD × D genotypes. “NM2” had 11% and 16% greater DIAMETER_{INC} than the D × D and TD × D genomic groups, respectively (Figure 4b).

The site and clone main effects were significant for Δ ($p < .0001$, $p < .0001$, respectively; Table 5). All three sites differed for Δ , with Ames being 2% and 4% greater than Waseca and Escanaba, respectively, and Waseca being 2% greater than Escanaba (Figure 4c). For clones, Δ ranged from 18.6 ± 0.1 (“NC13649”) to 19.9 ± 0.1 (“C916400”)‰, with an overall mean of 19.3 ± 0.1 ‰ (Figure 4d). Within genomic groups, “C918001” had 3% and 4% significantly less Δ than “C916000” and “C916400” for the D × D clones, respectively, while “NC13563” was 4% higher than “NC13649” for the TD × D genotypes. The relative magnitude of differences among genotypes was greater than for DIAMETER_{INC}, with D × D clones exhibiting 3% and 2% greater Δ than the TD × D genomic group and “NM2,” respectively (Figure 4d).

The site × age interaction was significant for DIAMETER_{INC} ($p = .0020$; Table 5), which was governed by age differences for Escanaba ($p = .0095$) and Waseca ($p = .0327$; Table 6). At Escanaba, DIAMETER_{INC} was consistent across years, with one exception. Five-year-old trees had 27%, 22%, and 33% significantly less DIAMETER_{INC} than their 3-, 6-, and 7-year-old counterparts, respectively (Figure 5a). In contrast, 5-year-old trees at Waseca exhibited the largest DIAMETER_{INC}, which was significantly greater than all other years. At age 5, DIAMETER_{INC} was 15% larger than the overall mean across years (Figure 5b).

The clone × age interaction was significant for DIAMETER_{INC} ($p = .0026$; Table 5), which was governed by age differences for “C916000” ($p = .0008$) and “NC13649” ($p = .0398$; Table 6). For “C916000,” DIAMETER_{INC} increased over time, with years 7–9 exhibiting 22% greater DIAMETER_{INC} than at 6 years and 52% larger trees than years 3–5. Only years 8 and 9 were greater than the overall mean, by 25% and 25%, respectively (Figure 5c). DIAMETER_{INC} was 22% smaller for “NC13649” than “C916000,” and values reached a maximum at year 5 followed by a decrease through year 9. None of the years exhibited DIAMETER_{INC} that was significantly different than the overall mean.

The site × clone × age interaction was significant for Δ ($p = .0001$). Sixteen, three-way combinations were different than the overall mean ($\Delta = 19.3$ ‰), with six being 8% greater and 10 being 9% less (Figure 6). Significantly

TABLE 6 Probability values from analyses of variance for tests of effect slices of two-way interactions between site and clone (slicing by clone), site and age (slicing by site), and clone and age (slicing by clone) for proportional difference in annual diameter increment relative to the overall mean ($\text{DIAMETER}_{\text{INC}}$) for seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. $\times$ *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. $\times$ *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray $\times$ *P. deltoides*) $\times$ *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]

	Site $\times$ Clone		Site $\times$ Age		Clone $\times$ Age
C916000	0.0027	Escanaba	0.0095	C916000	0.0008
C916400	0.0743	Waseca	0.0327	C916400	0.8080
C918001	0.0668	Ames	0.9845	C918001	0.6180
NC13563	0.5214			NC13563	0.0667
NC13624	0.0733			NC13624	0.4652
NC13649	0.6082			NC13649	0.0398
NM2	0.0014			NM2	0.4831

Note: Annual tree ages from 3 to 9 years were included in the analyses. Significant values are listed in bold.

greater interactions ranged from 20.5 (Ames, “C916400,” 8 years) to 21.4 (Ames, “NM2,” 9 years)%, while those lower than mean ranged from 17.2 (Escanaba, “C918001,” 3 years) to 18.1 (Escanaba, “C918001,” 4 years)%. There were two general trends: (1) With the exception of “C916400” at year 7 in Escanaba, all three-way combinations greater than the mean were from later in the rotation (i.e., 7–9 years) and southern latitudes (i.e., Waseca and Ames); and (2) combinations less than the mean were from years 3 and 4 at Escanaba (Figure 6).

3.3 | Relationships between $\text{DIAMETER}_{\text{INC}}$ and Δ with climate variables

For the regression analysis of $\text{DIAMETER}_{\text{INC}}$, the global model (pooled across clones) produced parameter estimates of $a_{\text{Dmax}} = 1.069$, $a_{\text{Topt}} = 20.77$, and $a_{\text{Tsens}} = 0.0225$ [which, based on its 95% confidence interval (0.0020, 0.0431), differed significantly from zero]. In other words, the mean predicted maximum $\text{DIAMETER}_{\text{INC}}$ was: (i) 6.9% above the average $\text{DIAMETER}_{\text{INC}}$, (ii) estimated to be optimized with a T_{max} of 20.77°C, and (iii) significantly sensitive to deviations away from this optimum value of T_{max} . Clonal model parameter estimates are shown in Table 7, and values falling outside the 95% confidence intervals for the global model parameters are considered significantly different (denoted with an asterisk). With regard to a_{Dmax} , three clones were found to have predicted maximum $\text{DIAMETER}_{\text{INC}}$ significantly higher than the global model (“C916000,” “NC13563,” “NM2”), while two clones were significantly lower (“C918001,” “NC13624”). For a_{Topt} , one clone was found to have a higher optimum value of T_{max} than the global model (“C918001”), while three clones were significantly lower (“NC13563,” “NC13624,” “NM2”). For “NC13563,” it should be noted

that its estimated optimum temperature ($a_{\text{Topt}} = 15.32^\circ\text{C}$) lies below the observed range of values in the study (i.e., 18.81–24.49°C), which means its predicted maximum $\text{DIAMETER}_{\text{INC}}$ across the observed range of T_{max} would be lower (i.e., 0.984–1.221) than its parameter estimate ($a_{\text{Dmax}} = 1.273$) would suggest. Regarding a_{Tsens} , two clones were found to have significantly higher sensitivity to T_{max} than the global model (“C916000,” “C916400”), while no clones were significantly less sensitive than the global model (e.g., “NC13563” had the lowest estimated a_{Tsens} value). The response curves for the seven clones illustrate the combined effects of the various parameter differences (Figure 7). “NM2” had the highest $\text{DIAMETER}_{\text{INC}}$ when T_{max} was below approximately 21°C, while “C916000” had the highest $\text{DIAMETER}_{\text{INC}}$ above that temperature. “NC13563” showed relatively high and stable performance across the range of observed T_{max} values. “C918001” had the lowest $\text{DIAMETER}_{\text{INC}}$ when T_{max} was below approximately 21.5°C, while “NC13624” had the lowest $\text{DIAMETER}_{\text{INC}}$ above that temperature.

For the regression analysis of Δ , the global model produced parameter estimates of $b_{\text{int}} = 16.40$ and $b_{\text{slope}} = 0.1857$ [which, based on its 95% confidence interval (0.1106, 0.2608), differed significantly from zero]. Clonal model parameter estimates are shown in Table 8, and values falling outside the 95% confidence intervals for the global model parameters are considered significantly different (denoted with an asterisk). Similar to the global model, all clones had positive slopes, so in general Δ increased as T_{avg} increased. Two clones (“C918001,” “NM2”) had b_{int} values that were significantly lower and b_{slope} values that were significantly higher than the global model, which indicates Δ for these clones was more sensitive than average to changes in T_{avg} . Conversely, two other clones (“NC13624,” “NC13649”) had b_{int} values that were significantly higher and b_{slope} values that were significantly lower than the global model. In fact,

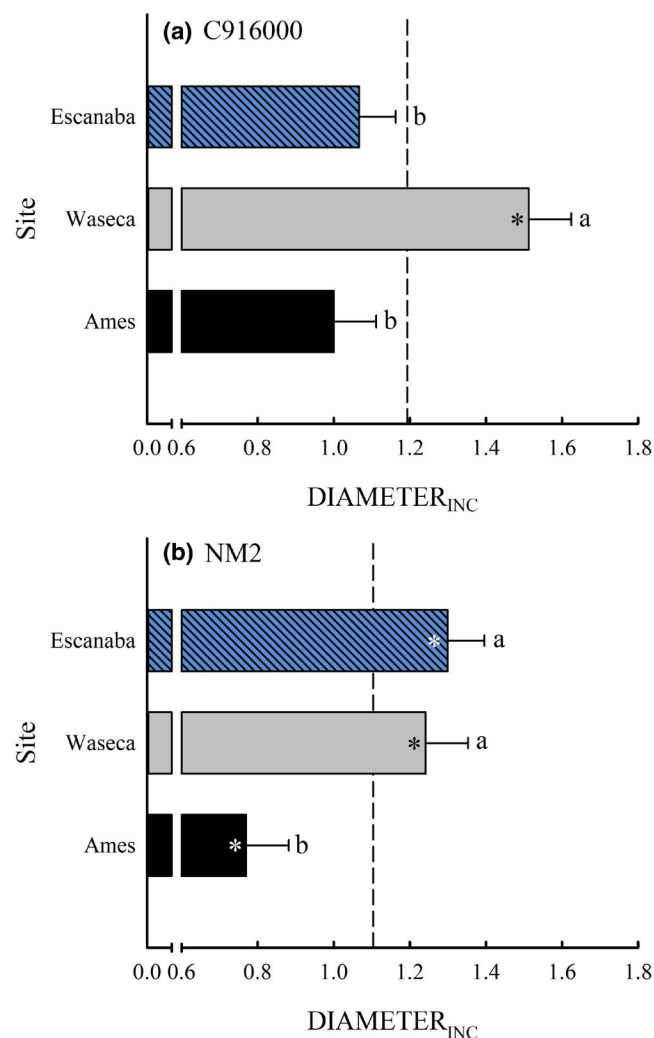


FIGURE 3 Proportional difference in annual diameter increment relative to the overall mean (DIAMETER_{INC}) for hybrid poplar clones *Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000” (a) and *P. nigra* L. × *P. maximowiczii* A. Henry “NM2” (b) grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]. The dashed line is the overall mean; means differing from the overall mean at $p < .05$ are indicated with asterisks. Bars with the same letters are not different at $p < .05$

these two clones had b_{slope} which did not differ significantly from zero, which indicates Δ (and by extension transpiration) was not significantly sensitive to T_{avg} for these particular clones. In general, Δ response to T_{avg} for the observed range of T_{avg} showed increased Δ with increased T_{avg} (Figure 8). However, the increase differed among the clones. “C918001,” “NM2,” “NC13624,” and “NC13649” had lower Δ values compared to global Δ at lower temperatures that was opposite of “C916400” and “C916000,” which had above average values across the entire observed range of T_{avg} . Δ values of “NC13624” and “NC13649” remained below global Δ values throughout the entire observed range of T_{avg} .

4 | DISCUSSION

In the current study, biomass production across sites and clones was highly variable, ranging from 3.13 to 14.01 Mg ha⁻¹ year⁻¹, which corroborated reported values from poplar testing in the Midwestern United States (Hansen, 1992; Netzer et al., 2002; Riemenschneider et al., 2001). Variability in biomass depends on the combination of broad genetic variation among clones, changing environment, and genotypic responses to conditions at contrasting field sites (Zalesny et al., 2009). Significant genotype × environment interactions were recorded for all tested biometrical parameters, especially for the TD × D hybrids (i.e., “NC13563,” “NC13624,” “NC13649”) that expressed specialist growth patterns. Significantly higher sensitivity of “C916000” and “C916400” to T_{max} compared to the global model indicated possible specialist behavior of clones whose performance depended highly on local temperatures. Although “C918001” and “NC13624” had the lowest DIAMETER_{INC} at opposite sides of T_{max} (21.5°C), both genotypes performed moderately well near their a_{Topt} values, suggesting that under certain conditions, they may be viable selections for deployment. Similar results were also recorded in studies testing productivity and performance of poplar clones across the Midwestern United States (Headlee, Zalesny, Donner, et al., 2013; Headlee, Zalesny, Hall, et al., 2013; Riemenschneider et al., 2001). In contrast, Zalesny et al. (2009) characterized “NM2” as a generalist with stable performance across Minnesota, Wisconsin, and Iowa, which was not the case in the current study. These differences were likely due to the fact that trees in the current study were at or near rotation age, while Zalesny et al. (2009) reported establishment-age results. Clone “NM2” often exhibits increasingly detrimental impacts from *Septoria musiva* stem canker (*Mycosphaerella populorum* Peck) as the trees age.

Site conditions substantially influenced poplar performance in the current study. Despite having the highest average annual precipitation, Ames had lowest productivity of all clones. These results can be explained by interpreting predominant soil properties and other climate factors. For example, Ames had greater soil proportions of sand coupled with higher temperatures and a lower water table than Waseca. In addition, total precipitation during the study period (i.e., sum of precipitation from tree ages 3–7 years) was lower for Ames than Waseca (results not presented). Differences in productivity between Ames and Escanaba were also driven by sand content and temperature, as well as higher precipitation and a lower water table in Escanaba. In particular, lower temperatures at higher latitudes likely contributed to lower vapor pressure deficit (VPD) and evapotranspiration (ET; Voelker et al., 2014). Although expected (Maisenhelder,

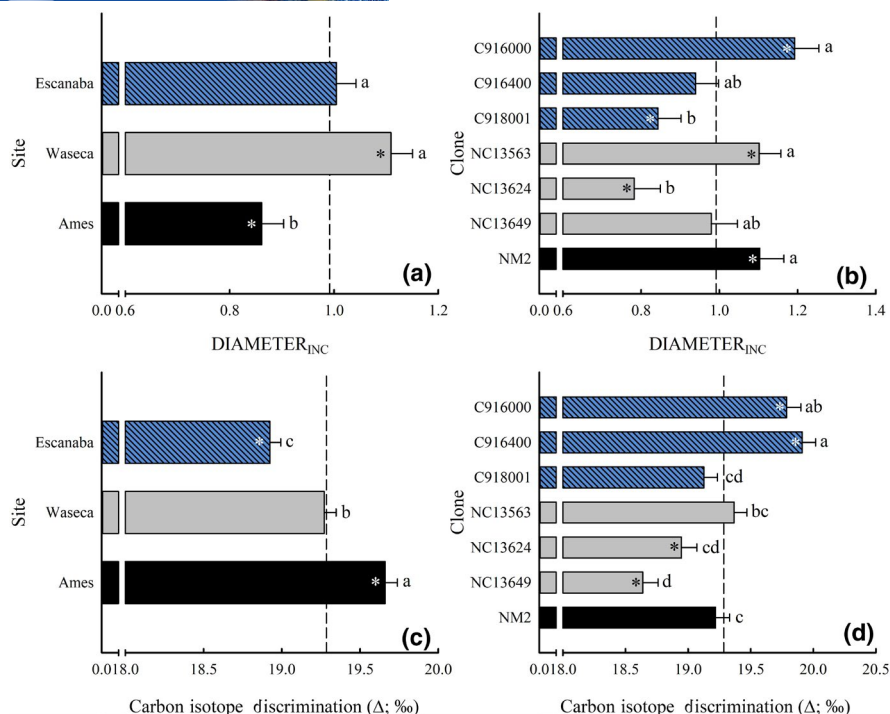


FIGURE 4 Proportional difference in annual diameter increment relative to the overall mean (DIAMETER_{INC}) (a; b) and carbon isotope discrimination (Δ) (c; d; ‰) for the site and clone main effects of seven hybrid poplar genotypes [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]. The dashed line is the overall mean; means differing from the overall mean at $p < .05$ are indicated with asterisks. Bars with the same letters are not different at $p < .05$

1970), latitudinal gradient effects were not fully expressed in our study from the standpoint of overall performance. Nevertheless, genomic grouping was evident for the *Aigeiros* × *Tacamahaca* hybrid “NM2” that decreased in productivity when grown further southward. These results can be explained by late spring bud break and early autumn bud set at lower latitudes (Zalesny et al., 2009), corroborating results from previous studies showing that temperature played significant roles in productivity and physiological activity (Dillaway & Kruger, 2010; Headlee, Zalesny, Donner, et al., 2013; Hozain et al., 2009). Regression analysis in our study confirmed clonal specialist performance where optimum temperature for DIAMETER_{INC} was significantly above average for “C918001” and significantly below average for “NM2,” “NC13563,” and “NC13624,” with “C916000,” “C916400,” and “NC13649” not differing from the global model parameters (Table 7). The resulting grouping of clones for optimum temperature (low: “NM2,” “NC13563,” “NC13624”; medium: “C916000,” “C916400,” “NC13649”; high: “C918001”) may be useful for deciding where an individual clone should be considered for use at a given site in the region (e.g., defining potential planting zones). “C916000” and “C916400” were especially sensitive to temperature (Table 7), indicating extra care should be taken to deploy them in correct environments. The

response curves for DIAMETER_{INC} (Figure 7) indicated “NM2” as the most suitable clone for the low end of the observed temperature range (<21°C) and “C916000” as suitable for the high end of observed temperatures (>21°C), while “NC13563” performed relatively consistently across the entire observed range. This type of information is useful for clonal selection within planting zones, as defined above. In addition to soil and climate, differences among clones likely resulted from pest and disease susceptibility (Coyle et al., 2008; Maisenhelder, 1970; Riemenschneider et al., 1992, 2001), although such testing was beyond the scope of the current study.

Climatic and site conditions also affected carbon isotope discrimination (Δ) values in growth rings of our tested clones. Voelker et al. (2014) emphasized that local environments, individual tree attributes, and fractionation processes affect $\delta^{13}\text{C}$ while being neglected by the original Farquhar model (Farquhar et al., 1982). Climatic conditions affect Δ, such as in Leffler and Evans (1999), who recorded genetic variability in carbon isotope accumulation in Frémont’s cottonwood (*P. fremontii* S. Watson) tree rings that followed patterns of changes under climatic conditions. Significant latitudinal effects were observed in the current study with increasing Δ in a southward direction. Higher values at lower latitudes agree with the

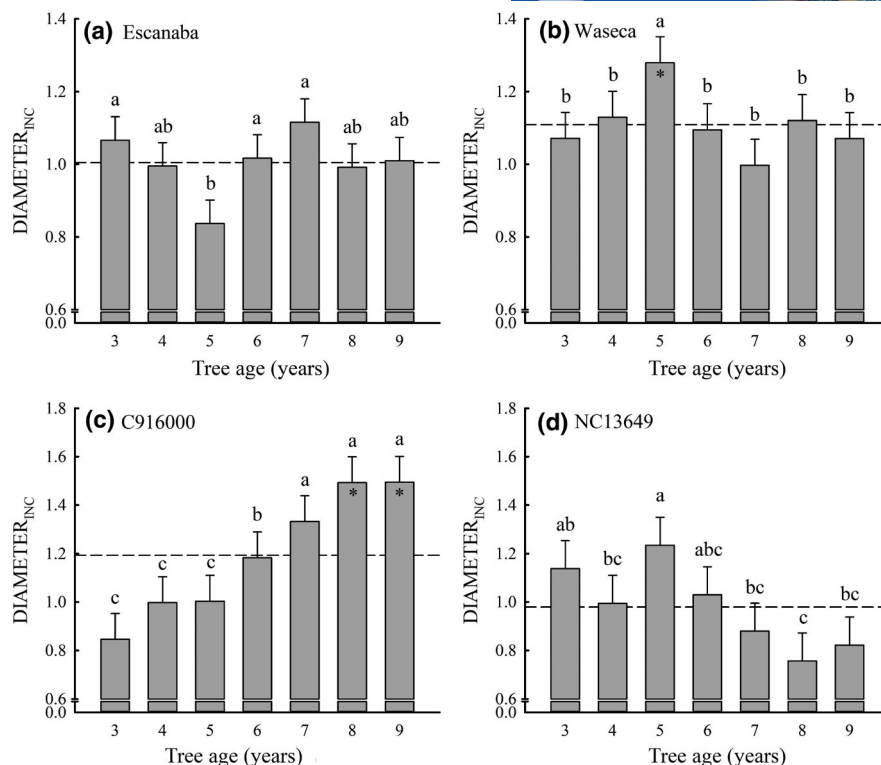


FIGURE 5 Proportional difference in annual diameter increment relative to the overall mean (DIAMETER_{INC}) for 3–9 years after planting: (1) across seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown at Escanaba, Michigan, United States (a) and Waseca, Minnesota, United States (b); and, (2) across three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa] for clones “C916000” (c) and “NC13649” (d). The dashed line is the overall mean; means differing from the overall mean at $p < .05$ are indicated with asterisks. Bars with the same letters are not different at $p < .05$

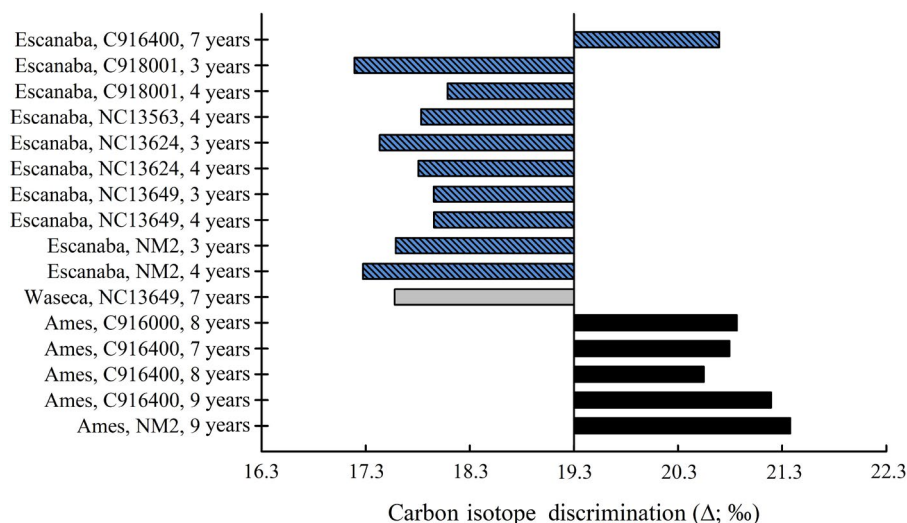


FIGURE 6 Carbon isotope discrimination (Δ) (‰) for three-way interactions that are significantly different than the overall mean (Δ = 19.3‰) at $p < .05$ for seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]

overall regression trend that for most clones, Δ increases with T_{avg} due to conditions where higher temperatures are associated with higher transpiration rates. Such results are

in accordance with Viger et al. (2016) who reported that northern populations of European black poplar (*P. nigra* L.) from France and the Netherlands had lower carbon

TABLE 7 Parameter estimates for the global and clonal models of proportional difference in the response of the annual diameter increment relative to the overall mean ($\text{DIAMETER}_{\text{INC}}$) for seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. $\times$ *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. $\times$ *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray $\times$ *P. deltoides*) $\times$ *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]

Model	a_{Dmax}	a_{Tsens}	a_{Topt}
Global	1.069	0.0225	20.77
95% CI	1.002–1.137	0.0020–0.0431	19.80–21.73
Clonal			
C916000	1.332*	0.0499*	21.68
C916400	1.050	0.0488*	21.54
C918001	0.937*	0.0261	23.37*
NC13563	1.273*	0.0035	15.32*
NC13624	0.944*	0.0268	19.32*
NC13649	1.072	0.0261	20.68
NM2	1.382*	0.0345	19.34*

Note: Clonal model parameters that differ from global model parameters are denoted with an asterisk (*).

a_{Dmax} , predicted maximum value of $\text{DIAMETER}_{\text{INC}}$; a_{Topt} , temperature that maximizes $\text{DIAMETER}_{\text{INC}}$; a_{Tsens} , sensitivity of $\text{DIAMETER}_{\text{INC}}$ to deviations from a_{Topt} .

isotope accumulation compared with southern populations from Spain and Italy. These authors discovered significant genotype $\times$ environment interactions when these populations were subjected to water stress in a common garden experiment (Viger et al., 2016), which was not the case in our study, as genotype $\times$ environment interactions for Δ were negligible.

It is important to consider soil conditions when interpreting the current results, because soil properties contributed to overall water use efficiency (WUE) of our tested clones. According to Vereecken et al. (1989), soil texture, bulk density, and soil C are the most important factors for soil water holding capacity and, therefore, enhance tree WUE (Verlinden et al., 2015). Lower organic matter of the Ames soils relative to Waseca likely contributed to higher Δ , which corroborated previous results of decreased Δ with increased soil organic matter (Ripullone et al., 2004), and the importance of fertility effects on Δ in drought-prone soils (Voelker et al., 2014). The highest Δ values at Ames (i.e., the driest site with the lowest productivity) can also be explained by the fact that low partitioning of $\delta^{13}\text{C}$ under drought conditions was due to stomatal regulation of the trees' water regime and expenditure of carbon for respiration processes instead of growth. Such carbon losses due to respiration have ranged from 30% to 50% of

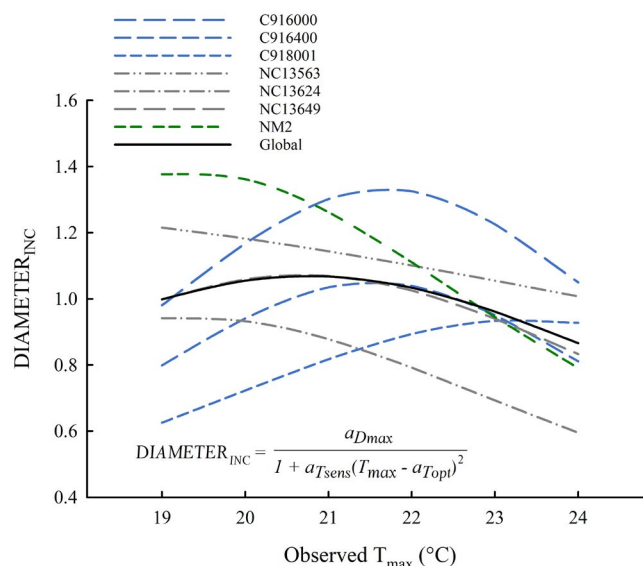


FIGURE 7 Response curves of seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. $\times$ *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. $\times$ *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray $\times$ *P. deltoides*) $\times$ *P. deltoides* “NC13563,” “NC13624,” “NC13649”] for proportional difference in the response of the annual diameter increment relative to the overall mean ($\text{DIAMETER}_{\text{INC}}$) to the maximum temperature (T_{max}), using the observed range of T_{max} from the current study. See Table 7 for parameter definitions

TABLE 8 Parameter estimates for the global and clonal models of carbon isotope discrimination (Δ) for seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. $\times$ *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. $\times$ *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray $\times$ *P. deltoides*) $\times$ *P. deltoides* “NC13563,” “NC13624,” “NC13649”] grown for 9 years at three sites in the Midwestern United States [Escanaba, Michigan; Waseca, Minnesota; Ames, Iowa]

Model	Intercept (b_{int})	Slope (b_{slope})
Global	16.40	0.1857
95% CI	15.23–17.57	0.1106–0.2608
Clonal		
C916000	16.32	0.2235
C916400	17.47	0.1572
C918001	14.83*	0.2765*
NC13563	16.23	0.2023
NC13624	18.35*	0.0383*
NC13649	17.79*	0.0547*
NM2	13.82*	0.3476*

Note: Clonal model parameters that differ from global model parameters are denoted with an asterisk (*).

assimilated carbon (Ripullone et al., 2004) and, therefore, can be an indicator of low $\delta^{13}\text{C}$ in sites and clones with lower productivity. In addition to nutrient availability in

soils, significant WUE variation has resulted from genetic variation among clones (Farquhar & Richards, 1984).

In the current study, Δ showed significant genetic variation ($p < .0001$) with values ranging from 18.6% to 19.9‰. Toillon et al. (2013) reported similar variability in biomass production, Δ , and related traits, although their range in variation for Δ was greater than 3‰. Despite differences between irrigated and non-irrigated trees, Coccozza et al. (2011) found a lack of differences among poplar clones for $\delta^{13}\text{C}$. Bonhomme et al. (2008) reported broad genetic variability along with site effects and genotype $\times$ environment interactions in growth, leaf traits, and $\delta^{13}\text{C}$ of D $\times$ N and T $\times$ D clones grown on alluvial and non-alluvial sites, with lower values for the latter areas. Regardless of the genotypic variation present for all traits in our study, there was an absence of genotype $\times$ environment interactions for Δ of all clones. Our results corroborated those of Voltas et al. (2006), who found negligible genotype $\times$ environment interactions for Δ among four *P. $\times$ euramericana* (i.e., D $\times$ N) hybrids.

Photosynthesis rate (A) and stomatal conductance (g_s) are major factors influencing genetic variation in Δ . Specific gas exchange traits that are primarily responsible for variation in Δ are species-dependent and have resulted in different correlations between Δ and productivity. In particular, variation in A has led to high productivity and low Δ , while stomatal variation has led to high productivity and high Δ (Voltas et al., 2006). Data from the current study showed different clonal responses, where productive clones acted either as “water consumers” (i.e., “C916000,” “NC13563”) or “water conservers” (i.e., “NM2,” “NC13649,” and “NC13624”), having exhibited different patterns of Δ control. Productive, water-consuming clones such as “C916000” were characterized by large diameter increment and low $\delta^{13}\text{C}$ accumulation (i.e., high Δ), indicating low stomatal control of water use. Similar results were also found for *Acacia* species (Treydte et al., 2001; Raddad & Lukkanen, 2006; Gebekirstos et al., 2011) where less conservative water use was expressed by high stomatal conductivity, wider rings, and decreased $\delta^{13}\text{C}$ values. In addition, Δ responses to temperature exhibited high levels of clonal specificity. The Δ results showed that “NM2” and “C918001” were more responsive than average to temperature, while “NC13624” and “NC13649” were less responsive (to the point of temperature having no significant effect), warranting further studies to better understand this clonal variability in temperature responses. Differences in water use strategies within dioecious species can also be sex related. Ward et al. (2002) reported that female box elder (*Acer negundo* L.) trees had higher growth rates and lower isotope ratios in wet years compared to male trees. Unfortunately, knowledge on the gender of the genotypes tested in the current study was not

available, yet this information could contribute to such interpretations in future studies.

Differences among genomic groups of the current study were evident for Δ , with D $\times$ D clones “C916000” and “C916400” exhibiting greater Δ values than TD $\times$ D backcross hybrids “NC13624” and “NC13649.” Dillen et al. (2008) reported similar results, with lower Δ values and higher WUE for pure *P. trichocarpa* genotypes and their T $\times$ D hybrids compared with pure *P. deltoides* clones and their D $\times$ N hybrids. In contrast, Pointeau and Guy (2014) reported higher $\delta^{13}\text{C}$ values in leaf tissues of *P. trichocarpa* relative to balsam poplar (*P. balsamifera* L.). In addition, they found species-specific correlation patterns between $\delta^{13}\text{C}$ and photosynthetic productivity, as well as $\delta^{13}\text{C}$ and growth, but not for all provenances within either species (Pointeau & Guy, 2014).

Carbon isotope discrimination in poplars is genetically driven, being a highly heritable trait (Monclus et al., 2006). Previous research highlighted broad clonal variability in both Δ and productivity of poplar clones, along with results showing that high heritability of carbon isotope accumulation can significantly contribute to future breeding and selection of superior genotypes, regardless of the magnitude and direction of correlations between Δ and growth (Dillen et al., 2008; Monclus et al., 2005, 2006). However, correlations were not always directly expressed, making interpretations difficult. For example, Monclus et al. (2005) reported that productive clones had low levels of Δ , while Monclus et al. (2006) highlighted positive correlations between Δ and number of stomata. Others reported non-significant or weak correlations between Δ and productivity (e.g., stem circumference) in a limited number of poplar families and provenances (Dillen et al., 2008; Toillon et al., 2013; Pointeau & Guy, 2014).

Overall, our results showed inverse relationships between Δ and productivity. The highest correlations were observed between Δ and ring width, along with Δ and $\text{DIAMETER}_{\text{INC}}$, for “NC13624” and “NC13649,” while much weaker correlations were recorded for “C916400,” “NC13563,” and “NM2.” Across our clones, “NC13649” had the greatest potential for WUE in moisture-limited environments, as “NC13649” maintained a relatively low rate of Δ (Figure 8) accompanied by a moderate $\text{DIAMETER}_{\text{INC}}$ (Figure 7) across the range of observed temperatures. In contrast, “C916000” had elevated Δ accompanied with elevated $\text{DIAMETER}_{\text{INC}}$, which suggested this genotype may be favorable for applications in which high throughput of water is desirable (e.g., riparian buffers, contamination plume control, treatment of wastewater, etc.). “NM2” had relatively low Δ and high $\text{DIAMETER}_{\text{INC}}$ in the lower half of the temperature range of this study, which was the opposite of the upper

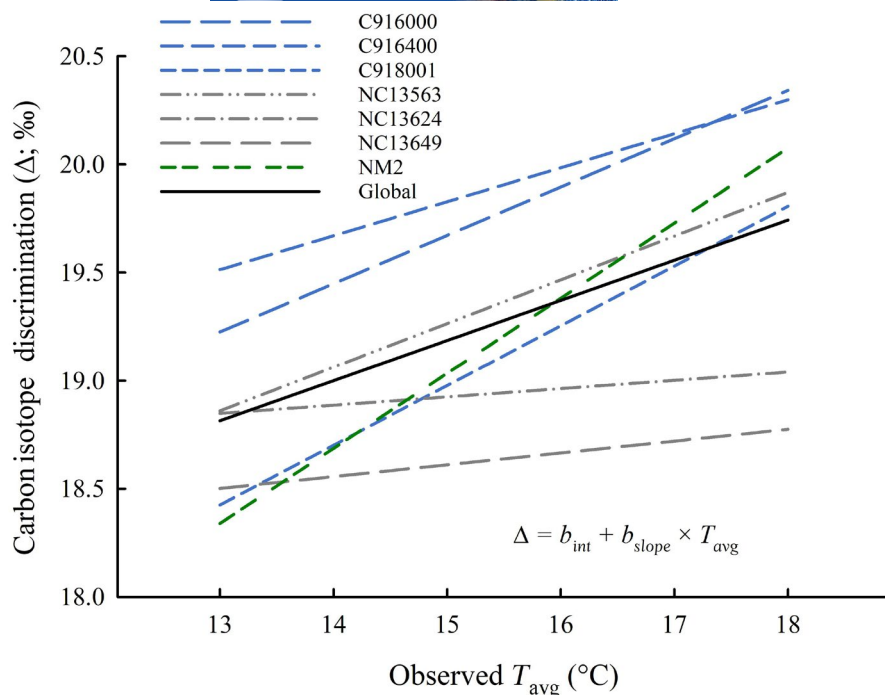


FIGURE 8 Response curves of seven hybrid poplar clones [*Populus deltoides* Bart. Ex Marsh. × *P. deltoides* “C916000,” “C916400,” “C918001”; *P. nigra* L. × *P. maximowiczii* A. Henry “NM2”; (*P. trichocarpa* Torr. & Gray × *P. deltoides*) × *P. deltoides* “NC13563,” “NC13624,” “NC13649”] for carbon isotope discrimination (Δ) (‰) to the average temperature (T_{avg}), using the observed range of T_{avg} from the current study. See Table 8 for parameter definitions

half, indicating high WUE near its optimum temperature and low WUE in warmer environments. Thus, temperature should play a major role for site selection of “NM2.” “C916000” had high Δ and high $\text{DIAMETER}_{\text{INC}}$ and others exhibited low Δ associated with low $\text{DIAMETER}_{\text{INC}}$ (“NC13624,” “C918001”), suggesting the two traits may be linked. Nevertheless, the high-efficiency examples above (i.e., “NC13649” and “NM2” at lower temperatures) along with “C916400” (which had high Δ and low $\text{DIAMETER}_{\text{INC}}$) indicate some independence between the two traits which presented potential for breeders to further explore. According to Toillon et al. (2013), negative correlations between Δ and growth are likely due to variations in photosynthetic capacities and have the following relationships: increased growth $\rightarrow$ increased canopy size $\rightarrow$ increased water demand. In addition, the lack of such clear relationships may result from reduced genetic expression under less favorable conditions, differences in timescales of integration of related traits, or the fundamental lack of physiological links among traits (Toillon et al., 2013). For example, the lack of correlations between Δ and growth within and among clones at Ames in the current study likely led to lower water availability as a consequence of less leaf area (which was visually observed), resulting in decreased transpiration and reduced soil water (Broeckx et al., 2014).

While such complexity of the interconnections among physiological parameters and processes complicates interpretation of the results, these relationships may provide useful information for future work. For instance, Broeckx et al. (2014) reported that variation in WUE resulted from both genetic factors and environmental stomatal control

in poplars, with negative correlations between net assimilation and both $\delta^{13}\text{C}$ and intrinsic water use efficiency (WUE_i ; i.e., the ratio of net photosynthesis to stomatal conductance). Relationships between WUE_i and $\delta^{13}\text{C}$ were non-existent (Broeckx et al., 2014). Similarly, there were weak relationships between water use efficiency of production (WUE_p) and $\delta^{13}\text{C}$ for three different poplar productivity groups, but significant correlations between WUE_p , growth, and leaf and stem Δ in faster growing clones indicated that photosynthesis-driven WUE was higher in productive clones (Maier et al., 2019). Verlinden et al. (2015) recorded genotypic differences in WUE_i from wood $\delta^{13}\text{C}$ of poplar hybrids belonging to four poplar species (*P. deltoides*, *P. maximowiczii*, *P. nigra*, and *P. trichocarpa*), while discrepancies existed for leaf $\delta^{13}\text{C}$ of one investigated clone (“Oudenberg”). Overall, correlations between $\delta^{13}\text{C}$ and aboveground biomass production were weak and highly variable, indicating that clonal selection focused on both growth and high WUE can enhance tree improvement efforts (Verlinden et al., 2015). In contrast, some researchers have reported positive correlations between leaf $\delta^{13}\text{C}$, sap $\delta^{13}\text{C}$, and wood $\delta^{13}\text{C}$ (Arndt & Wanek, 2002; Pointeau & Guy, 2014; Verlinden et al., 2015), suggesting that productive clones could have high accumulation of $\delta^{13}\text{C}$ during assimilation processes under drought conditions that are later used in wood formation processes. Therefore, $\delta^{13}\text{C}$ may be a useful trait when incorporating WUE and productivity into poplar breeding and selection schemes.

Three-way interactions among site, clone, and tree age in the current study showed higher Δ with increasing latitude and tree age. Similar results were recorded by

Voelker et al. (2014), who recorded positive correlations between Δ and growth for northern provenances of bur oak (*Quercus macrocarpa* Michx.) grown in Wisconsin, while southern provenances from Missouri and South Dakota exhibited negative relationships. From the standpoint of increased $\delta^{13}\text{C}$ accumulation by size and age, Schiffman et al. (2012) reported increased sensitivity by age over 3-year rotations of willow clones "SV1," "SX61," "SX64," and "Sherburne" in biomass plantations, suggesting that longer rotations could affect survival and productivity. Although the willows of Schiffman et al. (2012) may have been at different stages of canopy closure relative to the poplars in the current study, these results are somewhat contradictory to our observations, where Δ increased with age in some clones at less productive sites (data not presented). This increase of Δ in some clones and sites as trees got older may have resulted from modifications to metabolism and anatomy of the trees. For example, Galle et al. (2010) showed that even after a period of drought stress, values of $\delta^{13}\text{C}$ remained high. In addition, drought-induced modifications to photosynthetic processes and morphology prepared downy oak (*Quercus pubescens* Willd.) to survive future droughts and maintain viable hydraulic status (Galle et al., 2010). These responses for downy oak may be used to explain high Δ of some poplars, even after drought years. Arend and Fromm (2007) reported that similar adaptive responses for N $\times$ M hybrid "Kamabuchi" led to formation of smaller vessels in early summer caused by water-limited growth conditions. Therefore, drought adaptation in tree genotypes is not necessarily reflected in higher WUE (i.e., higher $\delta^{13}\text{C}$), but lower WUE may be related to adaptive mechanisms under genetic control (Tognetti et al., 2000).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

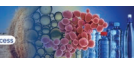
Data available on request from the authors.

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