

# Genetic variation in growth and leaf traits associated with local adaptation to climate in yellow birch (*Betula alleghaniensis* Britton)

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## Abstract

Understanding patterns of variation in functional traits of hardwood trees is crucial for conserving and managing North American temperate forests under climate change. This study examined provenance variation of yellow birch (*Betula alleghaniensis* Britton) in growth, biomass allocation, leaf morphology, and stable carbon isotope composition. Trees were grown from 10 seed sources originating from across Canada and the northern USA. Height and diameter were not significantly related to climate at seed origin, suggesting that variation may be better explained by site factors, such as soil pH and soil moisture. In contrast, carbon isotope composition and leaf morphological traits were significantly correlated to climate variables including temperature, precipitation, and solar radiation. Provenances from warmer, drier localities tended to have higher stable carbon isotope ratio ( $\delta^{13}\text{C}$ ), greater specific leaf area, and narrower leaf width than their counterparts from cooler, wetter climates. Thus, variation in leaf morphological traits appears to be involved in adaptation of yellow birch to variation in temperature and moisture availability across the species' range. Our results suggest that there may exist potential for selection and breeding of drought-resistant yellow birch genotypes to aid in reforestation under climate change.

**Key words:** *Betula alleghaniensis*, adaptive variation, leaf morphology, growth, biomass allocation, carbon isotope ratio

## Introduction

Understanding patterns of local adaptation in dominant tree species is crucial for projecting how forests may respond to climate change (Neale and Kremer 2011). Increased frequency and severity of extreme climatic events are expected to affect forest health and productivity negatively (Allen et al. 2010, 2015), with recent forest dieback linked to drought-induced mortality (Peng et al. 2011). However, as widespread trees typically exhibit significant adaptive variation (Savolainen et al. 2007), it should be possible to identify genotypes that are relatively drought-tolerant to aid reforestation under climate change (Taeger et al. 2013; Niemczyk et al. 2019).

Significant adaptive variation in functional traits, such as leaf area, width, and thickness, has occurred in many temperate tree species (Abrams 1988; Abrams et al. 1990; Pyakurel and Wang 2013; Kaluthota et al. 2015). Leaf morphology affects photosynthesis and transpiration and can affect how plants respond to water availability (Xu et al. 2009). Genotypes originating from drier environments often demonstrate reduced total leaf area and specific leaf area (SLA) compared to those from mesic environments, suggesting that reductions in leaf area may be an adaptation to water stress (Abrams 1994). Broader leaves are considered an adaptation to cool and wet environments (Westoby et al. 2004; Wright

et al. 2005; Warren et al. 2005). Adjustment of biomass allocation is another mechanism of plant adaptation to changes in soil moisture conditions (Hsiao and Xu 2000; Leuschner 2020). The root to shoot ratio (RSR) generally increases in response to decreases in soil moisture conditions (Boyer 1968; Westgate and Boyer 1985).

Stable carbon isotope analyses are commonly used to assess integrated water-use efficiency (WUE) in individual trees (Farquhar et al. 1989). The preferential photosynthetic assimilation of the two stable carbon isotopes, i.e.,  $^{12}\text{C}$  and  $^{13}\text{C}$ , changes with the stomatal control of transpirational water loss and the ratio of the two isotopes in plant biomass indicates the integrated, long-term WUE by plants (O'Leary 1988). A higher  $^{13}\text{C}$  discrimination ratio ( $\delta^{13}\text{C}$ ) indicates a higher WUE (Dawson et al. 2002; Canavar et al. 2014). Intraspecific variation in  $\delta^{13}\text{C}$  attributable to variation in the climate at seed origin has been reported for numerous temperate hardwood species (Lauteri et al. 1997; Knutzen et al. 2015). For instance, populations of pedunculate oak (*Quercus robur* L.) originating from drier climates have lower  $\delta^{13}\text{C}$  ratio values when growing at intermediate and moist sites, suggesting that they experienced less drought stress than provenances originating from more humid climates (George et al. 2020). Conversely, studies of silver birch (*Betula pendula* Roth) (Knutzen et al. 2015) and sweet chestnut (*Castanea sativa* Mill.) (Lauteri et al.

1997) have reported higher  $\delta^{13}\text{C}$  ratios for populations originating from drier environments compared to those from wetter climates.

Yellow birch is a widespread and ecologically important temperate hardwood tree species that ranges from the Canadian Atlantic Coast to southeastern Manitoba, across the Midwest states and south to the Appalachian Mountains (Erdmann 1990). The species is a component of several major temperate forest types (e.g., Hemlock–Yellow Birch, Sugar Maple–Beech–Yellow Birch, and Red Spruce–Yellow Birch) and is a source of food and shelter for many species of small mammals, birds, and insects. Economically, yellow birch is used for hardwood flooring, cabinets, doors, and furniture (Clausen 1973a). As a widespread, wind-pollinated tree species, yellow birch is expected to harbour significant genetic variation (Thomson et al. 2015). Provenance studies of yellow birch have revealed significant intraspecific variation in a variety of traits, including survival, height, growth cessation, catkin characteristics, leaf morphology, cold tolerance, and flowering times (Clausen 1968a, 1968b, 1975, 1977, 1980; Dancik and Barnes 1975; Sharik and Barnes 1975, 1979; Wearstler and Barnes 1977; Leites et al. 2019; Pedlar et al. 2021). Wearstler and Barnes (1977) found significant variation among provenances in height, seed weight, initial germination, and germination percentage in a common garden study. While a significant north-to-south cline in variation was observed, correlations of phenotypic measurements with climate at seed origin were not explicitly examined. Clausen (1968b) observed significant variation in height and growth cessation among yellow birch populations tested in common garden. The date of growth cessation was found to correlate significantly with growing season length, annual precipitation, and average July temperature, while height did not correlate significantly with any of the three climate variables tested. Dancik and Barnes (1975) and Sharik and Barnes (1979) examined variation in leaf morphology, including blade width, length, base shape, venation, and serration among yellow birch populations in their natural habitats. Dancik and Barnes (1975) found significant variation in all measured leaf traits, which were associated with soil pH, drainage, and climate of the sampling sites. Sharik and Barnes (1979) found that among-population variation in the number of lateral veins, blade length, width:length ratio, number of teeth, and petiole length were significantly correlated with either latitude or elevation of the sampling location.

The objective of this study was to investigate intraspecific variation in morphological and physiological traits in relation to the climate of seed origin in yellow birch seedlings originating from Canadian and northern United States populations. We analyzed traits that are of critical importance to understanding intraspecific differences in productivity and WUE, including growth, biomass allocation, leaf morphology, and stable carbon isotopes. This research will provide a more comprehensive understanding of the adaptive potential of yellow birch, which will be crucial for predicting the species' potential response to climate change, as well as for management and gene conservation under the change in climate (Nicotra et al. 2010; Aubin et al. 2016;

Leites et al. 2019). We have tested the hypotheses that (i) populations would exhibit significant variation in growth, leaf morphology, biomass allocation, and stable carbon isotope ratio when grown in common garden, and (ii) the variation would significantly correlate with climate at seed origin, with provenances from warmer, drier localities exhibiting trait combinations indicative of higher resistance to drought, including reduced leaf area and higher carbon isotope ratio.

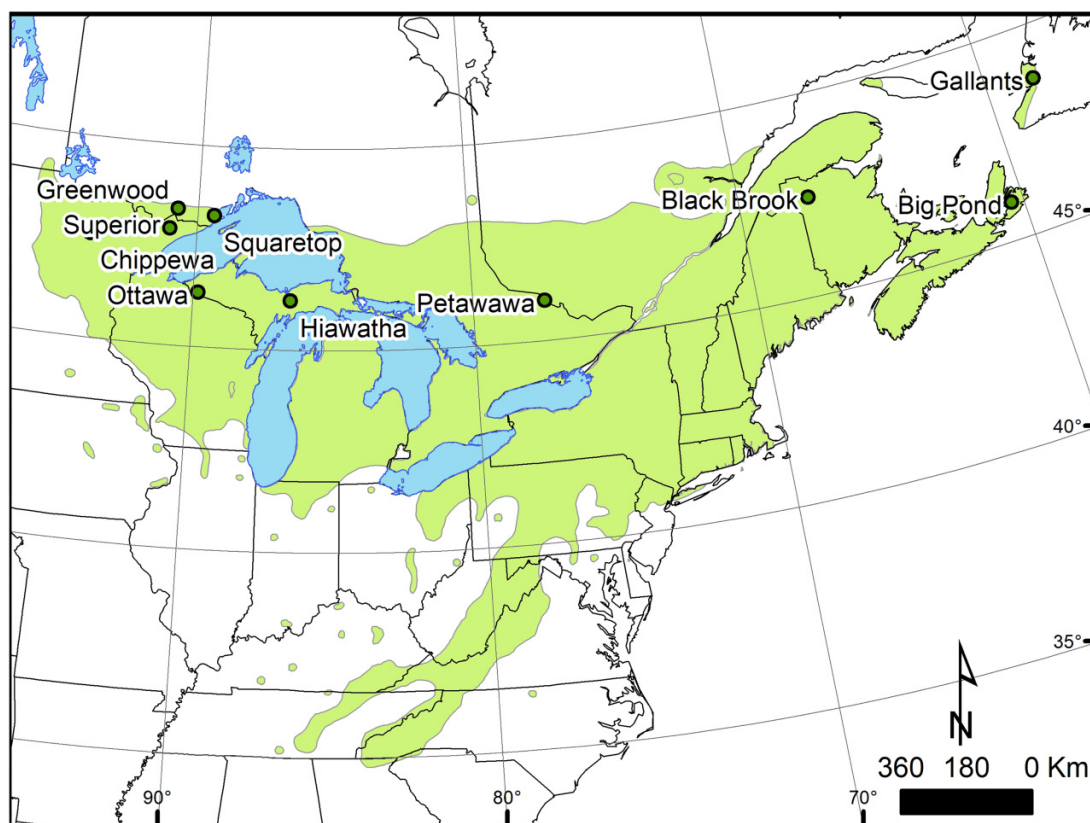
## Materials and methods

### Seed sources and experimental design

Seed was obtained from 10 yellow birch populations from Canada and Northern US states, including northwestern and eastern Ontario, New Brunswick, Newfoundland, Michigan, and Minnesota (Fig. 1, Supplementary Table S1). Wherever possible, we aimed to have each provenance represented by at least 10 maternal trees maintained as single-tree collections. However, in cases where these criteria could not be met, we accepted seed sources represented by at least five maternal trees preferring single tree collections but accepting bulked sources where single tree collections were not available. Sources obtained from the National Tree Seed Centre (four provenances) were represented by 5 to 10 single tree collections per provenance. Sources obtained from the JW Toumey Nursery (four provenances) were composed of single tree and bulk collections, each containing five trees per seed lot. We collected seed from two locations in northwestern Ontario (Greenwood and Squaretop) in October 2019 for the purpose of this study. For these sources, we collected seed from five dominant trees at least 50 m apart to minimize the chance of sampling full or half-sibs. The collected seeds were sent to the National Tree Seed Centre for cleaning and processing and a portion of the processed seed was obtained for this study. Thus, all provenances were represented by at least five maternal trees each. Research involving collection of specimens was conducted in accordance with all applicable laws, guidelines, and regulations.

A seedling common garden study containing the 10 provenances was established in the Lakehead University Greenhouse in May of 2020. Seeds were stratified prior to sowing and two seeds were sown per cell. The growing medium consisted of 60% peat and 40% vermiculite mixture in 28/340 mL Styrofoam SuperBlocks (Beaver Plastics Ltd.). After germination, the seedlings were thinned so that only one seedling remained per cell and the remaining seedlings were fertilized monthly with 15–15-18 Fertilizer (Plant Products, Soilless Feed). Seedlings were grown at ambient greenhouse conditions (min temp = 11.67 °C, max temp = 41.13 °C, and average temp = 22.72 °C) and watered daily throughout the growing season. In the fall of 2020, seedlings underwent dormancy associated with the naturally decreasing photoperiod. After all seedlings had hardened off in early November (assessed visually based on leaf abscission), they were transferred in their styroblocks to another greenhouse for the duration of the dormancy period. During this time, they were watered twice a week and maintained at ambient green-

**Fig. 1.** Provenance locations. Green dots indicate the location of seed origin, while text indicates the name used to track each source in the greenhouse experiment. The green shading indicates the natural range of yellow birch. Range shapefile of yellow birch obtained from <https://github.com/wpetry/USTreeAtlas>. Map created using ArcGIS Desktop 10.6 (ESRI, California) in Lambert Conformal Conic Projection (NAD\_1983\_Ontario\_MNR\_Lambert).



house conditions (min temp = 5.73 °C, max temp = 34.74 °C, and average temp = 13.11 °C) and natural photoperiod. In May of 2021, a total of 20 seedlings of each provenance were randomly chosen to be transplanted into 6.5 × 7 inch pots. The transplanted seedlings were then arranged into a randomized complete block design with four blocks containing one five-tree plot (1 × 5) per provenance. For sources represented by single tree collections (7 of 10 provenances), we kept each seed lot separate and tracked the family to which each seedling belonged. Once transplanted, the seedlings were fertilized with a slow release 16–14–18 granular fertilizer (Scott's Fertilizer, 2% added iron). Seedlings were watered daily throughout the growing season (May to October) and maintained at ambient CO<sub>2</sub> concentration with temperature ranging from 4.63 to 40.34 °C, with average daily temp of 20.74 °C. An aphid infestation occurred in September 2021 and so seedlings were treated with a neem oil solution in September and again in October 2021.

### Seedling measurements

Total seedling height and root-collar diameter were measured for all 200 seedlings in September 2021. In October 2021, a subset of 40 trees (one seedling per provenance per block) were randomly selected for biomass, leaf morphology,

and stable carbon isotope measurements. Seedlings were separated into leaves, branches, stem, and roots for biomass allocation measurements. The roots were gently rinsed to remove excess soil and debris. All the organs were oven-dried in an oven at 60 °C for 72 h and dry weights were taken using a precision balance. Leaves used for the morphometric analysis were first analyzed and then dried and weighed separately and then their biomass combined with the remaining leaves of the same seedling. For the leaf morphology analysis, we removed the top third, fourth, and fifth mature leaves of each seedling and scanned them using a high-resolution scanner. The leaf scan images were then analyzed using WINFOLIA software (Regent Instruments Inc.). Advanced and basic morphology were chosen for the analysis, which included measurements of leaf area, perimeter, vertical length (including petiole), blade length, width at 50% of leaf blade height (PerpWidth1), width at 90% of leaf blade height (PerpWidth2), maximum width perpendicular to blade length (MaxPerpWidth), lobe angle at 10% of leaf blade height (LobeAngle1), and lobe angle at 25% of leaf blade height (LobeAngle2) (Supplementary Fig. S1). The software also calculated several derived variables, including aspect ratio (width to length) and form coefficient, which is a measure of circularity based on perimeter to area ratio. Average horizontal width (AvgHorizWidth) was calculated as the average of all

width measurements. SLA was calculated as the ratio of total leaf area to leaf dry mass (Beaudet and Messier 1998). Derived variables

## Stable carbon isotope analysis

Dried leaves from each seedling were homogenized using a mortar and pestle in preparation for carbon stable isotope analysis. Approximately 30 mg of homogenized leaf tissue from each seedling was shipped to the Environment Isotope Lab (EIL) at the University of Waterloo for  $\delta^{13}\text{C}$  analysis. The  $\delta^{13}\text{C}$  data are the corrected delta value, reported in per mil (‰) units, against the primary reference scale of Vienna Pee Dee Belemnite (VPDB). Details of the methods used to conduct the stable-isotope analysis are provided in Appendix A1.

## Climate data

Climate NA v7.30 software (Wang et al. 2016) was used to obtain seasonal climate data describing conditions at seed source origin. In cases where bulk seed was provided for a provenance (i.e., provenances 7, 8, and 9), a single centralized coordinate was used to obtain climate information. Climate data for years 1961–1990 was chosen for the analysis as it describes the climate at seed origin prior to significant anthropogenic warming (Sebastian-Azcona et al. 2020). To reduce redundancy among correlated climate predictors, the CORR procedure in SAS software was used to identify and remove highly correlated predictor variables ( $r > 0.8$ ), leaving 10 climate variables (Supplementary Table S2) retained for use in statistical analyses.

## Statistical analysis

Values of height and diameter were averaged for the five trees in each plot to obtain provenance by block means. Normality of the data was evaluated by examining the homogeneity of variance, values of skewness and kurtosis, and Shapiro–Wilk tests. Several variables, including MaxPerpWidth, PerpWidth1, and root:shoot ratio, were transformed to meet the assumption of normal distribution. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of SAS software to determine if there were significant differences in provenance means according to the following linear model:

$$Y_{ij} = \mu + A_i + B_j + \varepsilon_{ij}$$

where  $i = 1-10$  seed sources,  $j = 1-4$  blocks,  $Y_{ij}$  is the plot mean of seed source  $i$  in block  $j$ ,  $\mu$  is the population mean,  $A_i$  is the random effect of seed source  $i$ ,  $B_j$  is the fixed effect of block  $j$ , and  $\varepsilon_{ij}$  is the random error effect of provenance  $i$  in block  $j$ . When ANOVA showed a significant effect, post-hoc Tukey HSD tests were performed to identify which provenances differed significantly from one another. Variance components were estimated using the restricted estimated maximum likelihood (REML) method in the SAS VARCOMP procedure and used to calculate the intraclass correlation coefficient (ICC) (Sokal and Rohlf 1995) as the proportion of variation due to provenance effects compared to the total variation (provenance and error) for that trait. All variables with ICC values greater than 5% were retained for further analysis. Fol-

lowing, we used Pearson's correlation analysis, implemented using the CORR procedure in SAS software, to identify phenotypic variables showing a significant relationship with one or more climate variables. Results of the correlation analysis were visualized using the *corrplot* package (Wei and Simko 2021) in R software.

Principal components analysis (PCA) was used to summarize the main components of variance in measured traits showing a significant relationship with one or more climate predictors. Variables were standardized to have a mean of zero and variance of one. Principal components with eigenvalues greater than one were extracted for further analysis, while those with eigenvalues less than one were discarded. A visual review of the resulting scree plot was also used to determine which principal components were appropriate for analysis. PCA results were visualized using the *factoextra* package (Kassambara and Mundt 2020) for R. Linear regressions were performed to examine the relationship between provenance factor scores and climate at seed origin. We used the maximum  $R$ -squared improvement (MAXR) selection method in SAS to identify the top models containing one to four climate predictors. The resultant models were then evaluated manually to identify the model with the highest  $R^2$  value, meeting the requirement that all predictor variables had variance inflation factor (VIF) values  $< 2$  to avoid over-fitting. For each of the selected models, we checked that the assumptions of regression were met by examining scatter plots of the residual versus predicted values, normal Q-Q plots, and histograms of the residuals.

## Results

No significant among-provenance variation ( $p > 0.05$ ) was detected in growth and biomass traits or  $\delta^{13}\text{C}$ . However, significant variation ( $p \leq 0.05$ ) was found for three leaf traits including AvgHorizWidth, PerpWidth1, and PerpWidth2 (Table 1). AvgHorizWidth was smallest for the Hiawatha seed source (3.68 cm) and greatest for Black Brook (4.59 cm), with an average of 4.26 cm (Fig. 2). PerpWidth1 was greatest for the population from Black Brook (6.85 cm) and smallest for the population from Hiawatha (5.19 cm), with a mean of 6.2 cm. Hiawatha had the smallest average PerpWidth2 (0.72 cm), whereas Squaretop had the largest (1.41 cm), with an overall average of 1.08 cm.

Provenance effects explained greater than 5% of the total phenotypic variation for 15 of the 28 measured variables (Table 2). Variables that did not differ substantially among provenances were mostly related to biomass partitioning, leaf length, and lobe angle. Variables related to leaf width generally had high ICC values, with the strongest provenance effects observed for PerpWidth1, PerpWidth2, and AvgHorizWidth with ICC values of 43.5%, 28.9%, and 28.0%, respectively. In total, six of the 15 retained variables were significantly correlated ( $p < 0.05$ ) with one or more climate variables (Fig. 3). Variables that were significantly related to climate include  $\delta^{13}\text{C}$ , leaf width variables, and SLA, while correlations with climate were not significant for any of the growth and biomass variables. Pearson's correlation coefficient ( $r$ ) values for significant correlations ranged from  $-0.87$

**Table 1.** General linear model results for three significant leaf traits.

Variable	Source	df	Sum of squares	Mean square	F value	Pr > F
AvgHorizWidth	Provenance	9	3.685	0.409	2.560	0.029
	Block	3	0.648	0.216	1.350	0.280
	Error	27	4.324	0.160		
PerpWidth1	Provenance	9	116039.630	12893.292	4.080	0.002
	Block	3	13563.316	4521.105	1.430	0.255
	Error	27	85282.776	3158.621		
PerpWidth2	Provenance	9	1.648	0.183	2.620	0.025
	Block	3	0.112	0.037	0.540	0.661
	Error	27	1.883	0.070		

for PerpWidth2 and average spring temperature ( $p = 0.001$ ) to 0.78 for SLA and spring precipitation ( $p = 0.0076$ ), reflecting that seed sources from warmer, drier localities generally had narrower leaf width and lower SLA than those from cooler, wetter localities.

The first two PCA axes cumulatively explained 89.4% of the variation in the six retained response variables and had eigenvalues greater than one. PC1 was determined by variables describing leaf size (AvgHorizWidth, MaxPerpWidth, PerpWidth1, and PerpWidth2), whereas PC2 was related to  $\delta^{13}\text{C}$  and SLA (Fig. 4). The best-fit regression model for PC1 included spring temperature (Tave\_sp) and winter solar radiation (Rad\_wt) ( $R^2 = 0.88$ ,  $p = 0.0005$ ) (Table 3). Provenances with positive eigenvalues on PC1 (e.g., Hiawatha and Ottawa) tended to originate from localities with higher spring temperature and lower winter solar radiation than provenances with negative eigenvalues (e.g., Black Brook and Squaretop). The best-fit regression model for PC2 included only spring precipitation (PPT\_sp) ( $R^2 = 0.49$ ,  $p = 0.0235$ ), with provenances from regions with high spring precipitation (e.g., Big Pond and Black Brook) tending to have higher eigenvalues than provenances from regions with relatively low spring precipitation (e.g., Greenwood and Superior).

## Discussion

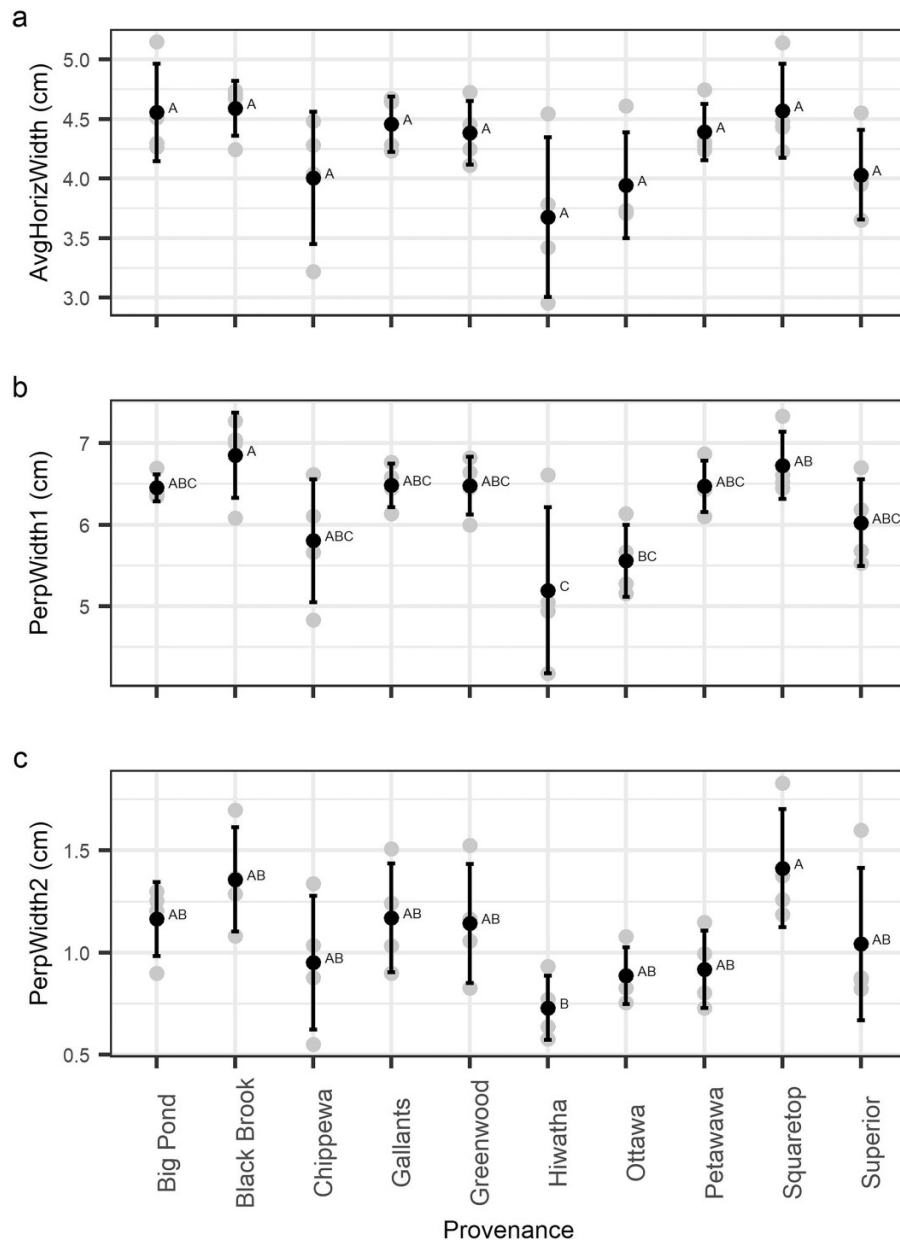
### Variation in growth traits and relationship with climate at seed origin

Variation in provenance mean height and diameter was not statistically significant. This was unexpected because previous provenance studies of yellow birch have reported significant variation in height (Clausen 1968b, 1973a, 1975, 1980; Clausen and Garrett 1969; Wearstler and Barnes 1977; Fowler and Park 1988; Leites et al. 2019) and diameter (Clausen 1973a; Fowler and Park 1988). Significant variation in height was reported for a nursery trial of 55 yellow birch provenances measured at 1 to 3 years of age (Clausen 1968b, 1973a), and for 5-year-old seedlings measured at five common garden locations (Clausen 1975). It is possible that the lack of significant provenance variation in this study might be related to the younger age of the seedlings, as maternal effects may obscure among-provenance differences in growth for several years after establishment (Roach and Wulff 1987;

Lopez et al. 2003; O'Brien et al. 2007). However, previous studies of 1- to 3-year-old yellow birch seedlings found significant variation in growth (Clausen 1968b, 1973a, 1975), suggesting that maternal effects do not adequately explain the lack of variation in our study. Instead, we suggest that the lack of significant variation in our study may be related to the small sample size and limited number of provenances tested ( $n = 10$ ) compared to previous studies that contained a larger number of provenances from across the range of yellow birch (Clausen 1968b, 1973a, 1975; Fowler and Park 1988). Our results are similar to those of a provenance-progeny test of 21 yellow birch seed sources that did not report significant variation in 5-year height among provenances tested at a field trial in northern Wisconsin (Clausen 1980). The author postulated that the lack of significant variation could be because a subset of provenances was tested compared to the previous studies that analyzed a total of 55 sources from across the range of yellow birch. Small sample sizes and high within-provenance variation in our study may explain the failure to detect differences among seed sources. The proportion of variation in height due to provenance effects was 12%, whereas the proportion of variation in height due to within-provenance effects was 88%. Thus, our results agree with previous studies reporting high within-provenance variation of yellow birch (Clausen 1973b) and lack of significant among-provenance variation in height and diameter (Clausen 1980).

The lack of significant correlation between height and climatic predictor variables indicates that variation in height growth is not clearly related to climate at seed origin, at least in the context of our study. Thus, our results are consistent with previous studies reporting weak patterns of yellow birch growth in relation to climate (Clausen 1968b; Leites et al. 2019; Pedlar et al. 2021). The overall weak clinal variation observed for yellow birch contrasts with provenance studies of numerous boreal and temperate trees that generally report strong clinal patterns of variation in height (Rehfeldt et al. 1999; Lesser and Parker 2004; Thomson and Parker 2008; Aitken and Bemmels 2016; Leites et al. 2019). Leites et al. (2019) suggested that the relatively weak growth clines observed in yellow birch compared to other temperate hardwoods could result from a high degree of physiological adjustment or a low degree of growth optimization in response to natural selection. However, common gardens examined in

**Fig. 2.** Provenance means for significant leaf traits. Black circles indicate provenance means, and letters next to each mean represent the Tukey post-hoc test group to which the provenance was assigned. Vertical lines represent standard deviation, while grey circles represent individual observations. (a) Average horizontal leaf width (AvgHorizWidth), (b) mean perpendicular leaf width at 50% of blade length (PerpWidth1), and (c) mean perpendicular width at 90% blade length (PerpWidth2).



that study were limited in terms of latitude and may not have been extreme enough to elucidate greater differences among the sources. Polyploids, such as yellow birch, are generally expected to demonstrate high phenotypic plasticity, which may allow them to respond efficiently to short-term environmental changes (Otto and Whitton 2000; Leitch and Leitch 2008; Parisod et al. 2010; Van De Peer et al. 2017). Alternatively, selection on height may operate at more local scales involving factors such as soil pH, site drainage, and elevation (Dancik and Barnes 1975; Moles et al. 2014).

While none of the correlations of biomass variables against climate predictors were statistically significant, moderate

correlations ( $r > 0.5$ ) were observed between root weight and several climate predictors. Biomass allocation to roots tended to be greater for provenances originating from warmer and drier conditions than for provenances originating from cooler and wetter environments, suggesting that variation in biomass allocation to roots among yellow birch provenances may reflect adaptation to divergent local environmental conditions. A previous study of yellow birch found that among-provenance variation in root, leaf, stem, above-ground, and total dry weight of nursery grown seedlings was significantly related to three tested climate variables including growing season length, average July temperature, and

**Table 2.** Variance components and intraclass correlation coefficient (ICC) for 28 measured variables.

Variable	Var (Prov)	Var (residual)	ICC (%)
Height	5.5910	40.9358	12.0
Diameter	0.0242	0.2132	10.2
LeafWeight	0.0823	2.9773	2.7
BranchWeight	0.0000	0.5196	0.0
StemWeight	0.2974	2.1559	12.1
RootWeight	0.3929	6.7699	5.5
AbvGrdWeight	0.8991	9.9469	8.3
TotalWeight	2.1232	28.1237	7.0
Leaf:Total	0.0000	0.0014	0.0
Branch:Total	0.0000	0.0010	0.0
Stem:Total	0.0000	0.0027	0.0
Root:Total	0.0000	0.0033	0.0
Root:Shoot	0.0000	0.0000	0.0
$\delta^{13}\text{C}$	0.0632	0.7891	7.4
LeafArea	10.1606	131.8927	7.2
AvgHorizWidth	0.0623	0.1602	28.0
BladeLength	0.0000	2.0023	0.0
PetioleLength	0.0235	0.0852	21.6
VerticalLength	0.0000	2.4022	0.0
AspectRatio	0.0001	0.0014	3.9
Perimeter	0.1104	38.4316	0.3
FormCoefficient	0.0006	0.0024	21.3
MaxPerpWidth	1924.7000	6957.2000	21.7
PerpWidth1	2433.7000	3158.6000	43.5
PerpWidth2	0.0283	0.0697	28.9
LobeAngle1	0.0000	26.5722	0.0
LobeAngle2	0.0000	26.6142	0.0
SLA	57.8152	754.1556	7.1

average January temperature (Clausen 1973a). Similarly, the two strongest predictors of root weight in our study were winter degree days above 5 °C ( $r = 0.59$ ) and summer minimum temperature ( $r = 0.57$ ). Our results are in contrast to those from a study of intraspecific variation in silver birch (*B. pendula*), which found that provenances from cooler climates had higher biomass allocation to roots compared to southern provenances (Tenkanen et al. 2020). However, our results are consistent with previous studies showing increased biomass allocation to roots as a mechanism of adaptation to dry conditions (Zhang et al. 2004; Markesteijn and Poorter 2009; Matías et al. 2019).

### Stable carbon isotope ratio

Our results of stable carbon isotope composition suggest that provenance effects explained a moderate proportion (ICC = 7.4%) of the total variation in  $\delta^{13}\text{C}$ . Significant variation in  $\delta^{13}\text{C}$  has been reported for other tree species growing under water-stressed conditions (Olivas-García et al. 2000). The seedlings in this study, however, were well-watered, which may not have provided the required conditions for the seedlings to realize their genetic potential to maximize their WUE. Although the provenance variation component

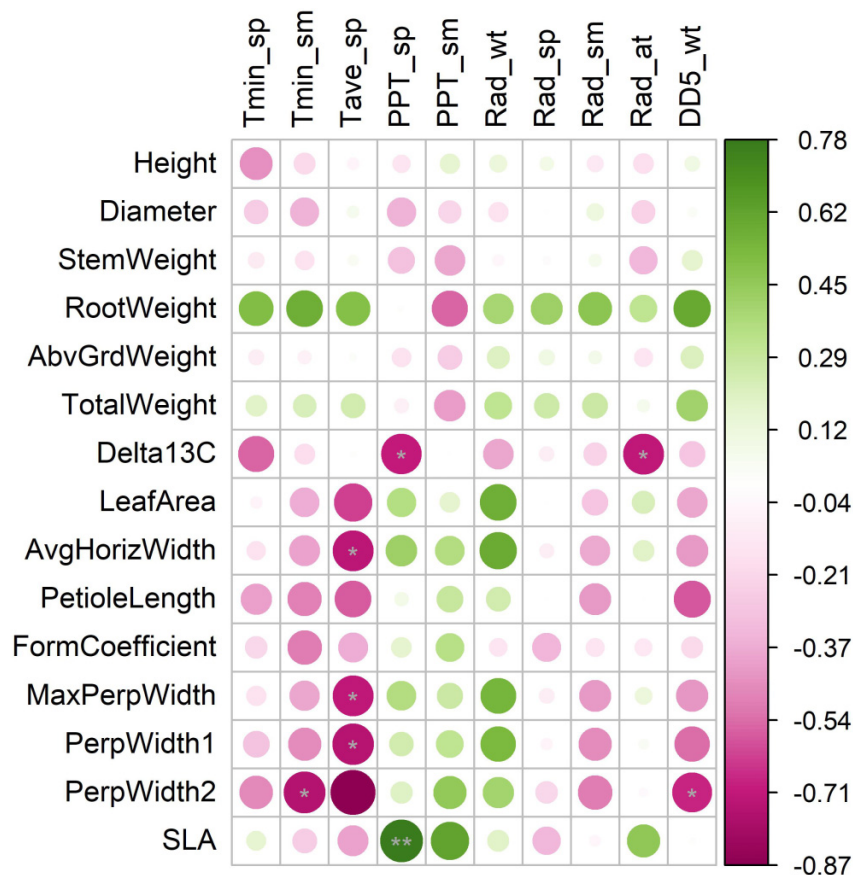
was not substantial, the significant negative relationship between  $\delta^{13}\text{C}$  and summer precipitation suggests that provenances from areas with higher summer precipitation have lower WUE compared to provenances originating from areas with lower summer precipitation. Our results agree with studies of other temperate broadleaf tree species in which higher WUE was found for populations originating from dry compared to wet environments (Lauteri et al. 1997; Zhang et al. 2004; Knutzen et al. 2015). In contrast, lower WUE of dry-adapted populations has been reported in some species, including *Populus balsamifera* (Soolanayakanahally et al. 2015) and *Quercus robur* (George et al. 2020).

Lower discrimination against  $^{13}\text{C}$  can reflect either lower stomatal conductance or higher photosynthetic capacity (Lambers et al. 1998). Higher carbon isotope ratios in dry-adapted populations of *Castanea sativa* was associated with higher stomatal and mesophyll conductance and higher photosynthetic capacity linked to greater leaf nitrogen content (Lauteri et al. 1997). In *Populus davidiana*, higher WUE of dry-adapted populations was linked to lower height, net photosynthesis, and transpiration, suggesting that these populations employ a conservative growth strategy to survive in dry conditions (Zhang et al. 2004). In our study, the carbon isotope ratio was positively correlated to stem mass (results not shown), which may suggest that higher WUE of dry-adapted yellow birch populations results from increased photosynthetic capacity rather than decreased stomatal conductance. However, variation in stem growth may also reflect variation in duration of height elongation associated with genetic differences in phenological timing of southern compared to northern provenances (Soolanayakanahally et al. 2015). As such, understanding of the physiological mechanism responsible for the increased WUE of dry-adapted yellow birch populations will require further studies incorporating measurements of additional parameters, including phenology, stomatal conductance, and photosynthetic rate.

### Leaf traits

As hypothesized, the study showed significant differences in leaf morphological traits among provenances from different geographic localities. Leaf width variables, including HorizWidth, MaxPerpWidth, PerpWidth1, and PerpWidth2, were found to be significantly negatively correlated to average spring temperature. Previous studies have reported significant clines in leaf morphology among yellow birch populations (Clausen 1973a; Dancik and Barnes 1975; Sharik and Barnes 1979). For example, a common garden study of 55 yellow birch provenances found variation in leaf size and shape significantly related to several climate variables, including growing season length, average July temperature, and average January temperature (Clausen 1973a). Overall, our results are consistent with studies of other temperate hardwoods that report decreasing leaf width moving from cooler and wetter to warmer and drier regions (Santini et al. 2004; Miljković et al. 2019; Stojnić et al. 2022). In contrast, maximum horizontal width of paper birch leaves increased with warmer and drier conditions at seed origin, which the authors concluded might have resulted from a plastic response

**Fig. 3.** Plot of correlation coefficient ( $r$ ) values of 15 measured variables with 10 climate variables. The area of the circles is proportional to the absolute value of the correlation coefficient, with larger circles indicating higher  $r$  values. Positive correlations are depicted in shades of green, while negative correlations are depicted in shades of pink. Asterisks denote significant correlations: \*, significant at the 0.05 level; \*\* significant at the 0.01 level. Refer to Supplementary Table S2 for climate variable abbreviations.



to the greenhouse growing environment (Pyakurel and Wang 2013). Narrower leaves are generally thought to be an adaptation to warmer, drier environments, as they dissipate heat more rapidly through convection than larger leaves that have a thicker boundary layer (Yates et al. 2010; Nicotra et al. 2011; Leigh et al. 2017). As such, plants with narrower leaves tend to have leaf temperatures more similar to ambient temperatures, whereas the difference between air temperature and leaf temperature is generally higher for broader leaves (Fonseca et al. 2000; Leigh et al. 2017).

The lack of significant genetic variation in leaf area, blade length, and SLA is in contrast with some previous studies of yellow birch (Clausen 1973a; Dancik and Barnes 1975; Sharik and Barnes 1979) and paper birch (Pyakurel and Wang 2013). For example, Dancik and Barnes (1975) found significant differences in blade length, blade width, and petiole length among 112 yellow birch populations from Michigan, Wisconsin, and Minnesota. One potential reason for the different results between studies is their different experimental approaches. Dancik and Barnes (1975) and Sharik and Barnes (1979) analyzed leaf traits sampled from populations grown in their natural environment (i.e., not in common garden) and so the variation in leaf morphology that they

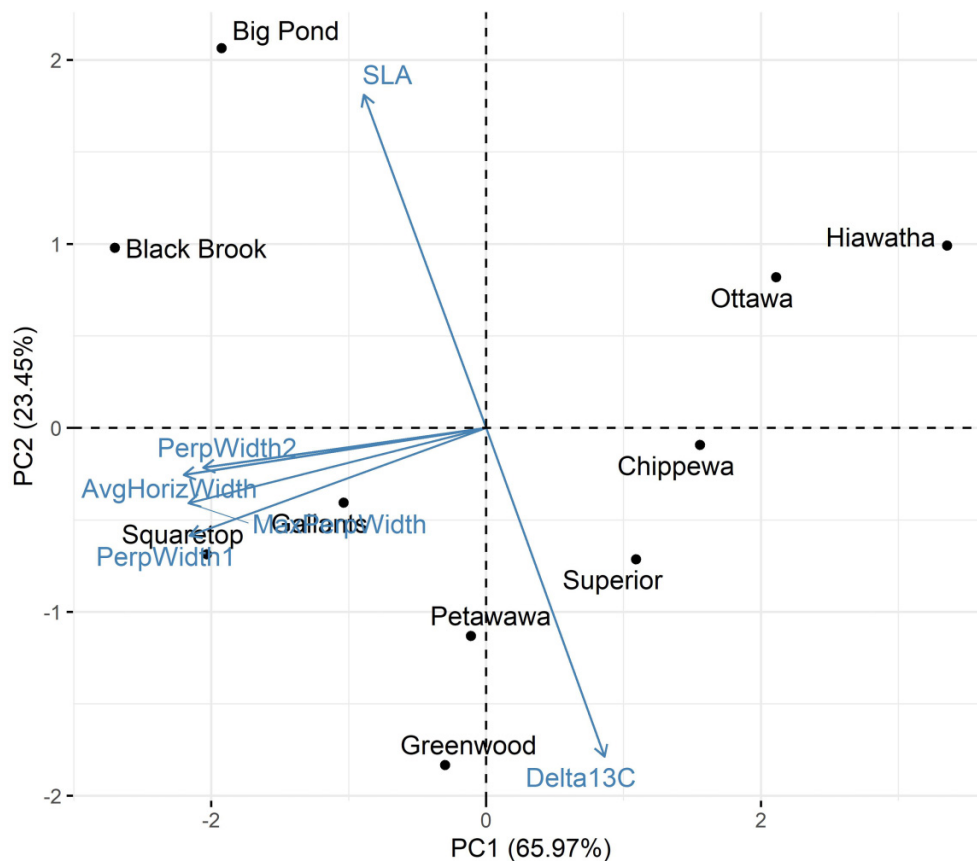
observed may be attributed to a confounded combination of phenotypic plasticity and genetic differences rather than just underlying genetic differences. Although SLA and petiole length did not vary significantly in our study, they were positively correlated with growing season precipitation at seed origin. Provenances originating from localities with higher spring and summer precipitation generally had longer petiole length and higher SLA than those originating from drier climates. Our results agree with other studies of temperate hardwoods reporting lower SLA for provenances originating from xeric compared to mesic environments, suggesting that smaller, thicker leaves may be an adaptation to dry environments (Donselman and Flint 1982; Abrams and Kubiske 1990; Abrams et al. 1990). Similarly, increased petiole length in moist environments may reflect increased investment in support structures for larger leaves (Niinemets et al. 2006; Xu et al. 2009).

### Research limitations and recommendations

This study is a short-term study on tree seedlings and thus has limited implementations to large trees in the field. Further research on genetic variation in yellow birch should measure multiple growing seasons to compare growth be-



**Fig. 4.** Results of the principal components analysis summarizing provenance variation in six functional traits with a significant relationship to climate at seed origin. Blue arrows indicate variable loadings while black dots indicate provenance factor scores.



**Table 3.** Parameter estimates for selected multiple regression models of principal component factor scores against climate variables.

Dependent variable	$P > F$	Independent variables	DF	Parameter estimate	Standard error	$t$ value	$Pr >  t $	VIF
PC1 $R^2 = 0.8836$	0.0005	Intercept	1	5.5028	2.2338	2.46	0.0432	0.0000
		Tave_sp	1	1.3221	0.2250	5.87	0.0006	1.0007
		Rad_wt	1	-1.6349	0.3931	-4.16	0.0042	1.0007
PC2 $R^2 = 0.4934$	0.0235	Intercept	1	-2.9695	1.1009	-2.7	0.0272	0.0000
		PPT_sp	1	0.0148	0.0053	2.79	0.0235	1.0000

**Note:** Abbreviations: Tave\_sp, average spring temperature; Rad\_wt, winter solar radiation; PPT\_sp, spring precipitation; VIF, variance inflation factor.

tween seedling ages. Past research has shown that younger seedlings may express higher levels of plasticity than older seedlings of the same species (Logan 1965; Clausen 1980; Gaucher et al. 2005). Therefore, collecting multiple growing seasons of measurements in growth, biomass, leaf characteristics, and carbon isotope values may provide insight to plasticity of yellow birch seedlings at varying ages grown under greenhouse conditions. Including measurements of leaf phenology and growth cessation may also uncover patterns of local adaptation within yellow birch, as past research has shown significant differences among provenances within yellow birch (Clausen 1968b) and other temperate hardwoods (Aspelmeier and Leuschner 2006; Vitasse et al. 2009; Etterson et al. 2020; Guo et al. 2020). Furthermore, including more

provenances and more replication per seed source would be beneficial for increasing statistical power and capturing a more robust view of the genetic variability among populations. Thirdly, a combination of controlled environment and field studies may provide more insight on genetic variations in phenological, morphological, and physiological traits that are important to the potential responses of the species to climate change. Lastly, a soil moisture deficit study using seed from the same 10 provenances would provide greater knowledge regarding the responses that yellow birch seedlings have toward changes in their environment. Including measurements of photosynthesis, transpiration, and carbon assimilation would also add significant value to a study involving WUE and resource usage. This study design would be time

consuming and labor-intensive. However, it is crucial for further understanding how yellow birch may respond to varying degrees of drought conditions.

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### Data availability

Data generated or analyzed during this study are available in the Dryad repository (<https://datadryad.org/>), doi:10.5061/dryad.8cz8w9gww.

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### Competing interests

The authors declare there are no competing interests.

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## Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjb-2023-0095>.

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reference material and in-house EIL standards that are calibrated using certified international reference materials (i.e., IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-40 + 41) with values provided through CIAAW. A mix of EIL in-house (i.e., EIL-72, EGC-3, and JSEC-01) and international materials are analyzed in each run.

A “like” material is also used when possible; usually a National Institute of Standards & Technology (NIST) material like NIST-1577b (Bovine Liver) is used when tissues or invertebrates are analyzed and NIST 2704 (River sediment) is used when analyzing soils and sediments. This provides a post-correction check throughout the analysis run for both isotopic and elemental % data.

In the case of  $^{15}\text{N}$  analysis in ammonia, three in-house ammonium sulfate materials were created and calibrated using the certified international reference materials IAEA-N1 + N2. These are incorporated within the ammonia disk sample preparation of each run, in duplicate to provide the information required to correct for any offset within the disk preparation technique.

Of the total sample number dropped in an analytical run, no less than 20% are Std/Ref materials. These Std/Ref measurements are used in data normalization and to ensure daily mass spec precision and accuracy, also to assess linearity issues or mass spec drift throughout the duration of the run. With these QA/QC checks, an error of 0.2‰  $\delta^{13}\text{C}$  and 0.3‰  $\delta^{15}\text{N}$  is required for reportable data.