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Stable Isotope Ecology of Temperate Conifers

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Isotope Ecology of Temperate Conifers

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ABSTRACT

Previous work has suggested that conifers could be more effective proxies for climatic changes than other plant groups, though taxonomic differences in isotopic C discrimination and leaf economics have not been studied in depth. Modern conifer shoot and dicot samples were collected from Hidden Lake Gardens (HLG; Michigan) and analyzed for isotopic and elemental composition; isotopic results are expressed in terms of Δ_{leaf} to allow for direct analysis of the consequences of biological processes. Significantly lower ($p < 0.05$) mean Δ_{leaf} values for conifer groups relative to the dicot outgroup are found, which indicates a difference in water use efficiency of the two groups. The deciduous conifer genus *Larix* more closely resembled the mean dicot Δ_{leaf}, which suggests that deciduous C_3 plants use water less efficiently than the evergreen conifers. Of the conifer genera in the data set, *Thuja* had the smallest range of Δ_{leaf}, which suggests that its isotopic composition is more heavily influenced by climatic conditions than by physiology. Given the important role of hydraulic architecture in C fractionation and the small range of Δ_{leaf}, *Thuja* is likely the best candidate for a climate proxy for future study. Global relationships of MAP and Δ_{leaf} compiled in meta-analyses of C_3 plants were not predictive of the mean Δ_{leaf} in HLG conifer and dicot samples, suggesting that climate inferences will require taxon-specific calibrations.

Keywords: Anthropogenic climate change, carbon isotopes, C:N ratio, conifer, dicot

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INTRODUCTION

Understanding climate sensitivity to greenhouse gases, such as carbon dioxide (CO_2), is of critical importance as atmospheric CO_2 ($CO_{2(atm)}$) has reached concentrations unprecedented over the last 800,000 years[1]. The economic and humanitarian risks associated with anthropogenic climate change highlight the need for robust predictions of future climate impacts. However, in order to improve these projections, it is necessary to be able to interpret effectively the variability in the climatic and ecological signals encoded in terrestrial ecosystems. One way to trace physical and environmental stresses on terrestrial ecosystems is through the use of terrestrial organic matter derived from plants, roots, and soils[2]. In particular, terrestrial organic matter is commonly used to examine the impacts of climate, as the isotopic carbon composition of leaves ($\delta^{13}C_{leaf}$), to a degree, reflects both the carbon composition of the atmosphere ($\delta^{13}C_{atm}$) as well as the combined environmental stresses experienced by a plant as it grows[3, 4].

The naturally occurring stable carbon isotopes, ^{13}C and ^{12}C, are unevenly distributed in compounds throughout the environment. Plants preferentially select for the lighter isotope, and in comparing ratios of $^{13}C/^{12}C$ in leaves with their abundance in standards (expressed as $\delta^{13}C_{leaf}$), it is possible to infer information about the physical, metabolic, and chemical processes associated with the carbon transformations that occurred[3]. The difference observed between $\delta^{13}C_{leaf}$ and $\delta^{13}C_{atm}$ (denoted as Δ_{leaf}) is the result of these carbon transformations, more specifically, the isotopic fractionation by the plant that occurs during photosynthesis[2,4]. As it stands, there are limitations when trying to interpret Δ_{leaf} across climatic gradients, especially given that Δ_{leaf} values are known to vary with environmental conditions and plant characteristics[3].

Recent studies have assessed the $\delta^{13}C_{leaf}$ values of various plant groups as they are influenced by different climatic conditions and have found that mean annual precipitation (MAP)

was the strongest predictor of Δ_{leaf} [2]. Preliminary work done by Sheldon and Smith[5] suggested that the predictability of MAP of Δ_{leaf} varies by region and plant group (**Figure 1**), where, for example, the regional slope relating Δ_{leaf} and MAP of conifers in arid to semi-arid Arizona is much steeper than in the global dataset of Diefendorf et al.[2]. The steeper slope not only reinforces the relationship between MAP and $\delta^{13}C_{leaf}$ found in previous studies[2,6] but also implicates MAP as a stronger predictor of $\delta^{13}C_{leaf}$ variability in conifers than other plant groups. In conjunction with the observed sensitivity to MAP, the ecological diversity between conifer species niche space, and longstanding (>300 myr) presence on Earth[7, 8] conifers could serve as an effective proxy for changing climatic conditions, such as MAP, over time. However, the efficacy of conifers as climate proxies has yet to be studied in depth, and little is known about the variability in leaf economics or Δ_{leaf} as a function of taxonomy. Here I address the potential taxonomic variability in Δ_{leaf} using conifers grown under the same environmental conditions.

MATERIALS AND METHODS

In order to assess conifer C, N, and $\delta^{13}C$ variance as a function of taxonomy, 163 conifer and 12 dicot samples (**Table 1**) were collected for analysis in August and September of 2014 from planting beds at Hidden Lake Gardens (HLG) in Tipton, Michigan (**Figure 2**). Michigan's continental climate varies across the state, with characteristically cooler temperatures and more severe winters in the Upper Peninsula, and warmer temperatures in the southern parts of the Lower Peninsula. The HLG collection site, which is approximately located at 42°01'45.59" N, 84°06'42.36" W, receives a MAP of 91.0 cm and has average temperatures that range from -3.5°C in the winter to 27.3°C in the summer [9]. These samples consisted of 15 conifer and five dicot genera (56 and seven species, respectively), the latter of which served as an "outgroup" to compare with the conifers (**Table 1**). The conifer specimens collected belong to the

Cupressaceae (n=14 species), Pinaceae (n=38 species), Sciadopityaceae (n=1 species), and Taxaceae (n=3 species) families, while the dicot samples belong to the Altingiaceae (n=1 species), Cornaceae (n=2 species), Rosaceae (n=1 species), Sapindaceae (n=2 species), and Lauraceae (n=1 species) families (**Table 1**).

In order to determine how conifers record changes in climate over time, the leaves of historic herbarium samples (n=23) were also collected from the University of Michigan Herbarium (MICH) to compare to the modern samples (**Table 2; Appendix 5**). The historic samples range in age from 1900 to 1986 and will provide a basis for future isotopic comparisons between historic and modern conifers. The historic samples were acid washed to remove any external debris, and then prepared for elemental analysis in the same way as the modern samples.

Collected samples from HLG were dried in a plant press in an oven at 40°C for at least 48 hours before being placed in envelopes and stored in bags with silica gel to prevent moisture reabsorption. After all of the samples had been dried, whole leaves from each specimen (n=175; plus 43 conifer replicates and six dicot replicates) were removed, representing multiple growth seasons along the shoot (except for deciduous species), finely ground using a mortar and pestle with liquid nitrogen, and stored in sealed glass vials. Herbarium samples were freeze-dried, ground with a mortar and pestle, and stored in sealed glass vials. For both HLG and herbarium samples, 1 mg aliquots were loaded into tin capsules and analyzed on a Costech ECS4010 Elemental Analyzer (EA) for their elemental composition, calibrated against acetanilide (71.09% C, 10.36% N) and atropine (70.56% C, 4.84% N) standards. The C and N data were then used to calculate more precise target weights for a sub-set of the samples (n=80, plus 14 replicate samples) that span the taxonomic range of the dataset, which were then re-loaded into a Costech ECS4010 EA linked to a Delta V+ Isotope Ratio Mass Spectrometer for isotopic analysis. The

results are reported as $\delta^{13}C$ values relative to the PDB scale, and calibrated against IAEA 600 Caffeine and IAEH-CH-6 Sucrose. External precision was maintained at <0.1‰ and duplicate samples were run on different trays to quantify uncertainty. The modern $\delta^{13}C_{leaf}$ values were calculated using Equation (1)[3], and converted to Δ_{leaf} values using Equation (2)[3]; the modern $\delta^{13}C_{atm}$ value (~-8‰) was obtained from the Institute of Arctic and Alpine Research (INSTAAR) at the University of Colorado via the GLOBALVIEW database[10].

Equation 1.

$$\partial^{13}C_{leaf} = 1000\times\left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1\right)$$

Equation 2.

$$\Delta_{leaf} = (\partial^{13}C_{atm} - \partial^{13}C_{leaf})$$

Taxonomic and Phylogenetic Comparison

Results were compared as a function of conifer taxonomy (family, genera, and species), with taxonomic groups requiring three or more samples to be included in comparisons; to aid analysis, a concatenated phylogenetic tree representing the sampled conifer species was constructed based on phylogenies recovered from previous studies (**Figure 3**)[11, 12, 13, 14]. To assess the relationships between phylogeny and C:N, Δ_{leaf}, C, and N, phylogenetic distance was determined by the number of divergences between a given "anchor" genus from all other genera sampled (**Appendix 1**) based on the phylogeny by Leslie et al.[14]. Isotopic similarity was determined by the absolute difference in mean Δ_{leaf} between the anchor genus and every other

conifer genus in the data set. Relationships were assessed using linear regression in SPSS, and reported as r^2 values (**Figures 4, 5**).

Statistical Analysis

To determine statistically significant differences ($p < 0.05$) between plant groups, independent samples t-tests and one-way ANOVA were run in SPSS. Assumptions of normality were tested and significant outliers removed prior to analysis (**Appendices 2, 3**)[15]. In the phylogenetic distance analysis, linear regressions were used to assess the relationships between phylogeny and the mean C:N, Δ_{leaf}, C, and N values for each genus; the strength of the relationships are reported as r^2 values (**Figures 4, 5**).

Characterization of Uncertainty

Conservative error estimates for elemental analysis were < ± 4.5% C and < ± 0.40% N between trays, although replicate samples run in the same tray typically had uncertainties < ± 3% C and < ± 0.15% N. In isotopic analysis, any machine error is likely small and consistent between samples, and would therefore not likely influence the results of the analysis.

RESULTS

Elemental Analysis

Modern Plants at Hidden Lakes Gardens

The conifer data set (n = 163, plus 43 replicate samples) contains four families, 15 genera, and 56 species; the dicot data set (n = 12, plus 2 replicates) is composed of five families, five genera, and seven species (**Table 1**). Overall, the conifer samples exhibited a narrower range of C:N (22.47–64.61) than the dicot samples (15.53–99.31). As single elements, N was more

variable than C for all of the plants and C was significantly higher for the conifers than for the dicot samples ($p < 0.05$) (**Figure 3**); there was no significant difference ($p > 0.05$) between conifer and dicot N.

Historic Herbarium Conifers

All of the historic samples (n = 23) belonged family Pinaceae, genus *Pinus*. Specimens were originally collected over the last century from a variety of locations in the Philippines, North and South Korea, Spain, Japan, China, Germany, France, and Mexico (**Table 2**; **Appendix 4**). The mean C and N compositions were 46.86 ± 0.46%, and 1.29 ± 0.07% with ranges of 46.07–55.59% and 0.72–85%, respectively. When analyzed together, the mean C:N was 47.25 ± 2.96. When samples were separated by location, specimens sampled from Mexico exhibited a pattern of increasing C and C:N and decreasing N over time (**Figure 6**).

Taxonomic Influence

Among families, C:N in Pinaceae (n=106, plus 26 replicate samples) and Cupressaceae (n=36, plus 5 replicates) had similar ranges of C:N (23.38–64.61 and 28.45–51.83, respectively), while Taxaceae (n=9, plus 3 replicates) had a comparatively smaller range (22.47–35.18). The mean C:N values for Pinaceae ($\mu = 40.83 \pm 0.84$) and Cupressaceae ($\mu = 38.82 \pm 0.80$) were similar, while Taxaceae ($\mu = 29.03 \pm 4.79$) was significantly smaller ($p < 0.05$) in comparison. When samples were grouped by genera, *Taxodium* (n=2, plus 1 replicate) had the smallest range of C:N (32.94–34.32) and *Pinus* (n=38, plus 8 replicates) the largest (23.38–62.13) out of the conifers.

Isotopic Analysis

In general, conifers exhibited a greater range of Δ_{leaf} (16.63–24.97‰) but smaller mean Δ_{leaf} ($\mu = 20.03 \pm 0.21$‰) than the dicots (19.45–24.14‰, $\mu = 22.08 \pm 0.50$‰) (**Figure 3**). When

examined by family, Pinaceae (n=46, plus 9 replicates) had the largest and Taxaceae (n=5, no replicates) the smallest range of Δ_{leaf} (16.58–24.97‰ and 18.40–23.12‰, respectively) of the conifer groups. Within the dicot plants, none of the genera were significantly different from one another ($p > 0.05$), but among the conifer families, Pinaceae Δ_{leaf} ($\mu = 20.65 \pm 0.22$‰) was significantly greater ($p < 0.05$) than Cupressaceae Δ_{leaf} ($\mu = 18.31 \pm 0.36$‰) (**Figure 3**).

When the specimens were separated by genus, there was greater isotopic distinction between the groups with the dicot *Acer* (n=2, plus 2 replicates) having the smallest (19.83–19.92‰) range overall; among the conifer groups *Juniperus* (n=6, no replicates) had the largest (14.63–21.98‰) and *Thuja* (n=4, no replicates) the smallest (18.48–19.38‰) ranges of Δ_{leaf}. All of the conifer genera within a given family did not significantly differ ($p > 0.05$) from one another. *Larix* (n=4, $\mu = 23.21 \pm 0.75$‰), however, was more similar to the dicot plants and was the only genus within Pinaceae that was significantly greater ($p < 0.05$) than all of Cupressaceae.

C:N and Δ_{leaf}

There was no relationship ($r^2 = 0.00$) between C:N and Δ_{leaf} for the HLG conifers (**Figure 7**). The HLG dicots exhibited a weak negative relationship ($r^2 = 0.27$) between C:N and Δ_{leaf} (**Figure 7**).

Phylogenetic distance, C:N, and Δ_{leaf}

There was no apparent relationship between C:N and taxonomic grouping; mean C values were relatively similar between genera while N behaved unpredictably (**Figure 3**). When both deciduous and evergreen conifer genera were included in the analysis, there was a weak to moderate correlation between phylogenetic distance and Δ_{leaf} with a majority of the r^2 values falling between 0.20 and 0.56; the more similar two given genera were phylogenetically, the more similar their mean Δ_{leaf} values (**Figure 4).** The strength of the relationship depended on

which genus served as the anchor being phylogenetically compared to the other conifer groups, with strongest relationship being exhibited by *Larix* ($r^2 = 0.82$) and the weakest by *Taxus* ($r^2 = 0.08$) (**Figure 4**). When deciduous conifers (i.e., *Larix*) were removed, the majority of r^2 values fell between 0.70 and 0.99; *Taxus* and *Juniperus* displayed the weakest and strongest relationships, respectively ($r^2 = 0.40$ and $r^2 = 0.99$) (**Figure 5**).

DISCUSSION

Elemental Analysis

Plants reflect the response of the terrestrial biosphere to the anthropogenically-induced changes in the atmosphere's chemical composition. Elevated levels of $CO_{2(atm)}$ increase the rate of photosynthetic C fixation by leaves, which produces more photosynthate, and results in faster growth rates[16]. As plants are able to maintain a higher rate of photosynthesis with relatively low stomatal conductance, water use efficiency (WUE) increases[16, 17]. The combined effect of the increased photosynthate and WUE is thought to explain decreases in plant tissue N composition; N within plant tissues is diluted by the extra non-structural carbohydrates produced during photosynthesis, and greater WUE means that fewer minerals are taken up from the soil as plants require less water [18]. The decrease in plant tissue N combined with the increase in tissue C composition would ultimately result in greater C:N as $CO_{2(atm)}$ increases.

While there were no significant differences in C:N or C between the modern and historic *Pinus* samples ($p > 0.05$), historic N was significantly greater ($p < 0.05$) than the modern. The insignificant difference in C composition between the historic and modern samples could be the result of simultaneously comparing specimens sampled from different climates across the globe. An analysis of the historic *Pinus* specimens by original sampling location affirms the climatic

influence on plant tissue composition; *Pinus* specimens sampled from various locations in Mexico from 1950 to 1980 showed increases C:N and C and a decrease in N over time (**Figure 6**). The increase in C in the Mexican herbarium tissue samples exhibits an upward trajectory similar to that of $CO_{2(atm)}$ observed from 1958 to 1982, which was potentially related to the increase in anthropogenic C emissions[1] and greater availability of CO_2. Given the nearly nonexistent ($r^2 < 0.00$) relationship between C:N and Δ_{leaf}, it is unlikely that the increase in C:N over time was simply a reflection of *Pinus* leaf economics, but was instead a reflection of a changing climate's influence on leaf elemental composition (**Figure 7**).

Unlike the C data, N was comparatively inconsistent. The inconsistency in tissue N observed across the modern plant specimens is likely a remnant of the environment from which the specimens were sampled; the majority of the HLG samples were collected from wood-mulched planting beds surrounded by fertilized lawn. The root systems of the conifers likely extend beyond the bed, making it possible that the N from the fertilizers was absorbed by the plants that were sampled. Because the historic specimens were wild-collected, it is not likely that anthropogenic N fertilization was a significant factor in each plant's N composition; it is much more likely that the different locations from where the shoots were sampled were naturally subject to different soil conditions, and thus different N compositions between samples.

Isotopic Composition

All of the plants in this study use a C_3 metabolic pathway where CO_2 is converted from a 5-carbon sugar into a 3-carbon sugar[3]. For C_3 plants Δ_{leaf} is a function of fractionation via diffusion in air (4.4‰), carboxylation of Rubisco during photosynthesis (~27‰), and the ambient and intercellular partial pressures of CO_2 [3]. Of the two stable C isotopes, ^{12}C is preferentially selected during photosynthesis as it is able to diffuse more quickly from the

atmosphere to the site of carboxylation and is more reactive than ^{13}C [4]. The intercellular pCO_2 is determined by the influx of CO_2 into the leaf, which is regulated by the plant's stomatal conductance and C assimilation rate, both of which are sensitive to changes in the chemical composition of the atmosphere [3, 16]. The most notable chemical changes are the result of anthropogenic burning of ^{12}C-rich fossil fuels which has led to unprecedented concentrations of $CO_{2(atm)}$ and an isotopically lighter atmosphere[1, 19]**.** Some of the consequences for plants are greater WUE, C assimilation rates, growth during photosynthesis, and a more negative isotopic signature [17].

Similar to other studies, these new results show that there is a difference between Δ_{leaf} between C_3 plant groups, but this study is the first to compare extensively conifers and dicots grown in the same environment[2, 5]. This study found that conifer Δ_{leaf} was significantly less than that of the dicots ($p < 0.05$). Within the conifer groups, Δ_{leaf} varied by taxonomy (**Figure 3**); Pinaceae, for example, had significantly greater mean Δ_{leaf} ($p < 0.05$) than Cupressaceae. Several studies have demonstrated a strong positive correlation between Δ_{leaf} and MAP; greater water availability leads to decreased stomatal limitations on C assimilation, which ultimately reduces WUE[2, 5]. As MAP was uniform for all modern samples in the data set, it seems likely that variations in WUE contributed to the differences between conifers and dicots as well as those among conifer taxonomic groups.

A similar WUE among deciduous taxa is likely what led to the greater isotopic similarity of the deciduous conifer, *Larix*, to the dicot Δ_{leaf} ($p < 0.05$). Similar to other studies, *Larix* Δ_{leaf} was significantly greater ($p < 0.05$) than the other evergreen conifers in the data set[20]. The significantly greater *Larix* and deciduous dicot Δ_{leaf} suggests that *Larix*, and perhaps deciduous C_3 species in general, use water less efficiently than the evergreen conifers[20]. Based on Δ_{leaf}, it is

likely that Cupressaceae have greater WUE than Pinaceae, which could have implications for their use in indicating climatic changes.

In order for a group to serve as an effective climate proxy, it would ideally exhibit a small range of Δ_{leaf} between specimens grown in the same environment. A smaller Δ_{leaf} range suggests that C fractionation was more strongly controlled by climatic conditions than by physiological or vital effects; of the conifer families studied, Pinaceae had the greatest and Cupressaceae the smallest range of Δ_{leaf}. As expected, the dicot plants showed the widest Δ_{leaf} range overall. Ranges in Δ_{leaf} were reduced when the groups were separated by genus; *Juniperus* had the greatest and *Thuja* the smallest ranges of Δ_{leaf} among the conifer genera (**Figure 3**), though the dicot *Acer* had the smallest range overall. The smaller range in *Acer* could perhaps be explained by the comparatively smaller number of specimens analyzed, as two of the samples were replicates.

Of the conifer genera in the data set, *Thuja* is the best candidate for a climate proxy. The small range in Δ_{leaf} values suggests that C fractionation is more heavily influenced by changes in climate than by plant physiology. Further study is needed to test this, but given the strong positive correlation between Δ_{leaf} and MAP observed in other studies[2, 5] comparison of historic and modern *Thuja* Δ_{leaf} using specimens sampled from the same region where the change in $\delta^{13}C_{(atm)}$ is known could test the applicability of *Thuja* as an environmental proxy.

Phylogenetic distance and Δ_{leaf}

The strength of the correlation between Δ_{leaf} and phylogenetic distance varied by genus and leaf type. When evergreen and deciduous conifers were analyzed together, correlations were generally weak to moderate with most r^2 values falling between 0.20 and 0.56 (**Figure 4).** When evergreen conifers were analyzed separately, however, the relationships between Δ_{leaf} and

phylogenetic distance were stronger, with most r^2 values falling between 0.73 and 0.99; the weakest relationship was exhibited when *Taxus* is the anchor genus ($r^2 = 0.40$) and the strongest when *Juniperus* is the anchor genus ($r^2 = 0.99$) (**Figure 5**). The distinction between the two analyses likely stems from the difference in the way that deciduous and evergreen conifers fractionate C; the deciduous *Larix*, for example, exhibited Δ_{leaf} values that were more similar to dicots than to conifers which likely skewed the initial regressions.

Comparisons were only made on a genus level due to insufficient sample sizes on the species level; a species-level comparison should be made to better constrain the relationship between phylogeny and Δ_{leaf}. It is possible that the comparatively weaker correlation shown by *Taxus* is related to the method of analysis; phylogenetic similarity was defined by the number of divergences between the conifer genera within the data set, but I lacked genera that were more similar to *Taxus* relative to the others in the data set [11, 12, 13, 14]. Plant characteristics, such as hydraulic architecture, influence Δ_{leaf} and vary by plant group; as such the poor data resolution across the range of phylogenetic divergences led to a correlation that is possibly weaker than the true value [21]. To better test the relationship between Δ_{leaf} and phylogeny, a greater variety and number of samples grown in the same environment should be collected and compared on the species-level.

Global Context

The HLG data did not show the same relationship as predicted by the Arizona transect conifers (*Juniperus*)[5] and global angiosperms[2] data; given the MAP at HLG (90.96 cm), the transect curve was more predictive of dicot Δ_{leaf} and the global curve more predictive of conifer Δ_{leaf} (**Figure 8**). This becomes more apparent when the data are separated into smaller taxonomic groups (**Appendix 3**). More data would need to be collected to better constrain conifer Δ_{leaf} at

different MAPs and evaluate the discrepancy between the HLG data and the global curves; in particular, Δ_{leaf} data collected from conifer and dicot samples from areas of varying MAP throughout Michigan.

CONCLUSIONS

Results of the elemental analysis indicate that conifer leaf tissues are significantly richer ($p < 0.05$) in C than non-conifers when grown in the same environment. Comparisons between modern and historic *Pinus* samples show increases in C and C:N while N decreases over time, which indicates greater WUE as $CO_{2(atm)}$ increases. Conifer Δ_{leaf} was significantly lower ($p < 0.05$) than dicot Δ_{leaf}, and significant differences ($p < 0.05$) in Δ_{leaf} between conifer genera suggest that hydraulic architecture varies taxonomically. Strong correlations ($r^2 > 0.73$) between Δ_{leaf} and phylogeny for all evergreen conifer genera except for *Taxus* ($r^2 = 0.40$) are further evidence of taxonomically variant WUE, though more study is needed on a species level to evaluate the predictability of the phylogeny-Δ_{leaf} relationship. The small range of Δ_{leaf} in *Thuja* indicates that C fractionation is more influenced by atmospheric conditions than by physiology, thus making *Thuja* the best candidate for a climate proxy in future study. Global curves relating MAP and Δ_{leaf} did not predict HLG Δ_{leaf} data as expected. More study is needed to determine why the Arizona conifer curve was more predictive of dicot Δ_{leaf} while the global angiosperm curve better predicted HLG conifer Δ_{leaf}, but the data presented here suggest that taxonomic grouping is important and better predictive relationships could be derived when these are taken into consideration. A better test to see if there were clear differences in the relationship of MAP and Δ_{leaf} between conifers and dicots would be to examine conifers growing under high water availability (where larger differences between Δ_{leaf}-MAP relationships would be predicted based upon the Arizona conifer transect and the global C_3 database; **Figures 1, 8**), or natural

populations of conifer species that are found under everwet regimes (e.g., *Taxodium distichum*, the bald cypress, which is a swamp species along the Gulf Coast of the USA).

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FIGURES

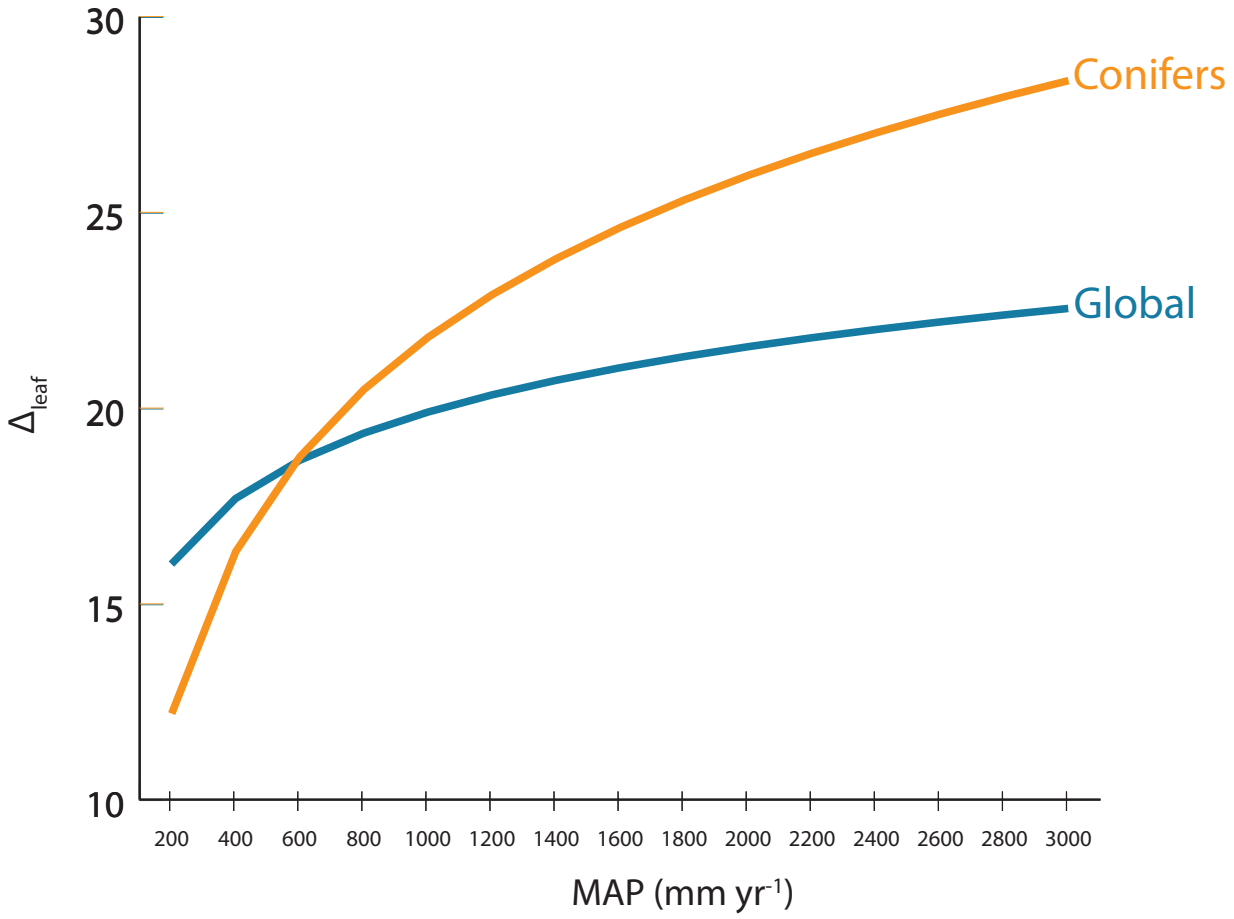

Figure 1 Global Δ_{leaf} data from Diefendorf et al.[2] shown in blue, plotted with Arizona conifer data, shown in orange, from preliminary work done by Sheldon and Smith.[5]

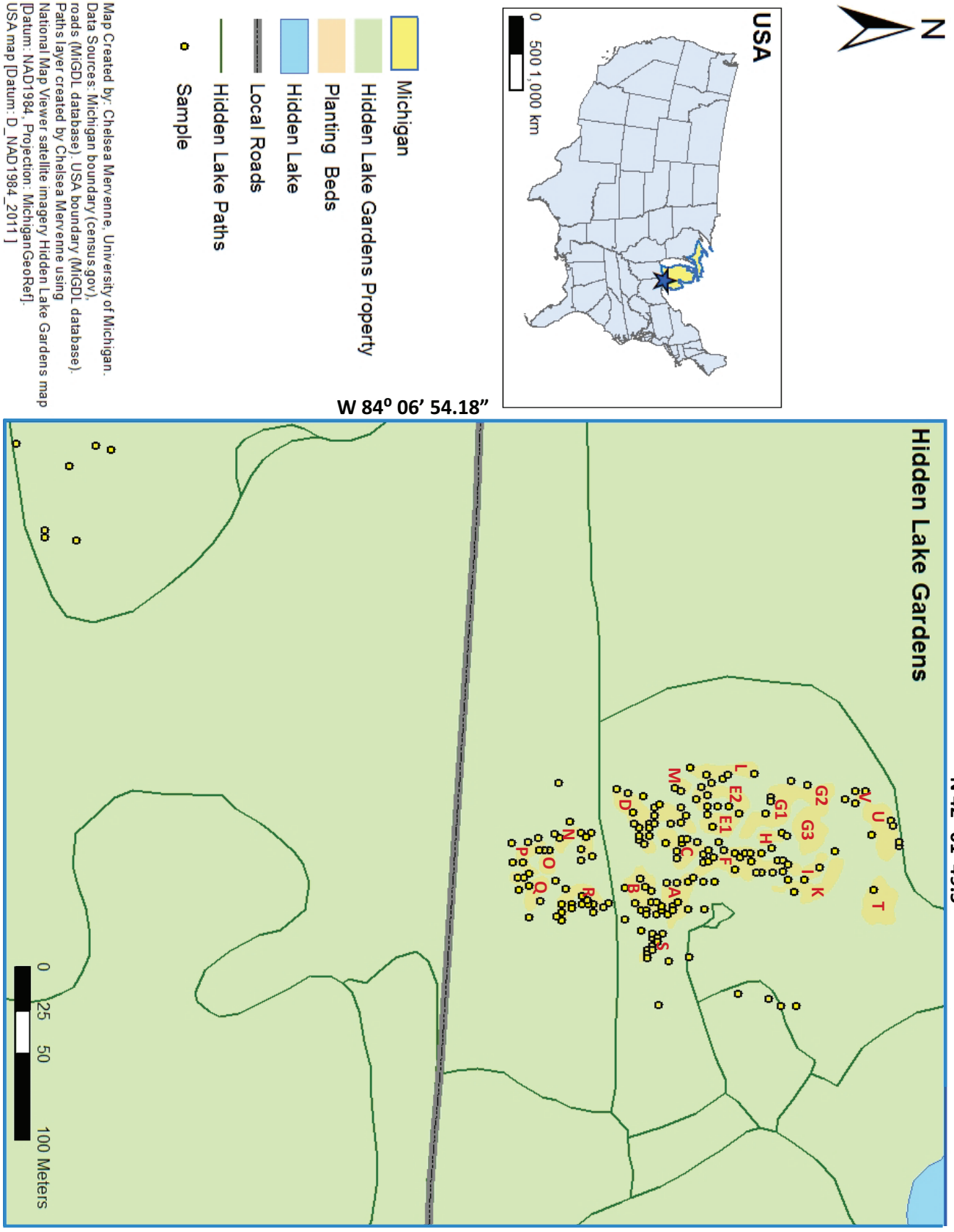

Figure 2 Sampling sites at Hidden Lake Gardens in Tipton, MI; the name of each planting bed is shown in red.

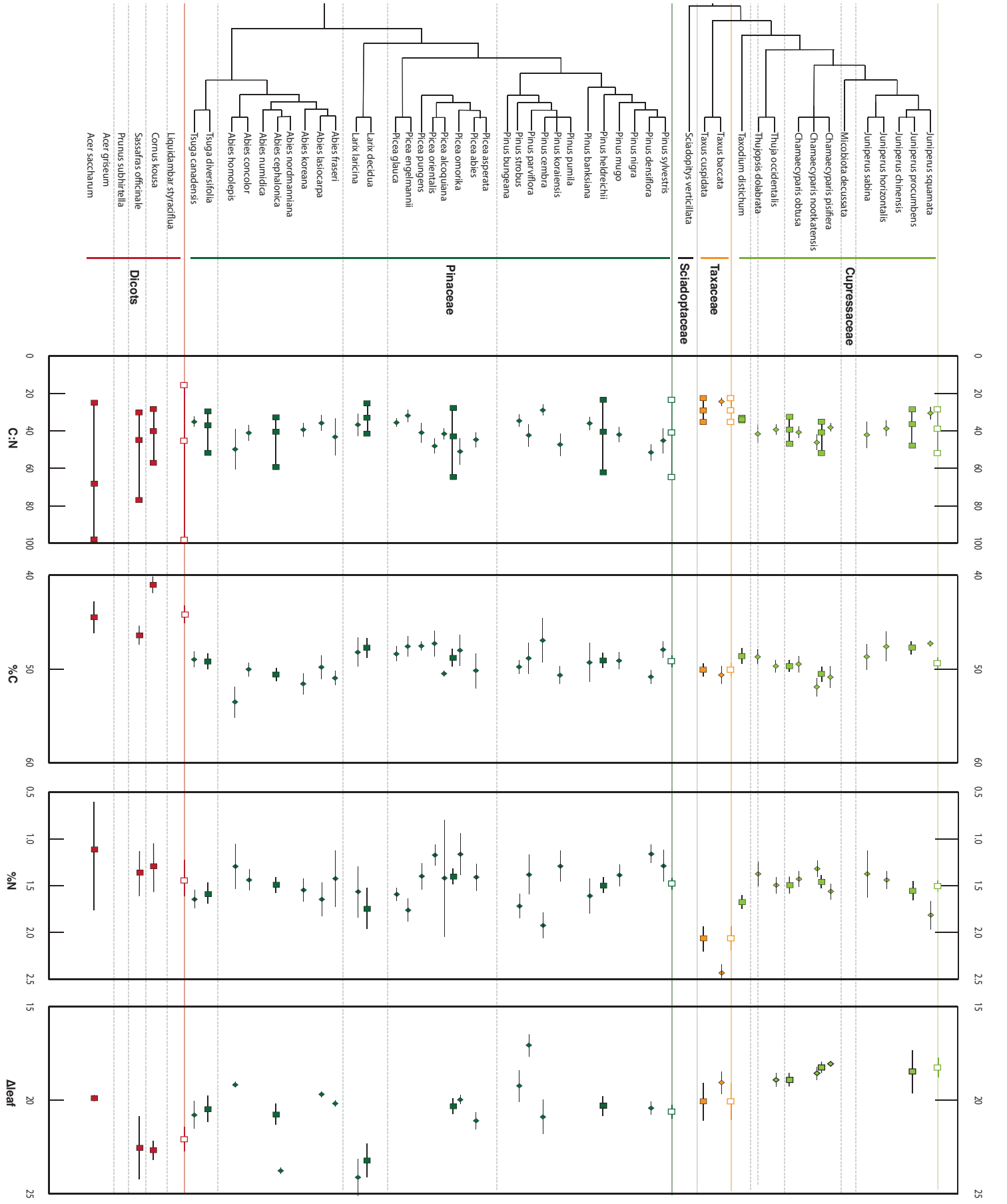

Figure 3 Phylogeny of modern conifer samples with corresponding family (hollow squares), genus (solid squares), and species (diamonds) C:N, %C, %N, and Δ_{leaf}. The dicots are treated as one group composed of multiple genera. Each family is plotted as minimum, mean, and maximum. Each species is plotted as the mean ± standard error.

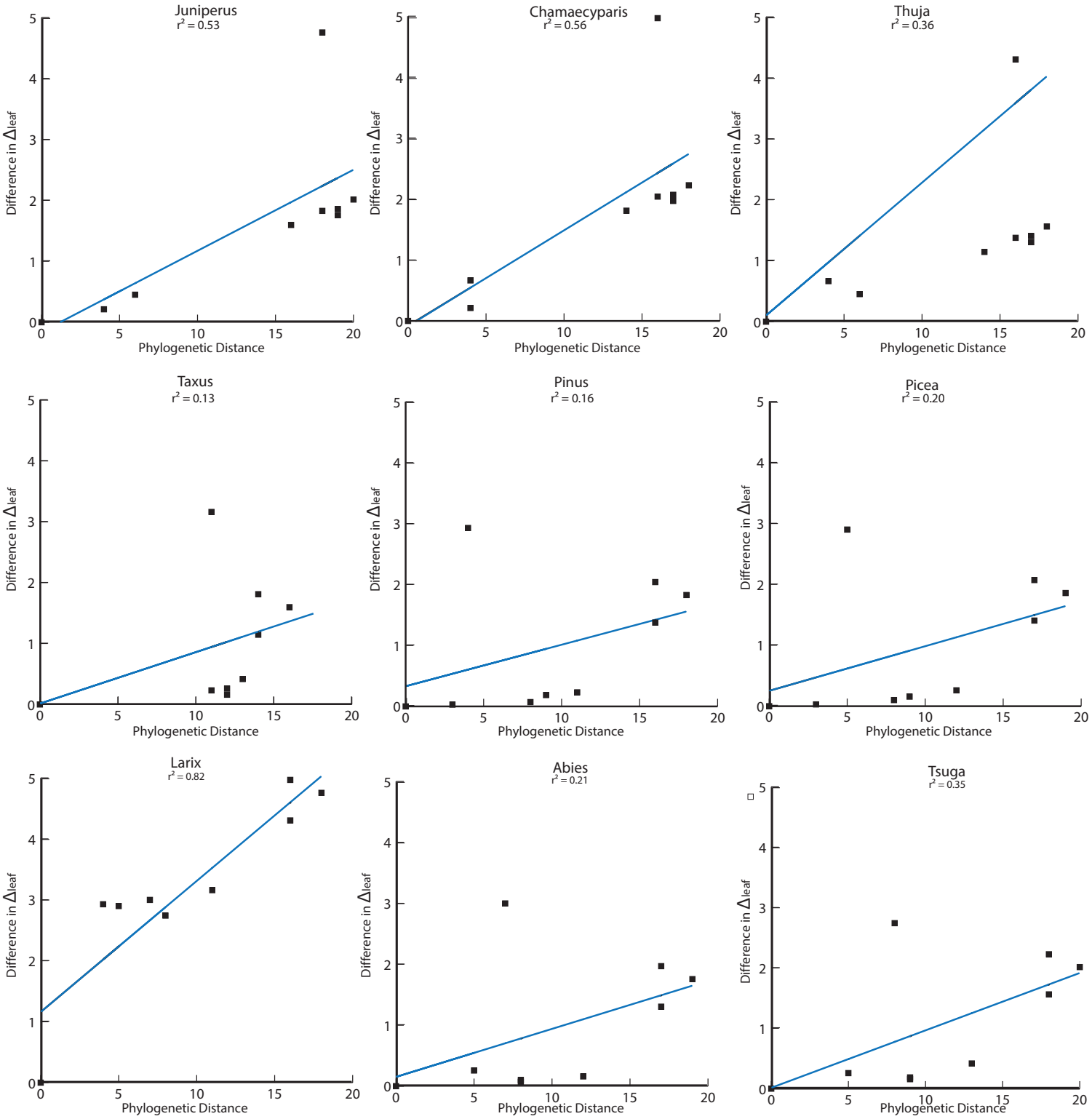

Figure 4 Plots of the difference in Δ_{leaf} versus phylogenetic distance (number of divergences) and the corresponding linear regression (blue) for each conifer genera. The difference in Δ_{leaf} represents the absolute difference Δ_{leaf} between the anchor genus (indicated by the title of each plot) and the other conifer genera in the data set.

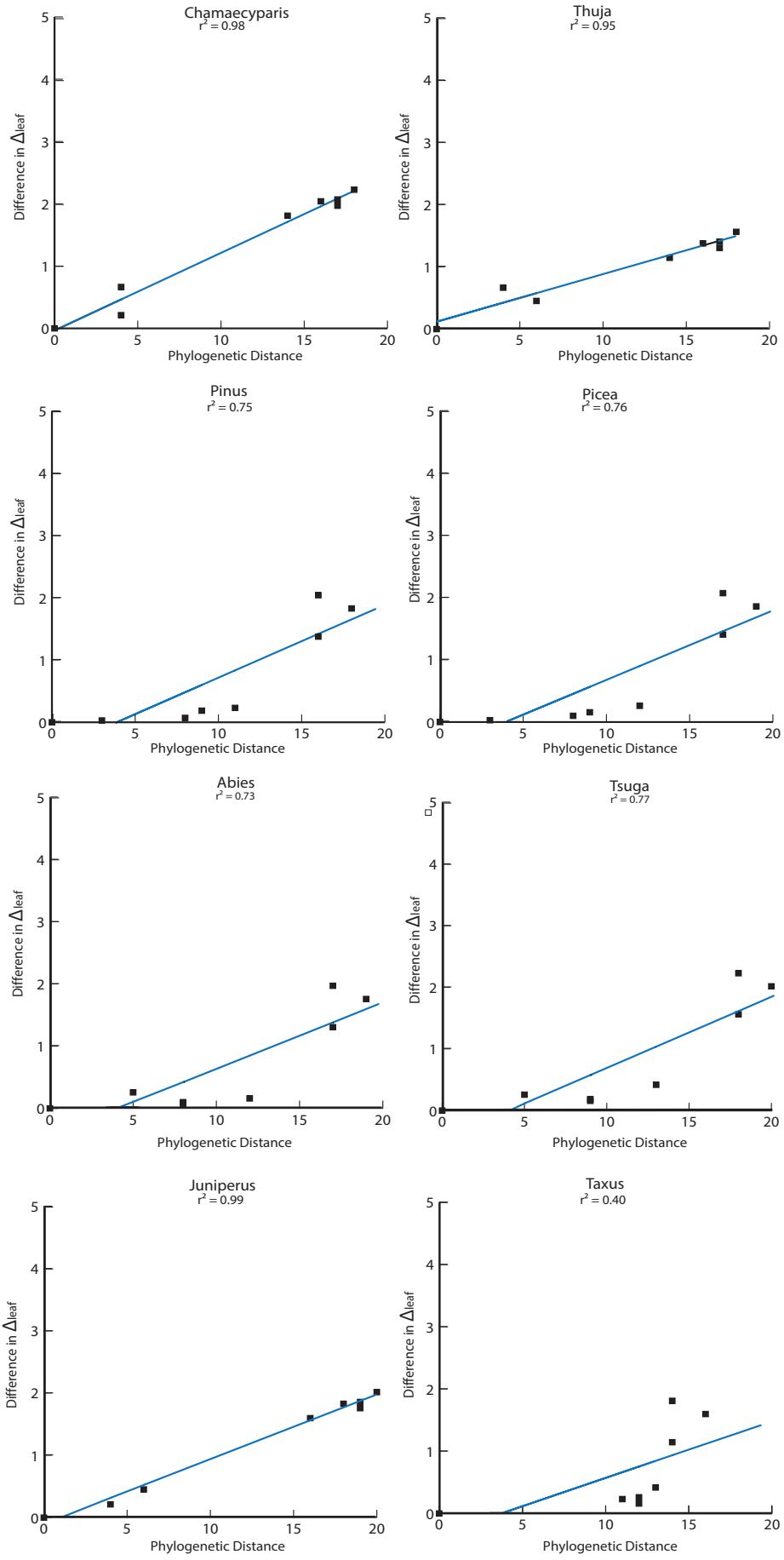

Figure 5 Plots of difference in Δ_{leaf} versus phylogenetic difference (number of divergences) and corresponding linear regression (blue) for evergreen conifer genera. The difference in Δ_{leaf} represents the absolute difference Δ_{leaf} between the anchor genus (indicated by the title of each plot) and the other conifer genera in the data set.

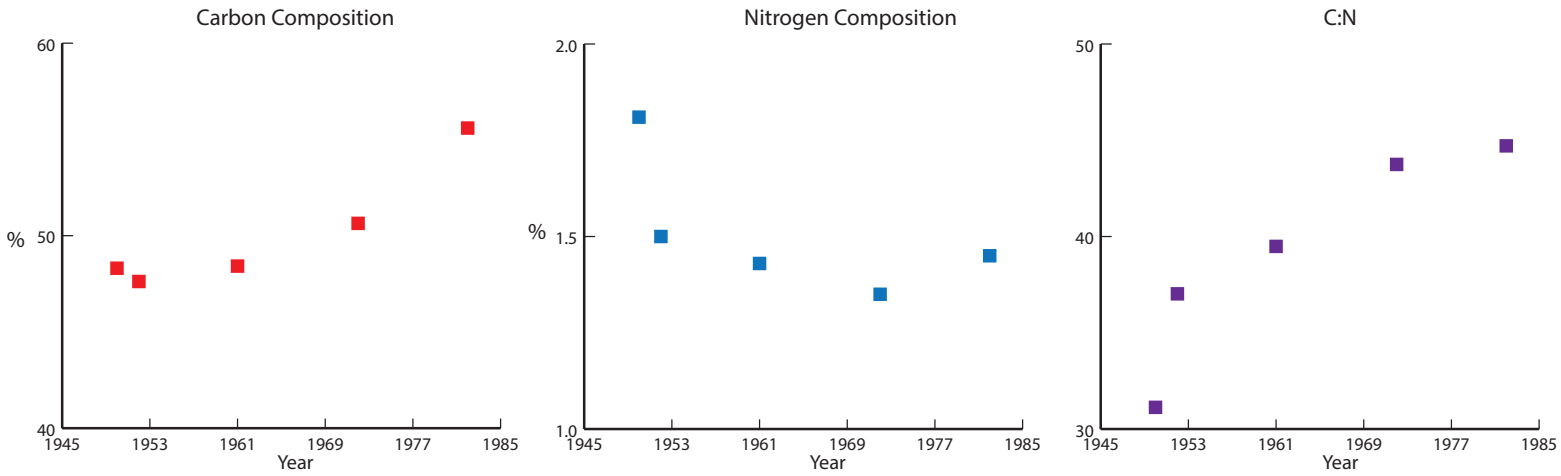

Figure 6 Historic Mexican herbarium C (red), N (blue), and C:N (purple) composition.

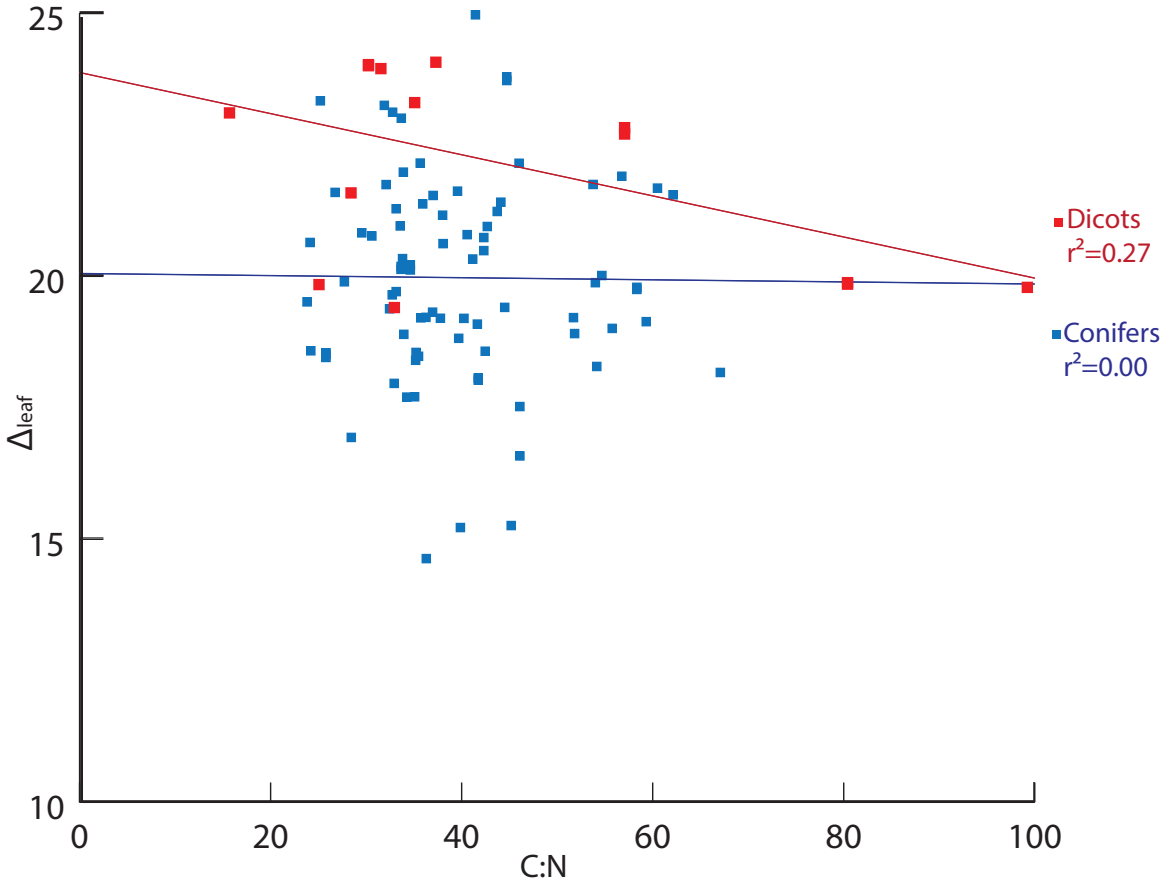

Figure 7 Plot of Δ_{leaf} versus C:N for all conifer and dicot samples.

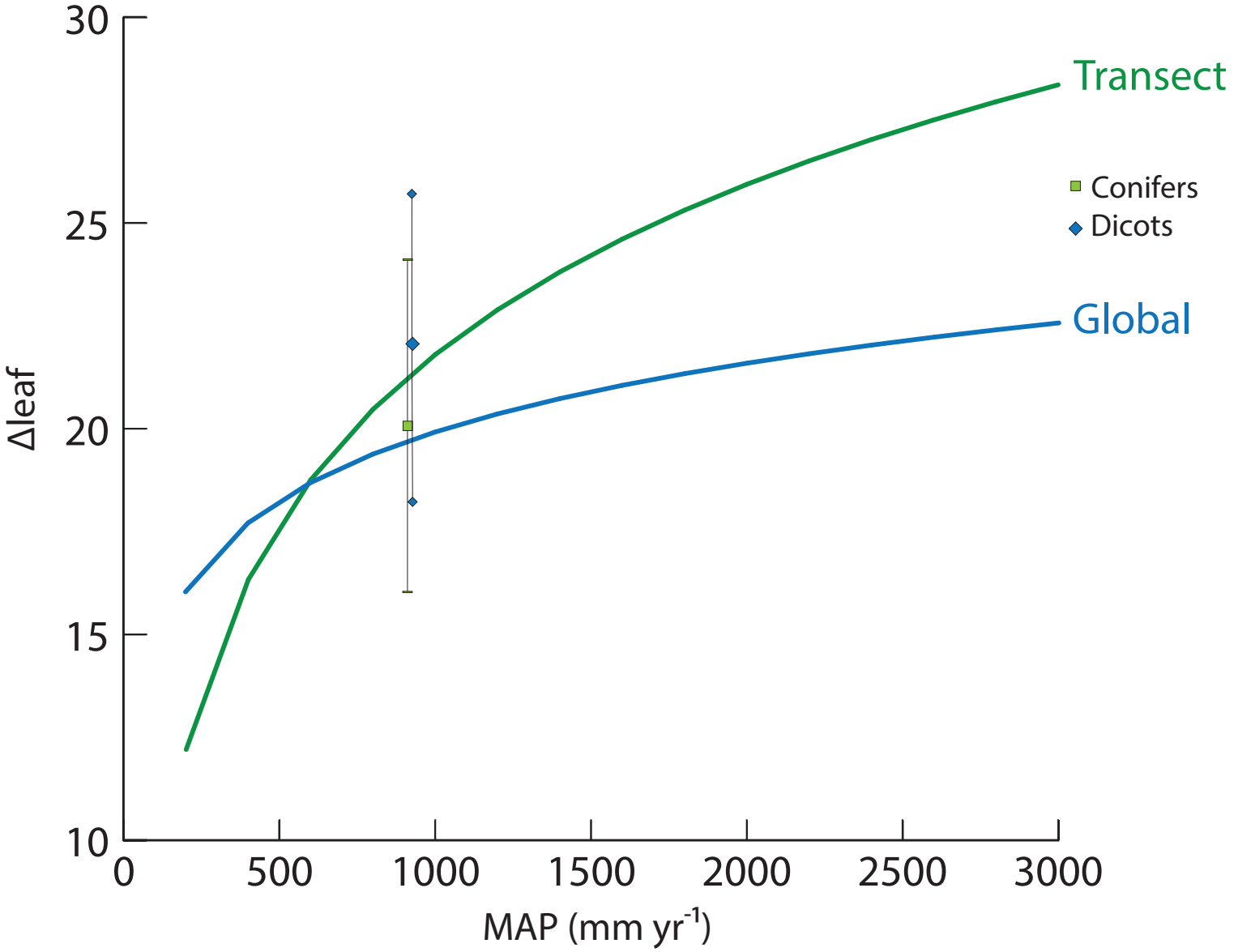

Figure 8 Plot of Δ_{leaf} versus MAP (mm yr^{-1}). HLG conifers (green squares) and dicots (blue diamonds) are plotted with Arizona transect conifers from Sheldon and Smith[5] and Global data from Diefendorf et al.[2]

TABLES

Table 1. List of HLG Samples Analyzed

	FAMILY	GENUS	SPECIES
Outgroup	**Altingiaceae**	***Liquidambar***	*styracifllua (n = 2)*
	Cornaceae	***Cornus***	*kousa chinensis (n = 1)*
			kousa (n = 2)
	Lauraceae	***Sassafras***	*officinale (n = 4)*
	Rosaceae	***Prunus***	*subhirtella (n = 1)*
	Sapindaceae	***Acer***	*griseum (n = 1)*
			saccharum (n = 2)
Conifers	**Cupressaceae**	***Chamaecyparis***	*nootkatensis (n = 1)*
			obtusa (n = 8)
			pisifera (n = 5)
		Juniperus	*chinensis (n = 1)*
			chinenis procumbens (n = 1)
			horizontalis (n = 3)
			media (n = 2)
			procumbens (n = 1)
			sabina (n = 2)
			squamata (n = 2)
		Microbiota	*decussata (n = 1)*

Conifers	**Cupressaceae**	***Taxodium***	*distichum (n = 1)*
		Thuja	*occidentalis (n = 8)*
		Thujopsis	*dolabrata (n = 1)*
		Xanthocyparis	*nootkatensis (n = 4)*
	Pinaceae	***Abies***	*cepalonica (n = 1)*
			chensiensis (n = 1)
			concolor (n = 3)
			fraseri (n = 2)
			homolepis (n = 2)
			koreana (n = 2)
			lasiocarpa (n = 2)
			nordmanniana (n = 1)
			numidica (n = 1)
		Cedrus	*deodara (n = 1)*
			libani stenocoma (n = 1)
		Larix	*decidua (n = 1)*
			kaempferi (n = 1)
			larcina (n = 2)
		Picea	*abies (n = 14)*
			asperata (n = 1)
			bicolor (n = 2)

Conifers	**Pinaceae**	***Picea***	*engelmannii* (n = 2)
			glauca (n = 5)
			omorika (n = 4)
			orientalis (n = 6)
			pungens (n = 5)
		Pinus	*banksiana* (n = 3)
			cembra (n = 6)
			densiflora (n = 4)
			densiflora x nigra (n = 1)
			heldreichii (n = 1)
			koraiensis (n = 3)
			mugo (n = 5)
			nigra (n = 1)
			parviflora (n = 3)
			pumila (n = 1)
			strobus (n = 7)
			sylvestris (n = 3)
			Unknown Hybrid (n = 1)
		Pseudotsuga	*menziesii* (n = 1)
		Tsuga	*canadensis* (n = 7)
			diversifolia (n = 1)

Conifers	**Taxaceae**	***Taxus***	*baccata (n = 2)*
			cuspidata (n = 3)
			media (n = 4)
	Sciadopityaceae	***Sciadopitys***	*verticillia (n = 1)*

Table 2. List of Historic Samples Analyzed.

GENUS	SPECIES	n
Pinus	*densiflora*	2
	halepensis	2
	insularis	1
	massoniana	1
	mugus	1
	oocarpa	3
	parviflora	3
	patula tecunumanii	1
	ponderosa	1
	pseudostrobus	1
	pseudostrobus oaxacana	1

	sibirica humistrata	1
	sylvestris	2

APPENDICES

Appendix 1. Phylogenetic Distances

	Number of Divergences								
	Juniperus	***Chamaecyparis***	***Thuja***	***Taxus***	***Pinus***	***Picea***	***Larix***	***Abies***	***Tsuga***
Juniperus	0	4	6	16	18	19	18	19	20
Chamaecyparis	4	0	4	14	16	17	16	17	18
Thuja	6	4	0	14	16	17	16	17	18
Taxus	16	14	14	0	11	12	11	12	13
Pinus	18	16	16	11	0	3	4	8	9
Picea	19	17	17	12	3	0	5	8	9
Larix	18	16	16	11	4	5	0	7	8
Abies	19	17	17	12	8	8	7	0	5
Tsuga	20	18	18	13	9	9	8	5	0

Appendix 2. Data Normality

The C:N and isotope data are treated as Gaussian based on the relatively normal behavior of the data exhibited in Q-Q plots and histograms created in SPSS (**Figures A–D).** Respective measures of skew and kurtosis the modern (0.846 ± 0.171 and 0.649 ± 0.340) and historic (0.936 ± 0.513 and 0.163 ± 0.992) conifer C:N data were within the acceptable range of -2 to 2 (**Figures A, C**) [21]. When the modern conifer data were separated according to taxonomic family, those with larger sample sizes, such as Pinaceae (n =140), more closely resembled a Gaussian distribution (**Figure E)**, while families with fewer samples, such as Taxaceae, did not behave as normally, which was likely due to the comparatively smaller sample sizes given the normal behavior of the larger data sets. Isotopic conifer data were within the acceptable ranges of skew and kurtosis and were thus treated as Gaussian (**Figure F**). The dicot data had more extreme values of skew and kurtosis, however this is likely due the comparatively smaller sample size.

Outliers, defined as points greater than two standard deviations from the mean, were removed from conifer datasets if present (based on a species-level comparison). Outliers were not removed from the dicot dataset because the specimens collected were not limited to any specific phylogeny; dicot specimens were simply non-conifers found at the Hidden Lakes sampling location, and thus any outliers are likely indicative of real-world variation.

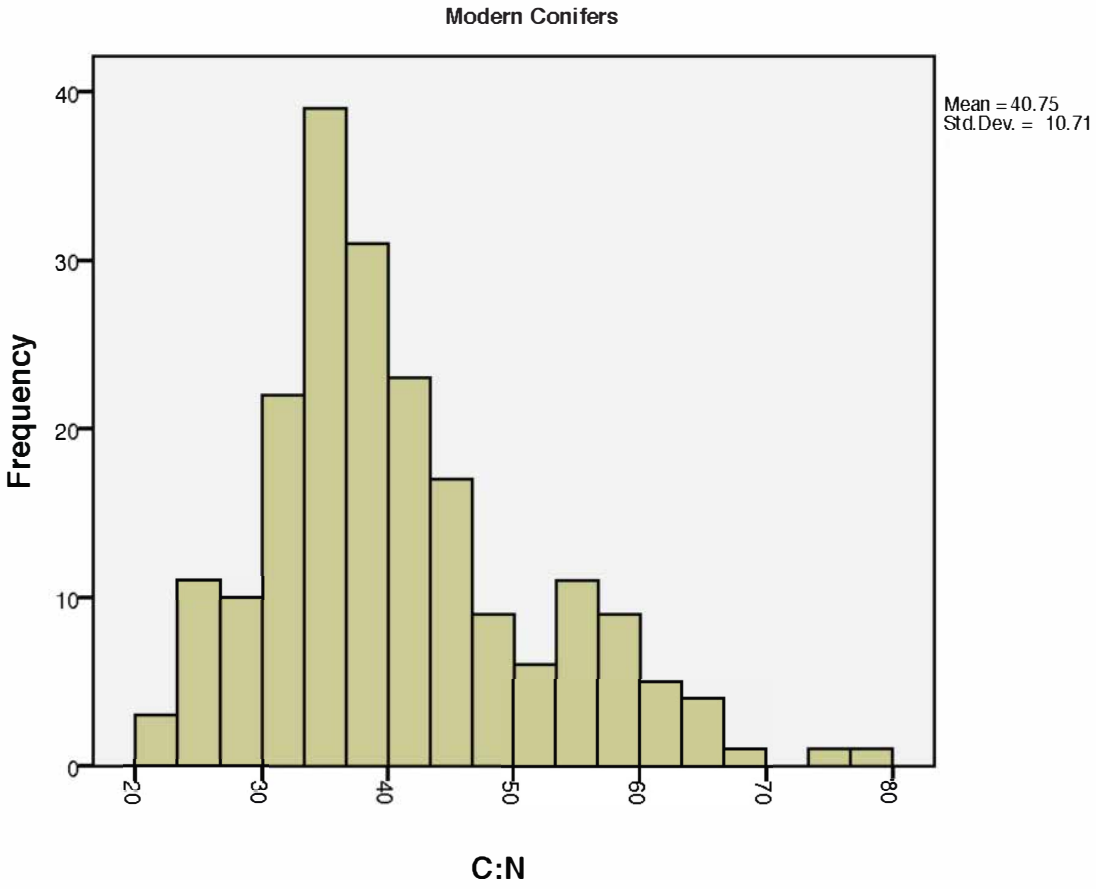

Figure A Distribution of C:N of all modern conifer samples.

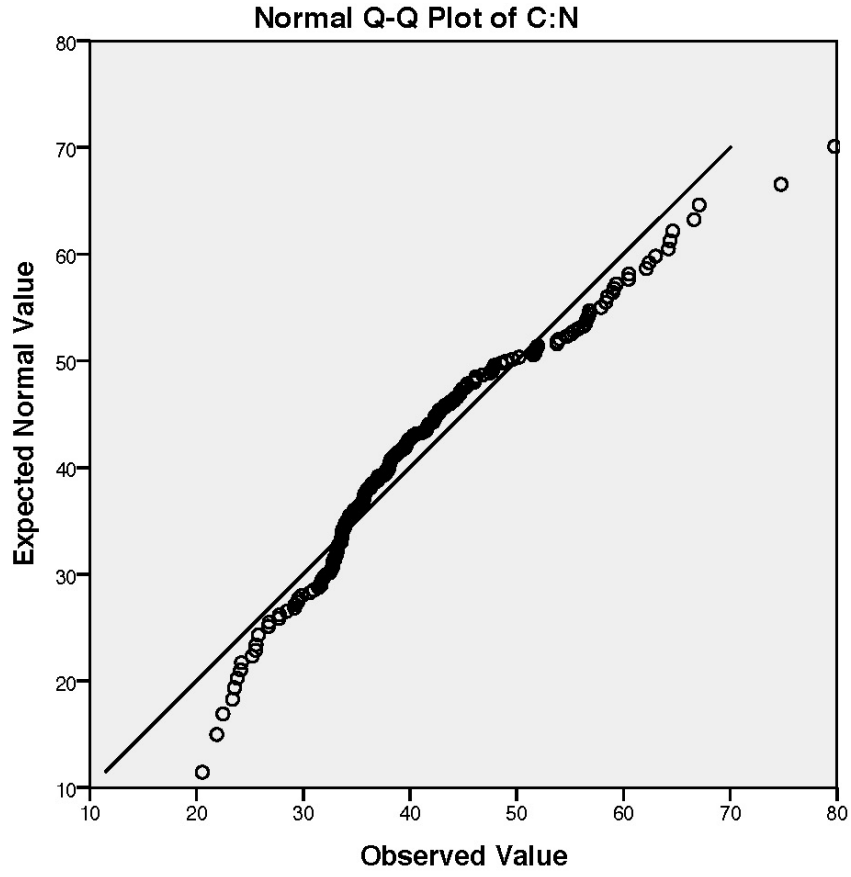

Figure B Q-Q plot of all modern conifer data.

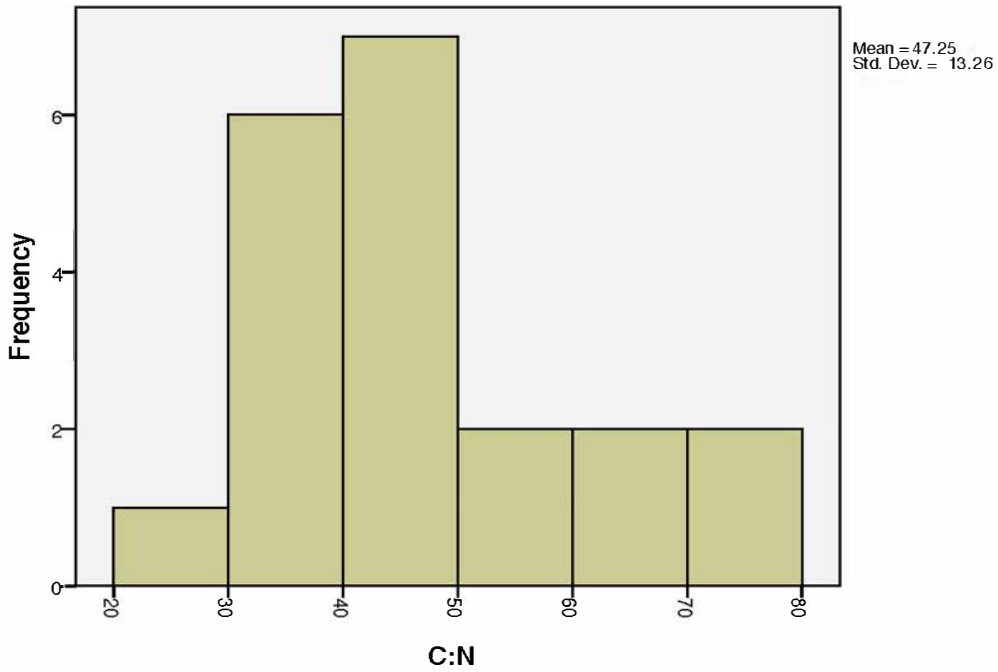

Figure C Histogram of historic Pinaceae C:N.

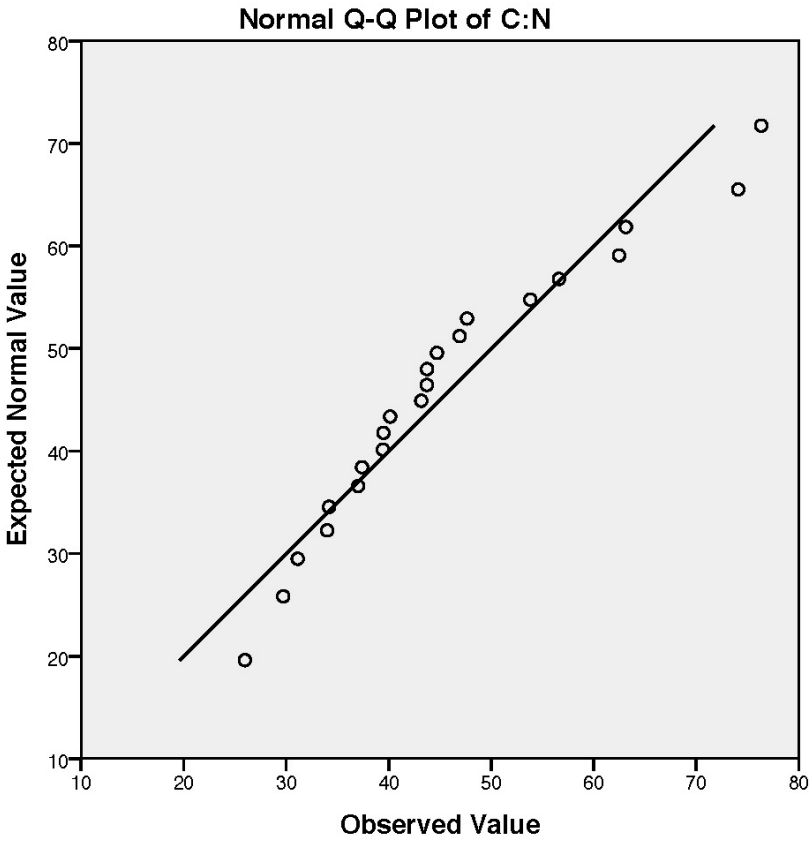

Figure D Q-Q plot of historic Pinaceae C:N data.

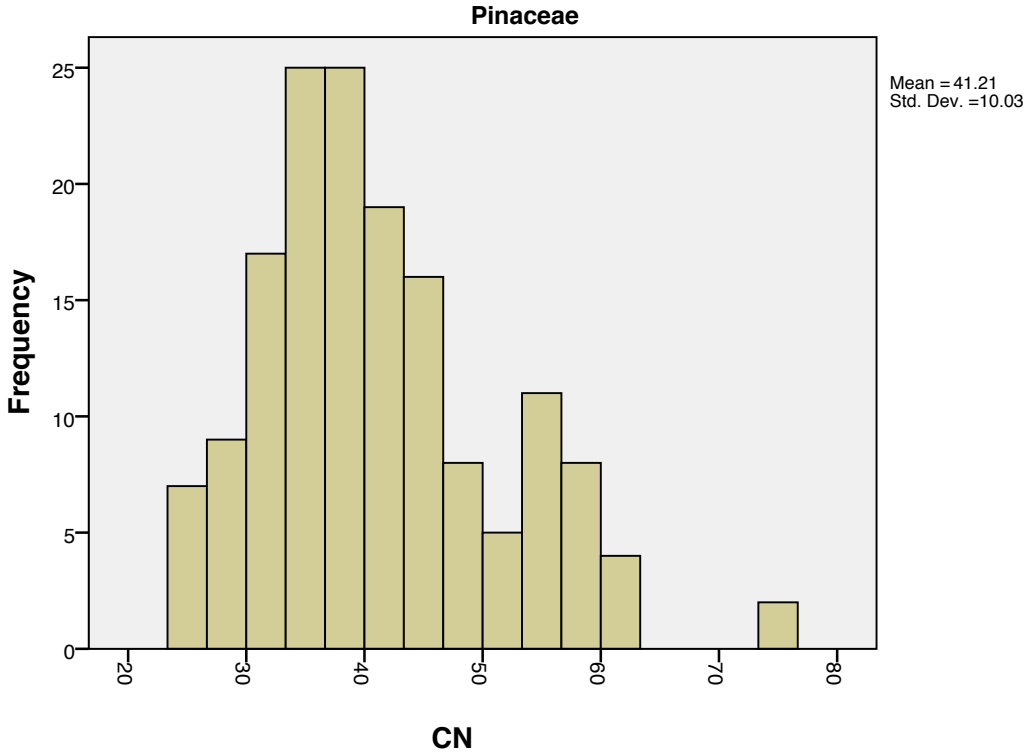

Figure E Histogram of Pinaceae C:N.

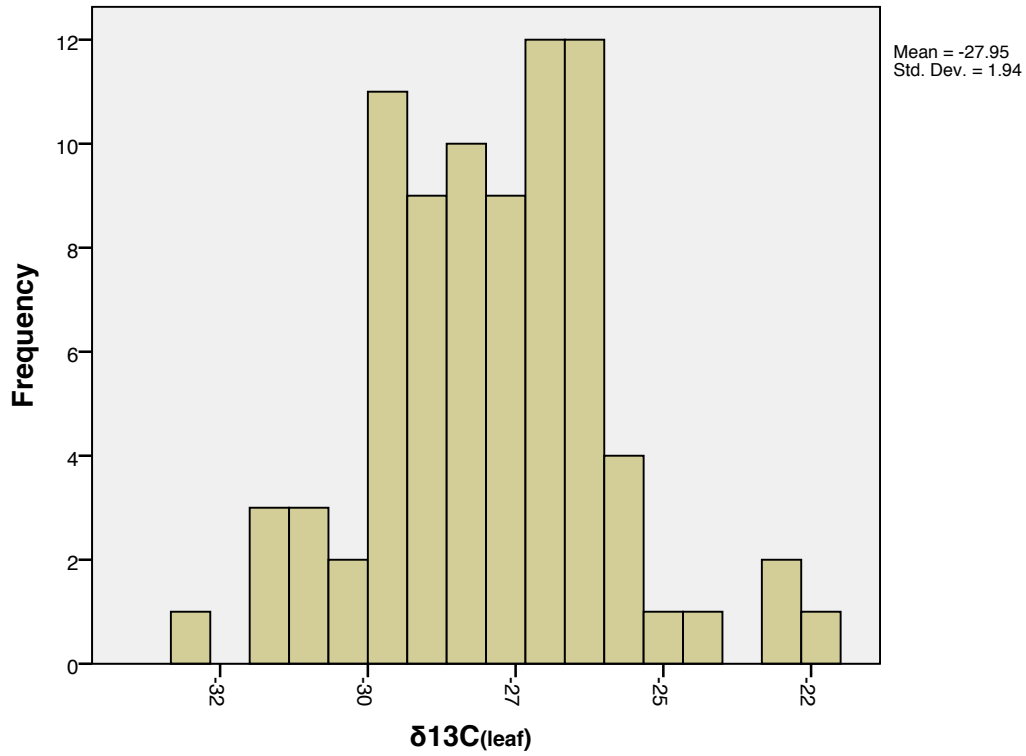

Figure F Histogram of modern conifer $\delta^{13}C_{(leaf)}$.

Appendix 3. Global Curve Predictions of Δ_{leaf}

HLG Group	HLG Group Mean Δ_{leaf} (Actual)	Standard Deviation	Predicted HLG Δ_{leaf} (AZ Transect Curve; MAP = 910 mm yr^{-1})	Predicted HLG Δ_{leaf} (Global Curve; MAP = 910 mm yr^{-1})
Conifers	20	1.51	21.24	19.69
Dicots	22.08	1.89		
Juniperus	18.53	1.07		
Thuja	18.91	0.46		
Chamaecyparis	18.24	1.74		
Pinus	20.28	1.74		
Taxus	20.05	1.93		
Picea	20.31	0.94		
Abies	20.21	0.74		
Tsuga	20.47	1.26		
Larix	23.21	1.16		

Appendix 4. HLG Data

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Altingiaceae	*Liquidambar*	*styraciflua*	NA	195	NA	NA	1.38	44.02	37.20	-32.14
Altingiaceae	*Liquidambar*	*styraciflua*	NA	196	NA	NA	1.6	43.12	31.43	-32.01
Cornaceae	*Cornus*	*kousa*	NA	193	42.02939167	-84.11148056	1.39	41.69	34.98	-31.36
Cornaceae	*Cornus*	*kousa*	NA	194	42.02955	-84.11143889	0.85	41.56	57.02	-30.89
Cornaceae	*Cornus*	*kousa*	NA	194	42.02955	-84.11143889	0.85	41.56	57.02	-30.76
Cornaceae	*Cornus*	*kousa chenensis*	NA	205	42.02969444	-84.11138889	1.64	39.79	28.29	-29.64
Cupressaceae	*Chamaecyparis*	*nootkatensis*	D	82	42.02888889	-84.11261111	1.2	53.33	51.83	-26.91
Cupressaceae	*Chamaecyparis*	*obtusa*	A	41	42.02902778	-84.11227778	1.65	49.63	35.08	-25.71
Cupressaceae	*Chamaecyparis*	*obtusa*	A	43	42.02902778	-84.11227778	1.44	47.75	38.67	
Cupressaceae	*Chamaecyparis*	*obtusa*	E2	108	42.02936111	-84.11280556	1.43	48.89	39.87	-23.22
Cupressaceae	*Chamaecyparis*	*obtusa*	F	60	42.02947222	-84.11241667	1.5	49.31	38.34	NA
Cupressaceae	*Chamaecyparis*	*obtusa*	F	70	42.0295	-84.11233333	0.75	48.07	74.74	NA
Cupressaceae	*Chamaecyparis*	*obtusa*	H	169	42.02966667	-84.11258333	1.27	52.19	47.92	NA
Cupressaceae	*Chamaecyparis*	*obtusa*	P	127	42.02841667	-84.11252778	1.04	50.42	56.54	NA
Cupressaceae	*Chamaecyparis*	*obtusa*	P	131	42.02830556	-84.11258333	1.29	49.09	44.38	NA
Cupressaceae	*Chamaecyparis*	*pisifera*	A	32	42.02902778	-84.11205556	1.32	47.24	41.74	-26.03
Cupressaceae	*Chamaecyparis*	*pisifera*	A	32	42.02902778	-84.11205556	1.32	47.24	41.74	-26.07
Cupressaceae	*Chamaecyparis*	*pisifera*	A	33	42.02905556	-84.11211111	1.6	49.91	36.38	
Cupressaceae	*Chamaecyparis*	*pisifera*	E2	113	42.02919444	-84.11286111	1.57	52.24	38.80	NA
Cupressaceae	*Chamaecyparis*	*pisifera*	E2	113	42.02919444	-84.11286111	1.48	50.22	39.57	NA

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Cupressaceae	*Chamaecyparis*	*pisifera*	F	48	42.02922222	-84.11227778	1.7	52.07	35.72	NA
Cupressaceae	*Chamaecyparis*	*pisifera*	L	163	42.02847222	-84.113	1.7	53.58	36.76	NA
Cupressaceae	*Juniperus*	*chinensis*	F	50	42.02922222	-84.11241667	1.67	48.61	33.94	-26.90
Cupressaceae	*Juniperus*	*chinensis procumbens*	R	152	42.02852778	-84.11211111	1.68	48.83	33.90	-29.98
Cupressaceae	*Juniperus*	*horizontalis*	K2	186	42.02969444	-84.11219444	1.51	46.29	35.75	NA
Cupressaceae	*Juniperus*	*horizontalis*	Shrub	207	42.02580556	-84.11480556	1.37	48.92	41.64	-27.09
Cupressaceae	*Juniperus*	*horizontalis*	Shrub	209	42.02580556	-84.11486111	0.85	48.55	66.61	
Cupressaceae	*Juniperus*	*media*	C	72	42.02922222	-84.11247222	2.56	45.08	20.54	
Cupressaceae	*Juniperus*	*media*	F	49	42.02916667	-84.11230556	1.55	47.19	35.50	
Cupressaceae	*Juniperus*	*procumbens*	S	11	-84.11191667	-84.11191667	1.4	45.35	37.78	-27.20
Cupressaceae	*Juniperus*	*sabina*	S	6	42.02894444	-84.11183333	1.6	49.8	36.30	-22.63
Cupressaceae	*Juniperus*	*sabina*	S	7	42.02894444	-84.11188889	1.16	47.57	47.82	
Cupressaceae	*Juniperus*	*squamata*	U	182	42.03025	-84.1125	1.94	47.32	28.45	-24.94
Cupressaceae	*Juniperus*	*squamata*	U	183	42.03025	-84.11252778	1.69	47.24	32.60	
Cupressaceae	*Microbiota*	*decussata*	R	146	42.02863889	-84.11213889	1.09	52.12	55.76	-27.01
Cupressaceae	*Taxodium*	*distichum*	J5	63	42.02947222	-84.11247222	1.63	47.9	34.27	-25.70
Cupressaceae	*Taxodium*	*distichum*	J5	63	42.02947222	-84.11247222	1.76	49.72	32.94	
Cupressaceae	*Taxodium*	*distichum*	P	134	42.02827778	-84.11244444	1.64	48.27	34.32	
Cupressaceae	*Taxodium*	*distichum*	R	151	42.02852778	-84.11213889	2.57	48.25	21.89	
Cupressaceae	*Thuja*	*occidentalis*	A	37	42.02908333	-84.11213889	1.58	49.13	36.26	-27.22
Cupressaceae	*Thuja*	*occidentalis*	A	38	42.02922222	-84.11208333	1.34	48.44	42.16	
Cupressaceae	*Thuja*	*occidentalis*	A	38	42.02922222	-84.11208333	1.4	49.94	41.60	
Cupressaceae	*Thuja*	*occidentalis*	D	96	42.02911111	-84.11280556	1.58	48.08	35.49	-26.48
Cupressaceae	*Thuja*	*occidentalis*	F	62	42.02947222	-84.11241667	1.67	50.44	35.22	-26.55
Cupressaceae	*Thuja*	*occidentalis*	F	62	42.02947222	-84.11241667	1.32	51.32	45.34	
Cupressaceae	*Thuja*	*occidentalis*	J6	67	42.02963889	-84.11233333	1.21	48.58	46.82	
Cupressaceae	*Thuja*	*occidentalis*	N	118	42.02861111	-84.11261111	1.55	50.22	37.78	
Cupressaceae	*Thuja*	*occidentalis*	Shrub	208	42.02597222	-84.11477778	1.86	51.76	32.45	-27.38
Cupressaceae	*Thuja*	*occidentalis*	R	156	NA	NA	1.44	49.06	39.73	
Cupressaceae	*Thujopsis*	*dolobrata*	A	40	42.02908333	-84.11227778	1.48	48.2	37.98	

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Cupressaceae	*Thujopsis*	*dolobrata*	A	40	42.02908333	-84.11227778	1.27	49.21	45.19	-23.26
Cupressaceae	*Xanthocyparis*	*nootkatensis*	A	34	42.02905556	-84.11208333	0.97	51.89	62.38	
Cupressaceae	*Xanthocyparis*	*nootkatensis*	A	35	42.02905556	-84.11208333	0.86	49.47	67.08	-26.17
Cupressaceae	*Xanthocyparis*	*nootkatensis*	G1	172	42.02955556	-84.11275	1.36	51.45	44.12	
Cupressaceae	*Xanthocyparis*	*nootkatensis*	I	187	42.02975	-84.11227778	1.4	50.98	42.47	-26.58
Lauraceae	*Sassafras*	*officinale*	NA	199	42.03021389	-84.11267778	0.69	45.49	76.88	
Lauraceae	*Sassafras*	*officinale*	NA	200	42.03021389	-84.11267778	1.48	46.37	36.54	
Lauraceae	*Sassafras*	*officinale*	NA	200	42.03021389	-84.11267778	1.71	48.16	32.84	-27.45
Lauraceae	*Sassafras*	*officinale*	NA	201	42.03022222	-84.11264444	1.85	47.79	30.13	-32.07
Lauraceae	*Sassafras*	*officinale*	NA	201	42.03022222	-84.11264444	1.85	47.79	30.13	-32.09
Lauraceae	*Sassafras*	*officinale*	NA	202	42.03022222	-84.11264444	1.08	44.25	47.78	
Pinaceae	*Abies*	*cepalonica*	C	80	42.02908333	-84.11244444	1.3	49.86	44.73	-31.79
Pinaceae	*Abies*	*cepalonica*	C	80	42.02908333	-84.11244444	1.3	49.86	44.73	-31.72
Pinaceae	*Abies*	*chensiensis*	J4	174	42.02969444	-84.11297222	1.49	48.56	38.01	-29.16
Pinaceae	*Abies*	*concolor*	E2	109	42.02927778	-84.11266667	1.55	49.19	37.01	
Pinaceae	*Abies*	*concolor*	E2	110	42.02941667	-84.11275	1.61	49.24	35.67	
Pinaceae	*Abies*	*concolor*	E2	110	42.02941667	-84.11275	1.36	50.99	43.72	-29.23
Pinaceae	*Abies*	*concolor*	V	178	42.03002778	-84.11288889	1.24	50.73	47.71	
Pinaceae	*Abies*	*fraseri*	D	104	42.02894444	-84.11261111	1.16	51.47	51.74	
Pinaceae	*Abies*	*fraseri*	E2	112	42.02922222	-84.11294444	1.7	50.48	34.63	-28.12
Pinaceae	*Abies*	*fraseri*	E2	112	42.02922222	-84.11294444	1.7	50.48	34.63	-28.22
Pinaceae	*Abies*	*homolepis*	K2	185	42.02969444	-84.11219444	1.51	52.11	40.24	-27.20
Pinaceae	*Abies*	*homolepis*	V	179	42.03008333	-84.11288889	1.08	54.94	59.32	-27.14
Pinaceae	*Abies*	*koreana*	B	21	42.02894444	-84.11213889	1.43	53.62	43.73	
Pinaceae	*Abies*	*koreana*	B	21	42.02894444	-84.11213889	1.35	49.44	42.71	
Pinaceae	*Abies*	*koreana*	D	102	42.02894444	-84.11266667	1.62	51.39	36.99	
Pinaceae	*Abies*	*koreana*	D	102	42.02894444	-84.11266667	1.79	51.9	33.81	-28.34
Pinaceae	*Abies*	*lasiocarpa*	L	161	42.02925	-84.11302778	1.81	50.8	32.73	
Pinaceae	*Abies*	*lasiocarpa*	L	161	42.02925	-84.11302778	1.81	50.8	32.73	-27.65
Pinaceae	*Abies*	*lasiocarpa*	L	161	42.02925	-84.11302778	1.79	50.87	33.14	-27.71

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Pinaceae	*Abies*	*lasiocarpa*	S	2	42.02891667	-84.11175	1.34	47.76	41.56	
Pinaceae	*Abies*	*nordmanniana*	V	177	42.03002778	-84.11280556	1.53	49.92	38.05	-28.62
Pinaceae	*Abies*	*numidica*	Q	140	42.02836111	-84.11216667	1.67	48.07	33.57	-28.96
Pinaceae	*Larix*	*decidua*	NA	16	42.02888889	-84.11194444	1.62	46.04	33.14	-29.29
Pinaceae	*Larix*	*decidua*	NA	16	42.02886111	-84.11213889	1.02	49.69	56.81	
Pinaceae	*Larix*	*kaempferi*	D	99	42.02911111	-84.11269444	2.24	48.42	25.21	-31.34
Pinaceae	*Larix*	*laricina*	J15	123	42.02844444	-84.11263889	1.81	49.52	31.91	-31.25
Pinaceae	*Larix*	*laricina*	J18	133	42.02822222	-84.11244444	1.32	46.91	41.44	-32.97
Pinaceae	*Picea*	*abies*	A	31	42.02902778	-84.11205556	1.8	47.23	30.60	-28.77
Pinaceae	*Picea*	*abies*	B	18	42.02886111	-84.11213889	0.91	47.18	60.46	
Pinaceae	*Picea*	*abies*	B	18	42.02886111	-84.11213889	1.36	49.83	42.73	
Pinaceae	*Picea*	*abies*	B	20	42.02891667	-84.11208333	1.52	49.57	38.03	
Pinaceae	*Picea*	*abies*	B	20	42.02891667	-84.11208333	1.51	50.62	39.09	
Pinaceae	*Picea*	*abies*	B	24	42.029	-84.11208333	0.98	48.64	57.88	
Pinaceae	*Picea*	*abies*	B	25	42.029	-84.11211111	0.87	48.2	64.61	
Pinaceae	*Picea*	*abies*	C	71	42.02922222	-84.11247222	1.32	47.8	42.23	
Pinaceae	*Picea*	*abies*	C	75	42.02919444	-84.11255556	1.45	47.38	38.11	
Pinaceae	*Picea*	*abies*	C	75	42.02919444	-84.11255556	1.49	48.84	38.23	
Pinaceae	*Picea*	*abies*	C	78	42.02902778	-84.11255556	1.32	48.74	43.06	
Pinaceae	*Picea*	*abies*	G2	168	42.02963889	-84.11261111	3.06	77.32	29.47	
Pinaceae	*Picea*	*abies*	J15	124	42.02852778	-84.11272222	1.17	46.33	46.18	
Pinaceae	*Picea*	*abies*	J6	47	42.02938889	-84.11236111	1	50.58	58.99	
Pinaceae	*Picea*	*abies*	J6	47	42.02947222	-84.11216667	0.92	50.77	64.36	
Pinaceae	*Picea*	*abies*	L	164	42.02936111	-84.11302778	1.73	46.99	31.68	
Pinaceae	*Picea*	*abies*	R	157	42.02847222	-84.11213889	1.68	48.33	33.55	
Pinaceae	*Picea*	*asperata*	D	87	42.02886111	-84.11277778	1.28	48.85	44.51	-27.41
Pinaceae	*Picea*	*bicolor*	J8	89	42.02883333	-84.11291667	1.48	50.56	39.84	
Pinaceae	*Picea*	*engelmanii*	E1	107	42.02925	-84.11275	1.92	45.64	27.72	-27.90
Pinaceae	*Picea*	*engelmanii*	E1	107	42.02925	-84.11275	1.94	48.55	29.18	
Pinaceae	*Picea*	*engelmanii*	E2	115	42.02925	-84.11288889	1.61	46.79	33.89	

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Pinaceae	*Picea*	*engelmanii*	E2	115	42.02925	-84.11288889	1.58	49.39	36.45	
Pinaceae	*Picea*	*glauca*	E2	111	42.02933333	-84.113	1.64	47.36	33.68	
Pinaceae	*Picea*	*glauca*	E2	111	42.02933333	-84.113	1.69	50.34	34.74	
Pinaceae	*Picea*	*glauca*	F	52	42.02925	-84.11241667	1.69	47.5	32.78	
Pinaceae	*Picea*	*glauca*	F	52	42.02925	-84.11241667	1.57	49.82	37.01	-29.54
Pinaceae	*Picea*	*glauca*	N	116	42.02858333	-84.11263889	1.56	47.57	35.56	
Pinaceae	*Picea*	*glauca*	P	128	42.02838889	-84.11252778	0.97	49.16	59.10	
Pinaceae	*Picea*	*glauca*	S	4	42.02894444	-84.11180556	1.41	47.78	39.52	
Pinaceae	*Picea*	*omorika*	A	39	42.02913889	-84.11227778	0.89	44.53	58.35	-27.75
Pinaceae	*Picea*	*omorika*	A	39	42.02913889	-84.11227778	0.89	44.53	58.35	-27.79
Pinaceae	*Picea*	*omorika*	B	30	42.02880556	-84.11225	1.75	50.5	33.65	-28.19
Pinaceae	*Picea*	*omorika*	B	30	42.02880556	-84.11225	1.75	50.5	33.65	-28.13
Pinaceae	*Picea*	*omorika*	D	86	42.02886111	-84.11269444	0.99	46.97	55.33	
Pinaceae	*Picea*	*omorika*	S	9	42.02894444	-84.11188889	0.88	47.54	63.00	
Pinaceae	*Picea*	*omorika*	S	9	42.02894444	-84.11188889	1.03	50.04	56.66	
Pinaceae	*Picea*	*orientalis*	A	36	42.02917361	-84.11200794	1.29	46.78	42.29	
Pinaceae	*Picea*	*orientalis*	A	36	42.02913889	-84.11208333	1.08	49.78	53.75	-29.75
Pinaceae	*Picea*	*orientalis*	C	73	42.02925	-84.1125	1.01	45.01	51.97	
Pinaceae	*Picea*	*orientalis*	D	100	42.02905556	-84.11269444	0.89	44.65	58.51	
Pinaceae	*Picea*	*orientalis*	D	104	42.02894444	-84.11261111	1.53	52.63	40.12	
Pinaceae	*Picea*	*orientalis*	E1	105	42.02930556	-84.11280556	1.36	47.03	40.33	
Pinaceae	*Picea*	*orientalis*	R	144	42.02869444	-84.11211111	1.06	45.1	49.62	
Pinaceae	*Picea*	*pungens*	C	79	42.02908333	-84.1125	1.65	47.45	33.54	
Pinaceae	*Picea*	*pungens*	D	83	42.02891667	-84.11255556	0.98	47.44	56.45	
Pinaceae	*Picea*	*pungens*	F	57	42.02938889	-84.11247222	1.41	46.98	38.86	
Pinaceae	*Picea*	*pungens*	J10	44	42.02913889	-84.11175	1.42	48.33	39.69	-26.82
Pinaceae	*Picea*	*pungens*	P	126	42.02841667	-84.11252778	1.54	47.54	36.00	
Pinaceae	*Pinus*	*banksiana*	K2	184	42.02966667	-84.11227778	1.77	51.12	33.68	-31.01
Pinaceae	*Pinus*	*banksiana*	S	3	42.02891667	-84.11180556	1.45	47.5	38.20	
Pinaceae	*Pinus*	*bungeana*	G2	166	42.02958333	-84.11286111	1.11	46.64	49.00	

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Pinaceae	*Pinus*	*cembra*	D	92	42.02897222	-84.11275	1.58	31.67	23.38	
Pinaceae	*Pinus*	*cembra*	D	92	42.02897222	-84.11275	2.29	50.21	25.57	
Pinaceae	*Pinus*	*cembra*	F	68	42.02958333	-84.11233333	1.39	44.05	36.96	-27.32
Pinaceae	*Pinus*	*cembra*	J14	17	42.02880556	-84.11202778	1.71	50.09	34.16	
Pinaceae	*Pinus*	*cembra*	J14	17	42.02880556	-84.11202778	1.84	50.63	32.09	-29.74
Pinaceae	*Pinus*	*cembra*	K1	189	42.02991667	-84.11247222	2.29	52.53	26.75	-29.60
Pinaceae	*Pinus*	*cembra*	Q	136	42.02825	-84.11233333	2.34	47.25	23.55	
Pinaceae	*Pinus*	*cembra*	S	14	42.02894444	-84.11180556	1.94	46.2	27.77	
Pinaceae	*Pinus*	*cembra*	S	14	42.02894444	-84.11180556	1.95	49.89	29.84	
Pinaceae	*Pinus*	*densiflor x nigra*	B	23	42.02897222	-84.11205556	0.87	47.9	64.21	
Pinaceae	*Pinus*	*densiflora*	B	29	42.02888889	-84.11230556	1.38	50.08	42.32	-28.74
Pinaceae	*Pinus*	*densiflora*	B	29	42.02888889	-84.11230556	1.38	50.08	42.32	-28.49
Pinaceae	*Pinus*	*densiflora*	D	103	42.02897222	-84.11269444	1.09	51.11	54.68	-28.02
Pinaceae	*Pinus*	*densiflora*	F	56	42.02933333	-84.1125	1.06	50.06	55.07	
Pinaceae	*Pinus*	*densiflora*	F	56	42.02933333	-84.1125	1.13	52.1	53.77	
Pinaceae	*Pinus*	*densiflora*	F	61	42.02947222	-84.11241667	0.73	49.94	79.78	
Pinaceae	*Pinus*	*heldreichii*	D	81	42.02888889	-84.11258333	0.94	50.08	62.13	-29.55
Pinaceae	*Pinus*	*koraiensis*	E2	114	42.02925	-84.11280556	1.2	51.67	50.21	
Pinaceae	*Pinus*	*koraiensis*	E2	114	42.02925	-84.11280556	1.17	51.73	51.56	
Pinaceae	*Pinus*	*koraiensis*	J15	125	42.02836111	-84.11261111	1.71	48.79	33.27	
Pinaceae	*Pinus*	*koraiensis*	J6	46	42.02930556	-84.11255556	1.09	50.47	54.00	-27.88
Pinaceae	*Pinus*	*mugo*	B	22	42.02891667	-84.11205556	1.24	48.92	46.01	-30.15
Pinaceae	*Pinus*	*mugo*	H	170	42.02958333	-84.1125	1.27	51.68	47.46	
Pinaceae	*Pinus*	*mugo*	N	120	42.02858333	-84.11244444	1.25	48.61	45.35	
Pinaceae	*Pinus*	*mugo*	R	142	42.02872222	-84.11213889	1.49	48.03	37.59	
Pinaceae	*Pinus*	*mugo*	R	149	42.02861111	-84.11216667	1.69	48.34	33.36	
Pinaceae	*Pinus*	*nigra*	R	159	42.0285	-84.11225	1.02	49.65	56.77	-29.90
Pinaceae	*Pinus*	*parviflora*	C	74	42.02925	-84.1125	1.13	46.26	47.74	
Pinaceae	*Pinus*	*parviflora*	G2	175	42.02977778	-84.11294444	1.74	49.32	33.06	
Pinaceae	*Pinus*	*parviflora*	M	94	42.02908333	-84.11294444	1.29	50.99	46.10	-25.52

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Pinaceae	*Pinus*	*parviflora*	M	94	42.02908333	-84.11294444	1.29	50.99	46.10	-24.58
Pinaceae	*Pinus*	*pumila*	J18	132	42.02822222	-84.11258333	1.44	50.82	41.16	-28.33
Pinaceae	*Pinus*	*strobus*	B	26	42.02897222	-84.11213889	1.99	49.89	29.24	
Pinaceae	*Pinus*	*strobus*	D	95	42.02911111	-84.11291667	1.34	51.32	44.66	
Pinaceae	*Pinus*	*strobus*	D	97	42.02913889	-84.11275	1.53	50.02	38.13	
Pinaceae	*Pinus*	*strobus*	D	97	42.02913889	-84.11275	1.64	50.52	35.92	-29.38
Pinaceae	*Pinus*	*strobus*	F	53	42.02927778	-84.11244444	2.3	50.88	25.80	-26.55
Pinaceae	*Pinus*	*strobus*	F	53	42.02927778	-84.11244444	2.3	50.88	25.80	-26.52
Pinaceae	*Pinus*	*strobus*	F	53	42.02927778	-84.11244444	2.3	50.88	25.80	-26.46
Pinaceae	*Pinus*	*strobus*	F	55	42.02927778	-84.11244444	1.71	49.32	33.64	
Pinaceae	*Pinus*	*strobus*	R	150	42.02858333	-84.11219444	1.73	47.1	31.75	
Pinaceae	*Pinus*	*strobus*	R	153	42.02847222	-84.11202778	1.52	49.17	37.72	
Pinaceae	*Pinus*	*sylvestris*	Q	137	42.02827778	-84.11233333	1.54	47.36	35.86	
Pinaceae	*Pinus*	*sylvestris*	R	145	42.02863889	-84.11208333	1.23	47.37	44.91	
Pinaceae	*Pinus*	*sylvestris*	R	145	42.02863889	-84.11208333	1.48	49.8	39.24	
Pinaceae	*Pinus*	*sylvestris*	S	8	42.02894444	-84.11183333	0.91	47.21	60.50	-29.68
Pinaceae	*Pinus*	Unnamed Hybrid	U	180	42.03011111	-84.11258333	1.38	51.22	43.28	
Pinaceae	*Pinus*	Unnamed Hybrid	U	180	42.03011111	-84.11258333	1.24	51.57	48.50	
Pinaceae	*Pseudotsuga*	*menziesii*	S	15	42.02891667	-84.11175	1.45	50.45	40.58	-28.79
Pinaceae	*Tsuga*	*canadensis*	B	27	42.02894444	-84.11222222	1.53	46.79	35.66	-30.15
Pinaceae	*Tsuga*	*canadensis*	D	91	42.02897222	-84.11280556	1.74	47.27	31.68	
Pinaceae	*Tsuga*	*canadensis*	D	91	42.02897222	-84.11280556	1.79	50.91	33.17	
Pinaceae	*Tsuga*	*canadensis*	D	93	42.029	-84.11283333	1.46	48.15	38.46	
Pinaceae	*Tsuga*	*canadensis*	D	93	42.029	-84.11283333	1.76	51.42	34.07	
Pinaceae	*Tsuga*	*canadensis*	F	58	42.02941667	-84.11244444	1.96	49.66	29.55	-28.83
Pinaceae	*Tsuga*	*canadensis*	F	64	42.02961111	-84.11241667	1.6	49.01	35.72	-27.21
Pinaceae	*Tsuga*	*canadensis*	N	119	42.02858333	-84.11252778	1.33	48.68	42.68	-28.95
Pinaceae	*Tsuga*	*canadensis*	P	129	42.02836111	-84.11252778	1.06	51.15	56.27	
Pinaceae	*Tsuga*	*diversifolia*	L	160	42.02916667	-84.11308333	1.15	50.99	51.71	-27.21
Pinaeceae	*Picea*	*abies*	J14	1	42.02902778	-84.11172222	1.29	48.79	44.11	-29.41

HLG Samples										
Family	**Genus**	**Species**	**Bed**	**Sample ID**	**Lat**	**Long**	**%N**	**%C**	**C:N**	**$\partial^{13}C$**
Pinaeceae	*Picea*	*bicolor*	J8	89	42.02883333	-84.11291667	1.36	50.44	43.25	
Rosaceae	*Prunus*	*subhirtella*	NA	197	42.0283	-84.11205556	3.23	43.02	15.53	-31.17
Sapindaceae	*Acer*	*griseum*	NA	204	42.02961111	-84.11138889	2.21	47.24	24.93	-27.88
Sapindaceae	*Acer*	*saccharum*	NA	192	42.02897222	-84.11141667	0.62	42.76	80.43	
Sapindaceae	*Acer*	*saccharum*	NA	192	42.02897222	-84.11141667	0.62	42.76	80.43	-27.92
Sapindaceae	*Acer*	*saccharum*	NA	192	42.02897222	-84.11141667	0.62	42.76	80.43	-27.89
Sapindaceae	*Acer*	*saccharum*	NA	192	42.02897222	-84.11141667	0.51	43.43	99.31	-27.83
Sciadopityaceae	*Sciadopitys*	*verticillia*	R	155	42.02844444	-84.11205556	1.07	49.68	54.15	-26.29
Taxaceae	*cedrus*	*deodara*	F	66	42.02966667	-84.11238889	1.64	46.32	32.94	-25.96
Taxaceae	*cedrus*	*libani var. stenocoma*	F	59	42.02944444	-84.11247222	1.43	48.52	39.57	-29.62
Taxaceae	*Taxus*	*baccata*	C	76	42.02911111	-84.11255556	2.49	51.69	24.21	-26.58
Taxaceae	*Taxus*	*baccata*	C	76	42.02911111	-84.11255556	2.25	51.71	26.80	
Taxaceae	*Taxus*	*baccata*	F	65	42.02963889	-84.11241667	2.53	48.75	22.47	
Taxaceae	*Taxus*	*baccata*	F	65	42.02963889	-84.11241667	2.47	50.44	23.81	-27.51
Taxaceae	*Taxus*	*cuspidata*	F	51	42.02922222	-84.11241667	2.33	48.2	24.12	-28.64
Taxaceae	*Taxus*	*media*	D	98	42.02913889	-84.11258333	1.84	48.86	30.97	
Taxaceae	*Taxus*	*media*	N	122	42.02847222	-84.11261111	1.9	51.18	31.41	
Taxaceae	*Taxus*	*media*	R	143	42.02869444	-84.11211111	2.26	49.48	25.53	
Taxaceae	*Taxus*	*media*	Shrub	206	42.02566667	-84.11547222	1.71	51.59	35.18	-26.40
Taxaceae	*Taxus*	*media*	Shrub	210	42.02594444	-84.11530556	1.64	48.27	34.32	
Taxaceae	*Taxus*	*media*	Shrub	210	42.02594444	-84.11530556	1.75	49.18	32.77	-31.12
Taxaceae	*Taxus*	*media*	D	98	42.02913889	-84.11258333	1.79	50.99	33.22	

Appendix 5. Herbarium Data

Genus	Species	UM Barcode	Collection Number	Year Collected	Collector	Locality	%N	%C	C:N
Pinus	*densiflora*	1214126	5752	1933	Tae-Hyon Chung	South Korea	0.86	46.07	62.47
Pinus	*densiflora*	NA	6416	1961	Chung In-Cho	South Korea	1.08	49.84	53.82
Pinus	*halepensis*	1214136	590	1974	Lorna F. Ferguson	Spain	0.88	47.64	63.13
Pinus	*halepensis*	1004001A	SN	1948	M.S. Clemens	Australia	1.23	50.25	47.64
Pinus	*insularis*	1214142	76	1903	Elmer D. Merrill	Philippines	1.03	50	56.61
Pinus	*massoniana*	1214161	1446	1986	Y.H. Xiang	China	1.45	49.03	39.43
Pinus	*mugus*	1214174	3885	Before 1980	Stohl	Germany	0.75	47.65	74.09
Pinus	*nigra*	1474986	50382	1900	Emma J. Cole	USA	1.43	48.42	39.49
Pinus	*oocarpa*	1155422	814	1961	J. Espinosa	Mexico	1.52	48.75	37.40
Pinus	*oocarpa*	1002215	803	1988	M. Fuentes	Honduras	1.45	55.59	44.71
Pinus	*oocarpa*	1155571	1130	1982	J. Bauml, J. Dice, G. Voss	Mexico	1.47	50.61	40.15
Pinus	*parviflora*	1214166	3209	1948	Chung In-Cho	South Korea	0.72	47.13	76.34
Pinus	*parviflora*	1214180	49	1982	Murata, Koyoma, Tabata (et al.)	Japan	1.64	48.08	34.19
Pinus	*patula tecunumanii*	1002249A	65	1983	P.S. McCarter	Honduras	1.29	47.76	43.18
Pinus	*ponderosa*	1213032	3157	1972	R.A. Bye	Mexico	1.35	50.64	43.74
Pinus	*pseudostrobus*	1002279A	9320	1950	Boone Hallberg	Mexico	1.81	48.31	31.13
Pinus	*pseudostrobus*	1002299A	13864	1952	Roger McVaugn & Joseph Sooby Jr.	Mexico	1.5	47.62	37.02
Pinus	*sibirica humistrata*	1214204	2548a	NA	NA	NA	1.85	47.14	29.72
Pinus	*sylvestris*	1214207	13842	1935	Ivar Tidestrom	France	1.23	49.49	46.92
Pinus	*sylvestris*	1214216	2598	1908	NA	NA	1.26	47.24	43.72