Effect of continuous light on leaf wax isotope ratios in *Betula nana* and *Eriophorum vaginatum*: Implications for Arctic paleoclimate reconstructions

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Highlights

- *E. vaginatum* (sedge) and *B. nana* (shrub) grown under continuous or diurnal light
- The effect of continuous light on δD_{wax} varies between species
- δD_{wax} is more negative for *E. vaginatum* than *B. nana* regardless of light
- $\delta^{13}C_{wax}$ is more negative in continuous light than in diurnal light

Abstract

Reconstructions of climate using leaf wax D/H ratios (δD_{wax}) require accounting for the apparent isotopic fractionation (ε_{app}) between plant source water and waxes. There have been conflicting publications on whether plants in the Arctic growing under 24-hour continuous light fractionate less than temperate and tropical plants. In this study, we examine the effect of diurnal light (DL) versus 24-hour continuous light (CL) on the isotopic composition of leaf *n*-alkanes and *n*-acids in greenhouse experiments using two common Arctic plants (*Eriophorum vaginatum*, or tussock cottongrass, and *Betula nana*, or dwarf birch). For *E. vaginatum*, the δD_{wax} values of various wax homologues were 5-11‰ more positive for CL plants relative to their DL counterparts, whereas for *B. nana*, CL waxes were 3-24‰ more negative, suggesting that daylight length is not a unifying control on leaf wax D/H ratios of Arctic plants. The $\delta^{13}C_{wax}$ of *B. nana* was more negative for plants grown in continuous light compared to diurnal light, reflecting lower water-use efficiency associated with prolonged stomatal opening in the CL treatment. We modeled the impact of increasing stomatal conductance and effective flow path lengths (mimicking variable leaf morphologies) on the isotopic composition of leaf waters (δD_{lw}) and find that variations in leaf-water enrichment may explain the variable δD_{wax} responses seen between *E. vaginatum* and *B. nana*. We suggest that between-species differences in the δD_{lw} response to light, and differences in the utilization of stored carbohydrates were important for governing δD_{wax}. Our greenhouse results suggest that Arctic plant leaf waxes do not consistently display reduced ε_{app} values as a result of 24-hour day light, providing additional support for field observations.

Keywords: Leaf waxes, hydrogen isotopes, carbon isotopes, growth experiment, Arctic, continuous light

1.1 Introduction

Hydrogen isotope ratios of terrestrial leaf waxes (δD_{wax}) are a powerful paleoclimate proxy, as waxes are abundant in sedimentary archives, geochemically stable, and δD_{wax} and can be used to infer rainfall D/H ratios ($\delta D_{precipitation}$) (Huang et al., 2004; Sachse et al., 2004; Hou et al., 2008; Garcin et al., 2012). Yet, δD_{wax} not only depends on the isotopic composition of source water, but also on the net apparent D/H fractionation (ε_{app}) between meteoric water and waxes. Apparent fractionation varies with changes in evaporative enrichment of soil water (δD_{soil}) and leaf water (δD_{lw}), as well as changes in biosynthetic isotope fractionations associated with different plant species, phenological stage, and environmental conditions (Roden and Ehleringer, 1999; Polissar and Freeman, 2010; Gao et al., 2014; Tipple et al., 2015; Freimuth et al., 2017). As ε_{app} can be sensitive to environmental factors and vegetation types, variations in ε_{app} must be considered and, if possible, corrected in paleoclimate reconstructions (Polissar and Freeman, 2010; Feakins, 2013; Konecky et al., 2016).

To this end, there are numerous surveys of δD_{wax} in modern vegetation and sediments in tropical and temperate regions that quantify ε_{app}. Leaf wax samples from living plants exhibit a large range in ε_{app}, from -34 to -202‰ (Gao et al., 2014), with monocotyledonous plants generally exhibiting greater biosynthetic fractionation than dicotyledons (Gao et al., 2014; Liu et al., 2016). D/H measurements of sedimentary waxes demonstrate that ε_{app} generally ranges from -100 to -150‰ at the integrated watershed scale (Garcin et al., 2012; Sachse et al., 2012), although some studies from the Arctic suggest a much weaker fractionation (ie. less negative ε_{app}). Waxes preserved in lake surface sediments from Baffin Island (Shanahan et al., 2013) and in ancient (ca. 30 ka) paleosols in western Canada (Porter et al., 2016) indicate ε_{app} of -60‰. Similarly, both grasses and woody plants from Baffin Island and Greenland appear to exhibit 20-50‰ less fractionation compared to temperate plants (Yang et al., 2011). These studies have led to suggestions of widespread weak apparent fractionation in the Arctic; however, they have relied on either modeled or reconstructed precipitation isotopic ratios for calculating fractionation factors, which, if incorrect, could bias estimates of ε_{app}. Indeed, a recent global synthesis of plant leaf wax isotopes shows no such latitude effect on ε_{app} (Liu et al., 2016). Furthermore, studies of lake sediments in Siberia (Wilkie et al., 2012), Alaska (Daniels et al., 2017), and northern Europe (Sachse et al., 2004) have demonstrated ε_{app} values that are similar to those of the mid-latitudes. Thus, it is uncertain if and why Arctic plant D/H fractionation differs from mid- and low-latitude plants, and, if so, what values of ε_{app} are appropriate for estimating ancient $\delta D_{precipitation}$ based on measurements of δD_{wax}.

The principal mechanism to explain small ε_{app} values in the Arctic posits that continuous daylight leads to increased/prolonged transpiration (E), and thereby enhanced evaporative D-enrichment of leaf water and correspondingly small apparent fractionation (Yang et al., 2009; Yang et al., 2011). An alternative leaf water theory, however, suggests that higher rates of E should result in less D-enrichment of leaf waters by increasing the advection of unfractionated xylem water to the site of biosynthesis (Barbour and Farquhar, 2004; Barbour et al., 2004; Song et al., 2013). Continuous transpiration, as is sometimes observed in plants transferred into continuous-light environments (Van Gestel et al., 2005), could therefore result in more D-depleted leaf waters, and by extension more negative δD_{wax}, relative to plants exhibiting diurnal

patterns in transpiration. A greenhouse experiment testing the effect of continuous light found that ε_{app} was, in contrast, 40‰ more positive for plants receiving continuous-light compared to diurnal-light (Yang et al., 2009). The experimental conditions of this prior study, however, may not have been ideal for testing the effect of light because two of the species studied, *Taxodium sp. and Metasequoia sp.,* have not been found in the Arctic since the Eocene and because treatment differences in temperature and humidity (Equiza et al., 2006) could have confounded the effects of continuous light. Furthermore, multiple studies have demonstrated that metabolic hydrogen isotope fractionation and the contribution of stored carbohydrates affect lipid D/H ratios, varying with plant type (Kahmen et al., 2013b), position within a leaf (Gao et al., 2015), environmental conditions (Cormier et al., 2018), and seasonality (Sessions, 2006; Newberry et al., 2015), although little is known about how this factor would be affected by the diel light cycle. An experimental growth study using modern tundra plants and identical environmental conditions could constrain the sensitivity of modern tundra vegetation δD_{wax} to a 24-hour light effect.

Carbon isotope ratios of leaf waxes also record vegetation and climatic information (Diefendorf and Freimuth, 2017), and so are useful in paleoclimate research (Aichner et al., 2015; Konecky et al., 2016). As in the case of hydrogen isotopes, little is known about how $\delta^{13}C_{wax}$ of plants grown in continuous light differs from plants grown in diurnal light. The $\delta^{13}C$ of leaf biomass depends on interfoliar CO_2 concentrations (Farquhar et al., 1982), and so an effect of light cycle on *n*-alkyl lipid $\delta^{13}C$ could emerge due to changes in photosynthetic rates or stomatal conductance. At a global scale, there is no correlation between latitude and $^{13}C/^{12}C$ discrimination (Diefendorf et al., 2010); however, the greenhouse study of Yang et al. (2009) revealed a decrease in $\delta^{13}C$ values of bulk leaf tissue from plants grown under continuous light. Thus, it remains uncertain if compound-specific measurements of $\delta^{13}C_{wax}$ are sensitive to continuous versus diurnal light.

In summary, empirical results from Arctic field studies are inconclusive as to whether the Arctic light environment affects the isotopic composition of leaf waxes. Using a controlled growth chamber experiment, we test the effect of 24-hour sunlight on leaf wax isotope ratios in two Arctic taxa with distinct growth forms. We evaluate the results in the context of a model examining how evaporative enrichment and leaf water circulation could influence δD_{wax} and ε_{app} under different light environments.

2. Methods

2.1 *Growth experiment*

To assess the effect of the diel light cycle on ε_{app} values, δD_{wax}, and $\delta^{13}C_{wax}$, we performed a growth chamber experiment at Brown University. We examined two common arctic tundra plants, the graminoid, *Eriophorum vaginatum var. spissum* (common name: tussock cottongrass) and the deciduous shrub, *Betula nana* (common name: dwarf birch). These two species together represent approximately 40% of above-ground biomass in tussock tundra of Northern Alaska (Chapin III et al., 1995), and are important components of tundra ecosystems across northern Eurasia and Canada (Walker et al., 2005). Eighteen specimens of *E. vaginatum* and ten specimens of *B. nana* were obtained in individual pots from the Welker's Grove Nursery in Connecticut, USA. Plants were divided equally into two E7/2 Conviron™ Plant Growth Chambers (height: 58 cm, area: 0.76 m^2), allowing control of environmental parameters. One chamber was configured with a diurnal light cycle (DL), emulating temperate photoperiod, and

the other with continuous light (CL) such as can be found in Arctic summers.

A light sensor was used to measure photosynhetically active radiation (PAR) levels at hourly intervals. In our study, PAR varied between 0 and 350 μmoles photons m^{-2} s^{-1} in the DL chamber and between 215 and 250 μmoles photons m^{-2} s^{-1} in the CL chamber (Fig. 1). The maximum PAR in our greenhouse experiment was approximately 50% of the maximum natural light in the Arctic (e.g. Williams et al., 2014) due to space and resource constraints in the growth chamber. Temperatures varied between 10 and 15 °C on a diel cycle (Fig. 1) and humidity was held at 75%. Plants were grown in loamy peat and were watered on average every 5 days during the experiment, to minimize water stress while keeping the soil from becoming anoxic. Irrigation water (degassed tapwater) was stored in sealed carboys over the course of the experiment, thereby maintaining constant isotope ratios throughout the experiment. The isotope values, measured on a Picarro cavity ring-down analyzer at Brown University, had a δD value of -39.0 ± 0.2‰ and $\delta^{18}O$ value of -6.1 ± 0.04‰, relative to VSMOW. To ensure we measured waxes produced only during the growth period, we trimmed the *E. vaginatum* specimens to approximately 2 cm length at the onset of the experiment. For *B. Nana*, we pruned branches and leaves, and marked remaining leaves so that they could be avoided in later harvesting. At the end of this growth period, we compared leaf wax hydrogen and carbon isotopes between the two treatments to determine the magnitude of the continuous light effect on δD_{wax} and $\delta^{13}C_{wax}$.

2.2 Leaf wax analysis

After 3 months of growth, new *E. vaginatum* growth (entire tillers) and new *B. Nana* leaves were harvested, composited for each individual specimen, and freeze-dried for processing and isotopic analysis. Leaf waxes from each plant were extracted and purified using standard procedures (Gao et al., 2014). The *n*-acids were derivatized to fatty acid methyl esters (FAMEs) using acidified methanol of known isotopic composition (Yang and Huang, 2003). The molecular composition of straight chain *n*-acids and *n*-alkanes were measured using a gas chromatograph (GC; 6890 gas chromatograph; Agilent Technologies) equipped with a split/splitless injector held at 320 °C and a flame ionization detector (FID) held at 325 °C. A 30 m fused silica column (HP-1MS, 0.32 mm i.d., 0.25 μm film thickness) was used with a helium carrier gas with a flow rate of 1.7 mL/min. The oven temperature program was: 60°C (1 min), ramping to 220 °C at 20 °C/min, then to 315 °C at 6 °C/min, where it was held for 15 min. Identification was done by comparison with a standard containing a suite of even-chain-length FAMEs ranging from *n*-C_{16} to *n*-C_{28}, and a second standard containing C_{25}, C_{27}, C_{29}, C_{30}, and C_{32} *n*-alkanes.

Carbon isotope ratios were determined using a Finnigan Delta V isotope-ratio mass spectrometer (IRMS) with the same GC configuration as in GC-FID analysis. Samples were run in duplicate and an external standard containing either FAMEs or alkanes of known isotopic composition was run in between every 6 injections. Not all *n*-acid and *n*-alkane homologues were measured for isotopic ratios due to prohibitively small abundances; as such, the maximum number of samples for a given compound and treatment was 9 for *E. vaginatum* and 5 for *B. nana*, but for some compounds, n was lower. The $\delta^{13}C$ values of FAMEs were mathematically corrected for the methyl groups added during derivatization using the following equation:

1) $\delta D_{real} = \left[(n+1)\delta^{13}C_{measured} + 36.52\right]/n,$

where n is carbon chain length of the compound and -36.52‰ is the $\delta^{13}C$ value of the added methanol. Compound specific hydrogen isotope ratios were determined in an identical fashion, in

triplicate, using a Finnigan Delta Plus IRMS and the δD of FAMEs were likewise corrected for the hydrogen added during methylation using:

2) $\delta D_{corrected} = [(2n + 2)\delta D_{measured} + 123.7 * 3]/(2n - 1)$,

where n is carbon chain length of the compound and -123.7‰ is the δD value of the added methanol. For δD measurements, only chromatogram peaks with areas between 2000 mV and 6000 mV are included in the analysis. Uncertainty was estimated from the results of replicate plant specimen.

Statistical comparisons were made using a 2-way ANOVA in R, with species and light treatment as independent factors. Statistical analyses were conducted independently for each lipid homologue. Data met the assumptions of normality (Shapiro-Wilk test, α=0.05) and homogeneity of variance (Levene's test, α=0.05) and no data required transformations. Where the 2-way ANOVA found statistical differences at the $p<0.05$ level, pairwise comparisons were performed using a Student's t-test to evaluate the effect of light for each test species, and to test the effect of species within each light treatment. To avoid type I error among the multiple comparisons, we only consider pairwise differences significant if the p-value was below a critical value of 0.01.

2.2 *Model sensitivity test*

We also modeled the isotopic composition of leaf waters to evaluate the potential effect of 24-hour daylight on the δD of leaf water. The model calculates steady state values of δD_{lw}, and is applicable for understanding the potential effect of diurnal light cycles on δD_{wax}. We adapt a commonly used leaf water $\delta^{18}O$ model to predict δD_{lw} across a range of stomatal conductance (g_s) values (Flanagan et al., 1991; Roden and Ehleringer, 1999; Barbour et al., 2004; Tipple et al., 2015; Cernusak et al., 2016). The basis of the model is a modified version of the Craig-Gordon evaporation model (Flanagan et al., 1991) that accounts for kinetic fractionation through both stomatal pores and across the leaf boundary layer. The model approximates the enrichment of evaporated water over source water (Δ_e) using Eq. 3 :

3) $\Delta_e = \varepsilon^* + \varepsilon_k + (\Delta_v - \varepsilon_k)\frac{e_a}{e_i}$

where ε* is the equilibrium fractionation between $HD^{16}O$ and $H_2{}^{16}O$, ε_k is the diffusive fractionation through the leaf boundary layer, Δ_v is the isotopic difference between external water vapor and source water, and e_a and e_i are the external and internal vapor pressures, respectively. We modified the values of the relevant parameters from Barbour et al. (2004) to describe the fractionation and diffusivities of $HD^{16}O$ rather than $H_2{}^{18}O$.

Equilibrium fractionation (ε*) is calculated as a function of temperature (Eq. 4; T is in kelvin) (Majoube, 1971).

4) $\varepsilon^* = exp(-0.052612 + \frac{76.248}{T} - \frac{24.844}{T^2})$

Diffusive fractionation (ε_k) is comprised of fractionation through the stomata and through the boundary layer (Eq. 3) and depends on the kinetic fractionation (ε_s and ε_b) and diffusive

conductance (g_s and g_b) through each layer. ε_s has an H/D fractionation of 1.025, while ε_b is calculated as ε_s to the two-thirds power, or 1.017 (Roden and Ehleringer, 1999). The boundary layer conductance is set to 1 mol m^{-2} s^{-1} following Barbour et al. (2004), although we note that slightly higher values have also been reported (Song et al., 2013). Values of g_s are allowed to vary from 0 to 1 mol m^{-2} s^{-1} in order to evaluate the sensitivity of δD_{lw} to this variable.

5) $$\varepsilon_k = \frac{{}^{25}/_{g_s} + {}^{17}/_{g_b}}{{}^{1}/_{g_s} + {}^{1}/_{g_b}}$$

The isotopic composition of mesophyll water from which waxes are biosynthesized, is a mixture of leaf water that has experienced evaporative enrichment at the leaf edge and xylem water that is isotopically similar to soil water and not yet undergone transpiration-related D-enrichment (Roden and Ehleringer, 1999; Barbour et al., 2004). The relative fraction of each component can be estimated empirically; however, advances in understanding water movement through leaves indicates that a Péclet-based model of the relative contribution of each pool can improve estimates of leaf water $\delta^{18}O$ and leaf cellulose $\delta^{18}O$ (Barbour et al., 2004). The Péclet number describes the ratio of advected xylem water to evaporated water diffused from the leaf surface at the site of biosynthesis, and is described by Eq. 6,

6) $$\wp = \frac{L*E}{C*D}$$

where L is the effective path length (m), E is the rate of transpiration (mmol m^{-2} s^{-1}), C is the molar density of water (55.6×10^3 mol m^{-3}), and D is the diffusivity of $DH^{16}O$ in water. D is set to 1.78×10^{-9} m^{-2} s^{-1} (Mills, 1973), a slight decrease compared to the parameter for $\delta^{18}O$ (3.4×10^{-9} m^{-2} s^{-1}). The resulting offset (Δ_{lw}) between the isotopic composition of the average leaf water and the xylem water is thus described as,

7) $$\Delta_{lw} = \frac{\Delta_e(1-e^{-\wp})}{\wp}$$

We use this model to explore how leaf physiology (described by L) influences the sensitivity of δD_{lw} to different illumination regimes. In this exercise, g_s serves as a proxy for illumination conditions, as it is thought to be the main mechanism connecting light cycles to δD_{wax} (Yang et al., 2009).

3. Results

3.1 *Greenhouse experiment*

We observed production of new foliar biomass over the three-month growth period in both study species and treatments. The cut *E. vaginatum* grew to approximately 20 cm in length. *B. Nana* showed less growth, but new leaves appeared and were sampled for analysis. No quantitative information on growth is available.

3.2 *Leaf wax distributions*

For both species, *n*-acids show a strong even over odd predominance, whereas *n*-alkanes show strong odd over even predominance (Fig. 2; Table 1). *B. Nana* has a higher concentration of waxes, except for the C_{30} and C_{32} *n*-acid homologues, of which *E. vaginatum* has more. Both species also produce a small amount of short-chain (C_{16} and C_{18}) *n*-acids, but no corresponding short-chain *n*-alkanes. The average chain length (ACL) was determined for the C_{20}-C_{32} *n*-acids and C_{21}-C_{32} *n*-alkanes. ACL for *B. nana* is 25.9 ± 0.4 for *n*-acids and 28.8 ± 0.4 for *n*-alkanes. ACL for *E. vaginatum* is 26.5 ± 0.5 for *n*-acids and 28.5 ± 0.2 for *n*-alkanes. No difference is observed in the wax distributions of either plant in response to light treatments.

3.3 *Hydrogen isotope ratios*

δD_{wax} ranges from -207‰ to -135‰ across all species, treatments and leaf wax homologues. The pooled analytical standard deviation of replicate standards and samples averages 2.6‰ across all homologues (Table 2). Calculated against the irrigation water δD value of -39‰, ε_{app} varies from -100‰ to -176‰ (Fig. 3, Table 3).

We observe an inconsistent effect of light treatment on δD_{wax}. For *E. vaginatum*, the C_{24} *n*-acid and C_{27} and C_{31} *n*-alkanes are 6-11‰ D-enriched in the CL treatment relative to the DL treatment ($p<0.01$). For other lipid homologues the effect of light was insignificant. In contrast, the CL waxes of *B. nana* are 3-24‰ more D-depleted than DL waxes ($p<0.01$), with the exception of C_{28} *n*-acid that showed no difference between light treatments. A species effect is apparent wherein *n*-acids and *n*-alkanes of *E. vaginatum* are D-depleted by, on average, 36 ± 16‰ relative to *B. nana* ($p<0.001$ for all homologues). The species effect is present in both the diurnal and continuous light treatments.

3.4 *Carbon isotope ratios*

$\delta^{13}C_{wax}$ ranges from -42.3 to -32.0‰ across all species, treatments, and leaf wax homologues (Fig. 3, Table 4), and the pooled analytical standard deviation averages 0.22‰ across all homologues (Table 2). For *B. nana*, $\delta^{13}C_{wax}$ averages 2.8‰ lower in the CL treatment than in the DL treatment, although no difference is observed for the C_{22} *n*-acid ($p=0.038$) and C_{28} *n*-acid ($p=0.021$). For *E. vaginatum*, $\delta^{13}C$ of wax homologues average 0.8‰ lower under CL than DL, although the differences are not statistically significant for any of the homologues ($p>0.01$ for all compounds). The difference in $\delta^{13}C_{wax}$ between the two species is insignificant for most wax homologues, with the exception that the C_{22}-C_{26} *n*-acids were ^{13}C-enriched in the DL *B. nana* compared to the DL *E. vaginatum*.

Upon combining data from both light treatments and various wax homologues, we find significant ($p<0.001$) correlations between $\delta^{13}C_{wax}$ and δD_{wax} for the *n*-alkanes for both taxa (Fig. 4). The correlation is positive for *Betula nana* ($r^2=0.85$) and negative for *Eriophorum vaginatum* ($r^2=0.50$). For *n*-acids, the $\delta^{13}C_{wax}$-δD_{wax} correlation is weakly positive for *B. nana* ($r^2=0.28$, $p=0.002$) and absent entirely for *E. vaginatum* ($r^2=0.03$,$p=0.200$), possibly reflecting slower regeneration rates of the *n*-acids (Gao and Huang, 2013) or more variable hydrogen exchange for that compound class.

3.5 *Leaf water model results*

We investigate the sensitivity of δD_{lw} to changing stomatal conductance using a modified

version of the Craig-Gordon model (Equations 1-5) in which the environmental parameters are similar to our growth chamber environments with $\delta D_{SourceWater}$ = -39‰, rH = 80%, and leaf temperature = 13 °C. The enrichment over source water is also a function of δD_{vapor}, which is unconstrained in our study, and was prescribed to -121‰ assuming equilibrium fractionation between the $\delta D_{SourceWater}$ (-39‰) and δD of vapor at the average incubation temperature (13 °C) (Majoube, 1971). The patterns of sensitivity are robust across different values of δD_{vapor}. It is important to note that in the model, E and g_s are positively correlated, which is only the case if vapor pressure is held constant. In field studies from the Arctic diel changes in temperature and rH can be more important factors controlling transpiration than stomatal conductance (Gebauer et al., 1998). In the current experiment, the diel changes in temperature and rH were identical between light treatments and so should not greatly interfere with a test of stomatal conductance.

Using the modified Craig-Gordon leaf water model, we estimate that at the site of evaporation, leaf water isotopes are 24‰ D-enriched over irrigation water under the conditions of our growth experiment, resulting in a δD_{lw} value of -15‰. The δD_{lw} at the site of evaporation is not strongly dependent on g_s, but after accounting for the mixing of evaporated and unevaporated water within the leaf (i.e. the Péclet number), we find that δD_{lw} of the bulk leaf water decreases with increasing g_s, especially for leaf morphologies with high effective path lengths (L) (Fig. 5). At high g_s and high Péclet number (i.e. large leaves or mixing path length), modeled δD_{lw} is only slightly D-enriched relative to the irrigation water because relatively D-depleted xylem water dominates sites of wax synthesis within leaf mesophyll. In low L cases (small leaves), δD_{lw} is insensitive to g_s, but shows consistently large D-enrichment relative to source water. As such, we might expect the largest leaves (high L) to be most responsive to a 24-hour light effect on δD_{lw} or δD_{wax}, if g_s varies as a function of the light cycle.

Song et al. (2013) showed that the effective path length can also vary as a function of gas exchange rates across the stomata. As E increases, L decreases due to a shift to more direct, extracellular, flowpaths of water through the leaf. In the model case where L varies with transpiration rate (dark red lines in Fig. 5), the Péclet number is small and relatively stable (Eq. 4), and δD_{lw} increases slightly (< 3‰) with increasing g_s.

4. Discussion

4.1 *The theoretical basis for δD_{wax} response to light duration*

Plants regulate their evapotranspiration rates to maximize the ratio of CO_2 uptake relative to water loss by controlling their stomatal conductance (Farquhar et al., 1989). There is also a rich body of evidence that stomatal opening and closure occurs directly in response to light availability (Darwin, 1898; Sharkey and Ogawa, 1987). Illustrating this point are multiple studies documenting that both angiosperms and gymnosperms, when transferred into a continuous light environments, will begin continuously photosynthesizing and transpiring over a 24-hour cycle (Van Gestel et al., 2005; Yang et al., 2009). Genetic differences between species, however, can occasionally maintain circadian rhythms in g_s despite continuous light or dark environments (Kerr et al., 1985; Hennessey and Field, 1991), and so we do not necessarily expect all species to respond in the same manner to alterations in daily light duration. Furthermore, the stomatal response to light availability depends on water availability – if soil water is limited, plants may be forced to close their stomata even in the presence of sunlight to avoid desiccation (Farquhar et al., 1989). It is not immediately obvious whether the integrated stomatal conductance (g_s) of

Arctic plants is in fact higher or lower than the same plants grown in low- or mid-latitudes (Gebauer et al., 1998; Llorens et al., 2009), especially given that the intensity of light in the Arctic during mid-summer can vary by over 1000 μmol photons m^{-2} s^{-1} on a diel cycle (Williams et al., 2014) and given that there are large variations in moisture across the Arctic (Shanahan et al., 2013).

The presence or absence of a diel light cycle could affect δD_{wax} by influencing the magnitude of evaporative enrichment at the leaf surface, by influencing the mixing ratio between unfractionated xylem water and fractionated leaf water at the site of biosynthesis, or by influencing whether the hydrogen atoms used in lipid synthesis are derived from stored or freshly produced organic substrates (Sessions, 2006). If δD_{lw} is the main control on δD_{wax}, then the specific stomatal response to the light cycle is a critical consideration. Indeed, the leaf water model results suggest that increased g_s in a CL environment would likely lead to more negative δD_{lw} values, similar to predictions by (Sullivan and Welker, 2007), but at odds with the experimental results of Yang et al. (2009).

We might expect that in our growth experiment, CL plants express more negative δD_{wax} than DL plants because the daily integrated δD_{lw} values decrease with prolonged periods of transpiration. Alternatively, CL plants might express more positive δD_{wax} than DL plants because the daily peak light intensity (Fig. 1) and, correspondingly, the daily peak instantaneous values of g_s, are lower in the CL treatment. That is, the daily duration of stomatal opening and photosynthesis may be less important than the instantaneous values of g_s during the period of photosynthesis, particularly given that δD_{lw} reaches steady state values rapidly (<2 hours) upon stomatal opening or closure (Roden and Ehleringer, 1999). The magnitude of leaf water enrichment during the dark hours are irrelevant because photosynthesis is not occurring. Variations in δD_{wax} should thus only be discernible where there are non-linear relationships between photosynthesis and g_s, or where there are variations in biosynthetic strategies.

4.2 *D/H fractionation under varying light in growth experiments*

Few studies on the diurnal patterns of transpiration in continuous light have focused on changes in the hydrogen isotopic composition of leaf biomass. Previous observations (Yang et al., 2009; 2011) have suggested a relatively large (15-40‰) effect of CL on plant wax hydrogen isotopic compositions (Fig. 6). Our greenhouse experiment demonstrates that the effect of CL versus DL can vary substantially between Arctic-dwelling species, pointing to a need to better constrain spatial and species differences in ε_{app} across light regimes. The opposing δD_{wax} responses between our two study species to the light treatment is surprising and indicates that day length is not a unifying control on the δD_{wax} of plants.

The magnitude of D/H fractionation observed for *B. nana* and *E. vaginatum* in our greenhouse study agrees almost perfectly with field studies of the same species conducted in the Alaskan Arctic (Daniels et al., 2017). Comprehensive analyses of the isotopic composition of soil water, leaf water, and waxes showed that, in the field, *B. nana* has an ε_{app} of -100 to -110‰ for various *n*-acid and *n*-alkane homologues, compared to -100 to -147‰ for our greenhouse specimens. The ε_{app} values for *n*-alkanes ranges from -100 to -121‰, almost identical to the range of -102 to -118‰ reported for *n*-alkanes from a related species, *B. pubescens,* sampled across a wide range of latitudes, including latitudes that receive 24-hour daylight in summer (Sachse et al., 2006). Likewise, ε_{app} of waxes from *E. vaginatum* in Alaska range from -155 to -170‰

(Daniels et al., 2017) and from -145 to -176‰ in this growth experiment. Additionally, our greenhouse experiments yield ε_{app} values that are similar to those from studies of plants within the same families and growth forms grown in DL environments (Gao et al., 2014), indicating that our study is broadly representative for these two species in a range of environmental conditions. This is noteworthy, as the light intensity in the growth chambers is somewhat less than occurs in nature. The consistency of ε_{app} values observed for these plants, in the field and in experiments, and across very different light cycles and intensities strongly suggests that light is not the principle control on ε_{app}.

Previously, Liu et al. (2016) showed that the ε_{app} values of dicots and monocots exhibit similar latitudinal gradients (a proxy for day length, among other variables). Our data suggest these two plant groups might display differing sensitivities to day length. For *E. vaginatum*, CL waxes are the same or D-enriched (6-11‰) relative to DL waxes, but CL waxes are D-depleted (3-24‰) for *B. nana*. The model results imply that either the two species altered their g_s in opposite directions in response to continuous light, that leaf morphology modulates the impact of g_s variability in governing δD_{lw}, or alternatively that the two species exhibited contrasting biosynthetic responses to light. There is no *a priori* reason to assume the stomatal response to light treatment differ for *E. vaginatum* and *B. nana*. Thus, the contrasting responses likely originate from morphologic or biosynthetic differences in these plants.

A possible explanation for why the two species responded differently is that δD_{wax} is less sensitive to environmental changes in monocotyledonous plants, such as *E. vaginatum*, than it is in dicotyledonous plants such as *B. nana* (Kahmen et al., 2013b). These authors demonstrate that dicots express the full magnitude of leaf water enrichment in their waxes, whereas only 18-68% of leaf water variability is expressed in the waxes of monocots. In this framework, *B. nana* specimens in the CL treatment may have experienced a decrease in Δ_{lw} relative to the DL treatment, associated with an increase in stomatal conductance (Fig. 5). This shift in Δ_{lw} was then expressed as more negative ε_{app}. A similar decrease in Δ_{lw} in *E. vaginatum* would not have been completely expressed in the lipid δD values.

The slight increase in ε_{app} for some of the *E. vaginatum* wax homologues in response to continuous light suggests that specific plant traits might affect not only the sensitivity, but the direction, of the δD_{wax} response to light. Our isotope modeling results suggest that leaf morphology, reflected in L, could contribute to the species-specific differences (Barbour and Farquhar, 2004; Cernusak et al., 2016). In particular, the model predicts that for plants in which L=f(E) (Song et al., 2013), changing leafwater flowpaths could potentially alter the direction of the δD_{lw} response to continuous light. Unfortunately, we do not have sufficient species-specific information on L to assess the importance of this factor directly in our experiments, and future work could assess leaf water dynamics, particularly within grasses, under different light regimes. In general, both monocots and dicots exhibit a gradient of increasing δD_{lw} and δD_{wax} from the base or center of the leaf to the leaf edge (Gao and Huang, 2013; Liu et al., 2016). While our leaf water model does not account for variations in Δ_{lw} along the leaf blade, the positive shift in ε_{app} for *E. vaginatum* is a possible indication that back-diffusion of D-enriched water from the terminus of the leaf may increase as stomatal conductance increases under CL, i.e. L=f(E), as in the dark red line of Fig. 5. Likewise, the large δD_{wax} response to CL observed by Yang et al. (2009) could derive from morphological traits of the conifer needles they studied, wherein the low-

intensity/long-duration light gave rise to greater D-enrichment. In conifers needles, there is a resistant physical barrier between the xylem and mesophyll that limits water transport between these two components (Roden et al., 2015; Cernusak et al., 2016) which could make the leaf water used in biosynthesis more sensitive to evaporative enrichment and a 24-hour light effect. Analogous morphological factors could be important for *E. vaginatum*, which showed the same direction of δD_{wax} change as the conifers, albeit to a lesser degree.

Experimental differences in temperature regimes and watering protocols may have also contributed to the constrasting results between our results and those of Yang et al. (2009). For comparison, in order to mimic Eocene Arctic conditions, Yang et al. (2009) kept soils saturated throughout the growth phase in both treatments such that water stress did not limit transpiration (Equiza et al., 2006; Yang et al., 2009), whereas our intermittent watering protocol may have resulted in occassional stomatal closure as might occur in naturally dry conditions. That said, none of the plants shows outward signs of water stress in our experiment, and so other factors most likely cause the results of our experiment to differ from those of Yang et al. (2009). In general, the contrasting δD_{wax} responses between *E. vaginatum* (a graminoid), *B. nana* (a broadleaf angiosperm), and conifers (Yang et al., 2009) (Fig. 6) suggest that leaf morphology could modulate the daylength response of δD_{wax}. Future study across a broader array of species could further elucidate a relatinship between plant morphology and a light effect on δD_{wa}.

In addition to the role of δD_{lw} as a control on long-chain *n*-alkane and *n*-acid δD values within leaves and across environmental gradients (Sachse et al., 2006; Gao et al., 2015; Tipple et al., 2015), there is abundant evidence that biosynthetic fractionation (ε_{bio}) is an important control on δD_{wax}. ε_{bio} is determined by the D/H of wax precursors and the D/H fractionation in various biosynthetic pathways, and it varies between monocots and dicots (Gao et al., 2014; Liu et al., 2016), throughout the season (Sessions et al., 1999; Newberry et al., 2015; Freimuth et al., 2017), and as a function of light availability and pCO_2 (Cormier et al., 2018). It remains difficult to disentangle the joint effects of ε_{bio} and Δ_{lw} in our study without direct measurements of photosynthesis rates or leaf water isotope measurements. In general, waxes that are produced from recent photosynthate tend to be D-depleted compared to waxes derived from stored sugars (Sessions, 2006; Cormier et al., 2018). As such, a relatively greater utilization of newly produced carbohydrates for *B. nana* wax production under CL relative to DL could explain the more negative δD_{wax} values in the CL treatment. Greater utilization of fresh photosynthate under CL is also supported by the lower $\delta^{13}C$ values (Helle and Schleser, 2004). In *E. vaginatum*, on the other hand, no such effect is apparent. The lower concentrations of waxes in the *E. vaginatum* specimens (Fig. 2) indicate lower requirements for wax production such that lipid precursors may be present in sufficient quantity in both the CL and DL treatment and no shift in metabolic pathway is required. If differences in photosynthesis rates or wax formation pathways differed between light treatments, it will be important to perform perennial growth experiments for determining if continuous light could eventually affect δD values of the stored carbohydrates and NADPH pools, thereby resulting in a different effect of light than that observed over the 3 months of this experiment.

Lastly, we highlight the important role that vegetation plays as a determinant of ecosystem-scale ε_{app}. The considerable difference in ε_{app} between *E. vaginatum* and *B. nana* agrees with findings showing that grasses fractionate between D and H more strongly than shrubs (Hou et al., 2007; Gao et al., 2014), and that species more basal in their evolutionary histories tend to

show smaller ε_{app} than later-derived plants (Gao et al., 2014). The three conifers grown by Yang et al. (2009), *Taxodium distichum*, *Larix laricina,* and *Metasequoia glyptostroboides*, had ε_{app} values from -95 to -106‰ in the DL treatment, somewhat smaller than the angiosperms *B. nana* (-110 ‰) and the *E. vaginatum* (-150 ‰) grown here. While these differences do not directly explain the response of *Larix* and *Metasequoia* to changes in light conditions, the differences in ε_{app} for DL-grown plants between our study and that of Yang et al. (2009) are not unexpected. The between-species difference in ε_{app} eclipses leaf water enrichment effects, pointing to a need to further quantify ε_{app} for a broader range of species in order better constrain vegetation effects on paleoclimate reconstructions using δD_{wax}.

4.3 *$^{13}C/^{12}C$ fractionation under varying light in growth experiments*

Variations in light regime have previously been documented to influence the $\delta^{13}C$ of bulk leaf biomass (Smith et al., 1976; Ehleringer et al., 1986; Pearcy and Pfitsch, 1991; Yang et al., 2009) but changes of $\delta^{13}C$ of leaf wax compounds in response to day length have not been described. We observe an average $\delta^{13}C_{wax}$ depletion of 1.8‰ for plants in the CL treatment relative to the DL treatment, although the effect is smaller and non-significant in the *E. vaginatum* specimens. The direction and magnitude of the observed $\delta^{13}C_{wax}$ shift is similar to those observed in several other studies that examined bulk leaf $\delta^{13}C$ and found that higher light intensity induces less carbon isotope discrimination. Yang et al. (2009) document a negative shift of 1.8-4.6‰ for plants grown under low-intensity/continuous light compared to high-intensity/diurnal light; both Pearcy and Pfitsch (1991) and Lockheart et al. (1997) document a negative shift of 1-2‰ for shade leaves relative to sun leaves; and, Ehleringer et al. (1986) report $\delta^{13}C$ shifts on the order of 4‰ across a light intensity gradient.

A likely explanation for the shift in $\delta^{13}C_{wax}$ is that the light treatment gives rise to changes in the intercellular concentration of CO_2 (C_i), and thereby $^{13}C/^{12}C$ discrimination, by impacting photosynthesis rates and/or stomatal conductance (Farquhar et al., 1982; Diefendorf et al., 2010). $\delta^{13}C_{wax}$ generally decreases as water-use efficiency decreases, reflecting for example, an increase in g_s in response to greater water availability or light, or a reduction in photosynthesis, A. It is difficult to partition the effects of varying A versus varying g_s (Sullivan and Welker, 2007) without direct measurements of these variables. Yang et al. (2009) observed sustained transpiration by plants in CL, and, given that plants were not water stressed in either experiment, we propose that increased/sustained g_s can explain lower $\delta^{13}C_{wax}$ in our CL plants. This carbon isotope-inferred change in g_s further suggests that stomatal regulation likely plays some role in governing leaf water D-enrichment and δD_{wax} in response to light availability, particularly for *B. nana*. The correlations between $\delta^{13}C_{wax}$ and δD_{wax} (Fig. 4), have not previously been noted for our study taxa. The correlation is positive for *B. nana* (*n*-acid R^2=0.28, *n*-alkane R^2<0.85) but negative for *E. vaginatum* (*n*-acid R^2 = 0.03, *n*-alkane R^2=0.50). A similar contrast in the $\delta^{13}C$-δD relationships was previously reported between C_3 trees and C_4 grasses (Bi et al., 2005). Our results, from two C_3 species, suggest that growth form (i.e. woody vs herbaceous), in addition to photosynthetic pathway, may be an important determinent of whether the slope is positive or negative. Tandem measurements of the two isotopes could provide a useful means of identifying wax sources in Arctic lakes.

Given the experimental results showing that $\delta^{13}C$ of *B. nana* leaf wax is sensitive to the

intensity and diurnal cycle of light, we might expect there should be a latitude effect on $\delta^{13}C$ of foliar biomass at a global scale. The apparent absence of correlation between $\delta^{13}C$ and latitude (Diefendorf et al., 2010), however, suggests that other environmental factors mask the effect of latitude, or that the gradient in light intensity and day length is weaker across latitudes than it was between our experimental treatments. Thus, caution is necessary in applying these results to field studies.

4.4 *Application to Field Studies*

How applicable are our growth experiments to understanding the isotopic composition of leaf waxes in the Arctic? Although light is available continuously during polar summer, daily rhythms in temperature, plant photosynthesis, soil moisture, and even light availability exist at high latitude sites and may lead to diel patterns of gas exchange in plants. For example, at Toolik Field Station in Alaska (69°N latitude), hourly PAR measurements during the summer solstice from 1999 to 2013 show a strong daily cycle from 30 μmol photons m^{-2} s^{-1} at 'night' up to 980 μmol photons m^{-2} s^{-1} at midday.

For *Betula sp*. and *Eriophorum sp*., the relationship between light and g_s is ill-defined (Gebauer et al., 1998). Jagels and Day (2000) indicate that g_s and E can be as much a function of temperature, water availability, and species as it can be of light. Moreover, diurnal studies from the Arctic show day/night differences in E despite continuously available PAR. Gebauer et al. (1998) found that E decrease during "night" in the arctic sedge *Eriophorum*, similar to results from Bliss (1960) for *Betula* and *Salix* shrub specimens in northern Alaska. Diel patterns in E have also been observed in eddy flux measurements in the tundra and are attributed to a stomatal conductance control (Vourlitis and Oechel, 1999). These measurements demonstrate that g_s is most sensitive to vapor pressure deficit and water stress in the tundra and show that in the field, under continuous light, many Arctic plants experience diurnal cycles in key physiologic processes that would influence the isotopic compositions of their leaf waters. Given these results, it is not surprising that continuous light does not have a consistent effect on the hydrogen isotopic composition of Arctic plant waxes.

In the Arctic, day length changes substantially over the course of the year. Given the differences we observe between CL and DL treatments, the annual cycle in day length could possibly impart a small seasonal signal in ε_{app}, species dependent. Over long time scales, however, the annual cycle of day length at a particular location is approximately unchanging. As such, the interpretation of sedimentary δD_{wax} records at a given location is not contingent on the effect of day length. More important is the identification of accurate fractionation values in the Arctic and information on the latitude and light dependencies of ε_{app} at ecosystem or plant scales.

Our data, based on leaf-level measurements, do not conclusively support a 24-hour light effect to explain small ε_{app} values (~ -60‰) observed in some studies in the Arctic. Furthermore, Arctic sites, which experience similar day lengths, exhibit highly variable ε_{app} signatures indicating that day length likely plays a secondary role to vegetation and climate effects (Sachse et al., 2006; Yang et al., 2011; Wilkie et al., 2012; Shanahan et al., 2013; Porter et al., 2016; Thomas et al., 2016). These other factors, or possibly inaccuracies in ε_{app} estimates, are important for explaining the ε_{app} differences across Arctic sites, particularly with regards to the small fractionation inferred at Baffin Island (Shanahan et al., 2013) and central Canada (Porter et al., 2016) which were previously attributed to a 24-hour continuous light effect.

Species differences in our greenhouse and field experiments could implicate vegetation type as an important control on ε_{app} variations across the Arctic. Likewise, relative humidity, which we did not test in this experiment, has been frequently identified as a critical influence on ε_{app} through its control on leaf evaporation (Kahmen et al., 2013a; Tipple et al., 2015). At present, however, Baffin Island, Lake El'gygytgyn, and the North Slope of Alaska all have growing season rH values similar to the 75% rH used in our growth experiment (average 74%, 78%, and 75%, respectively), yet field studies at those sites (Wilkie et al., 2012; Shanahan et al., 2013; Daniels et al., 2017) document difference in ε_{app} of approximately 40‰ between the sites. Likewise, variable soil conditions may contribute to pan-Actic ε_{app} variations by influencing the magnitude of evaporative enrichment of soil waters, but at present, there is insufficient information on the D/H or $^{18}O/^{16}O$ ratios of soil waters at sites with leaf wax measurements to quantitatively assess the importance of soil evaporation. We cannot rule out inaccuracies in the estimates of δD of source water in the Arctic studies, which are difficult to constrain and strongly influence the field estimates of ε_{app}. Shanahan et al. (2013) relied upon modeled, rather than measured, source water isotopic composition and used modeled mean annual $\delta D_{precipitation}$. There is evidence, however, that Arctic plants derive much of their biosynthetic water from precipitation received during the growing season (Wilkie et al., 2012; Daniels et al., 2017). The precipitation-weighted mean annual $\delta D_{precipitation}$ at Hall Beach, Baffin Island is -161‰ and the growing season (JJA) $\delta D_{precipitation}$ is -134‰ (IAEA/WMO, 2017), so the assumed seasonality of source water could contribute as much as 27‰ toward the approximately 40‰ difference in ε_{app} estimated at Baffin Island relative to Alaska or Siberia. Likewise, Porter et al. (2016) compared the D/H of fossil waxes to fossil (permafrost) water to estimate ε_{app}, but the seasonality and the age of the fossil water is not strictly known, creating uncertainty in the calculated ε_{app}. Continued work constraining ε_{app} at both the whole-ecosystem scale for a range of arctic bioclimatic subregions, as well as at the scale of individual plants, will help guide interpretations of Arctic sedimentary δD_{wax} records, particularly where δD_{wax} is paired with pollen or biomarker-based vegetation/bioclimatic information (Feakins, 2013).

5. Conclusion

Our greenhouse experiments and modeling studies establish that day length has an inconsistent and relatively small influence on apparent hydrogen isotopic fractionations between leaf wax lipids and source water for two prominent plant species found in high-latitude northern locations. Variable responses of δD_{wax} to light regime suggest that the effect of increased stomatal conductance in a 24-hour light environment may be modulated by leaf water dynamics or changing biosynthetic pathways, possibly explaining divergent results from previous growth experiments. Furthermore, the ~40‰ difference in ε_{app} between the monocotyledon, *E. vaginatum*, and the dicotyledon, *B. nana*, supports previous research suggesting that vegetation differences are an important factor governing δD_{wax} across the modern Arctic (Wilkie, 2013; Gao et al., 2014; Daniels et al., 2017). In contrast to hydrogen isotopes, carbon stable isotope ratios are sensitive to the light regime, with more negative $\delta^{13}C_{wax}$ values apparent in a continuous light environment. Overall, these results will guide reconstructions of $\delta D_{precipitation}$ across vast areas of tundra where *B. nana* and *E. vaginatum* have been prominent in the plant communities for

millennia.

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8. Tables:

	n-acids		*n*-alkanes	
	C_{20}-C_{32} CPI	C_{20}-C_{32} ACL	C_{21}-C_{33} CPI	C_{21}-C_{33} ACL
B. nana	15.8 ± 2.7 (9)	25.9 ± 0.4 (10)	11.9 ± 3.7 (10)	28.8 ± 0.4 (10)
E. vaginatum	23.7 ± 4.3 (18)	26.5 ± 0.5 (18)	11.7 ± 0.7 (18)	28.5 ± 0.2 (18)

Table 1. Chain length distributions for *E. vaginatum* and *B. nana*. Uncertainties represent 1 standard deviation of the replicate plant specimens, while the numbers in parentheses are the numbers of samples analyzed.

	n-acids				*n*-alkanes			
	C_{24}	C_{26}	C_{28}	C_{30}	C_{25}	C_{27}	C_{29}	C_{31}
Hydrogen								
Standards		2.8 (41)	2.7 (43)		1.9 (22)	1.2 (23)	1.5 (24)	
Samples	2.3 (97)	3.1 (98)	3.0 (107)	2.0 (62)	3.2 (52)	1.8 (57)	3.6 (80)	4.9 (81)
Carbon								
Standards		0.1 (20)	0.1 (20)		0.1 (25)	0.2 (25)	0.3 (25)	
Samples	0.5 (62)	0.3 (40)	0.4 (56)	0.2 (38)	0.1 (22)	0.3 (60)	0.1 (63)	0.2 (44)

Table 2. Pooled standard deviation (1σ) and number of injections for δD_{wax} and $\delta^{13}Cwax$ analytical measurements. Error is calculated at daily time steps and the pooled standard deviation s is calculated as $\sigma = \sqrt{\frac{\Sigma((N_i-1)*s_i^2)}{\Sigma N_i}}$, where N_i is the number of replicate standards for each day i, or the number of replicate injections for each sample i.

	B. nana (n=5)		*E. vaginatum* (n=9)	
	DL	CL	DL	CL
n-acids				
C_{20}	-124.6 ± 9.0	na	-170.8 ± 8.1	-161.6 ± 6.1
C_{22}	-122.9 ± 9.3	-147.1 ± 1.8	-167.8 ± 7.3	-160.5 ± 8.3
C_{24}	-116.4 ± 4.8	-129.7 ± 1.1	-171.2 ± 8.7	-159.8 ± 4.9
C_{26}	-112.4 ± 4.2	-120.1 ± 2.7	-149.8 ± 7.2	-145 ± 5.3
C_{28}	-103.7 ± 6.5	-106.5 ± 1.6	-170.7 ± 4.9	-165.8 ± 6.5
C_{30}	na	na	-175.8 ± 6.4	-170.3 ± 5
n-alkanes				
C_{25}	-110 ± 2.5	-116.5 ± 1.3	-131.6 ± 3.3	-123.3 ± 4.6
C_{27}	-109.4 ± 2.3	-121.3 ± 1.6	-147.1 ± 3.9	-140.8 ± 3.8
C_{29}	-100.3 ± 2.5	-110.6 ± 3.1	-157.4 ± 5.7	-148.9 ± 6.9
C_{31}	-102.5 ± 3.5	-111.9 ± 2	-158.1 ± 4	-149.4 ± 5.4

Table 3. Net apparent fractionation (ε_{app}) for *B. nana* and *E. vaginatum* under different light conditions. DL = Diurnal Light; CL = Continuous Light. Fractionations are calculated relative to irrigation water with a δD value of -39‰, and the uncertainties represent 1σ based on replicate plant specimens.

	B. nana (n=5)		*E. vaginatum* (n=9)	
	DL	CL	DL	CL
n-acids				
C_{20}	-37.5 ± 1.2	-39.7 ± 1.2	-38.2 ± 0.7	-38.9 ± 1.0
C_{22}	-35.5 ± 1.2	-39.2 ± 0.8	-37.3 ± 0.7	-38.3 ± 1.1
C_{24}	-36.6 ± 0.6	-38.5 ± 0.8	-38.4 ± 0.6	-39.2 ± 1.1
C_{26}	-34.9 + 0.8	-37.2 ± 0.5	-36.6 ± 0.6	-38.0 ± 1.0
C_{28}	-35.8 + 1.0	-37.4 ± 0.5	-36.6 ± 0.7	-37.3 ± 1.0
C_{30}	-35.5	-37.1 ± 1.4	-36.6 ± 0.8	-37.2 ± 1.1
n-alkanes				
C_{25}	-36.2 ± 0.5	-40.4 ± 0.6		-38.6 ± 0.7
C_{27}	-39.4 ± 0.7	-42.3 ± 0.7	-41.1 ± 0.5	-42.3 ± 1.2
C_{29}	-38.1 ± 1.1	-40.5 ± 0.7	-38.1 ± 0.6	-39.2 ± 1.0
C_{31}	-37.5 ± 1.1	-40.5 ± 0.6	-38.1 ± 0.5	-39.1 ± 1.2

Table 4. Carbon isotope ratios ($\delta^{13}C_{wax}$) for *B. nana* and *E. vaginatum* grown under different light conditions. DL = Diurnal Light; CL = Continuous Light. The uncertainties represent 1σ based on replicate plant specimens.

9. Figures

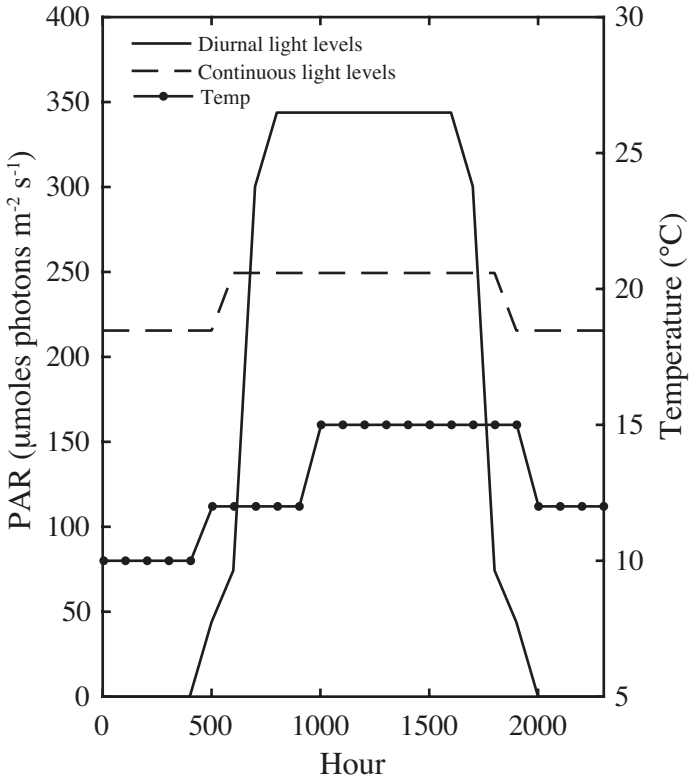

Figure 1. Growth chamber light and temperature conditions.

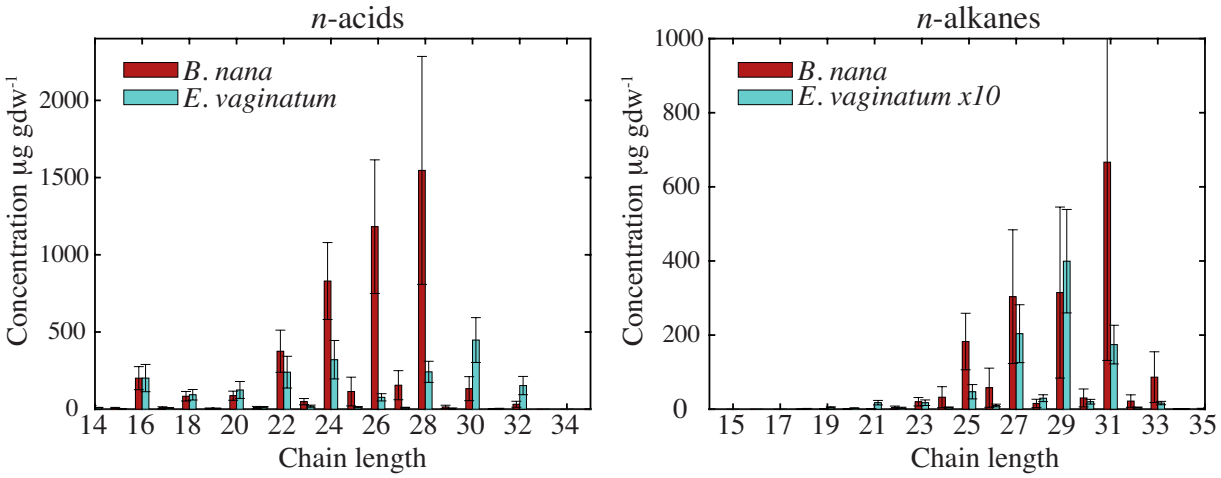

Figure 2. The *n*-alkane and *n*-acid distributions for *B. nana* and *E. vaginatum*. No differences in wax distributions were observed between light treatments, and so data represent an average of both treatments. Note scale difference between *n*-acids and *n*-alkanes. Error bars represent 1σ uncertainty of the concentrations based on replicate plant specimens (*n*=5 for *B. nana* and *n*=9 for *E. vaginatum*).

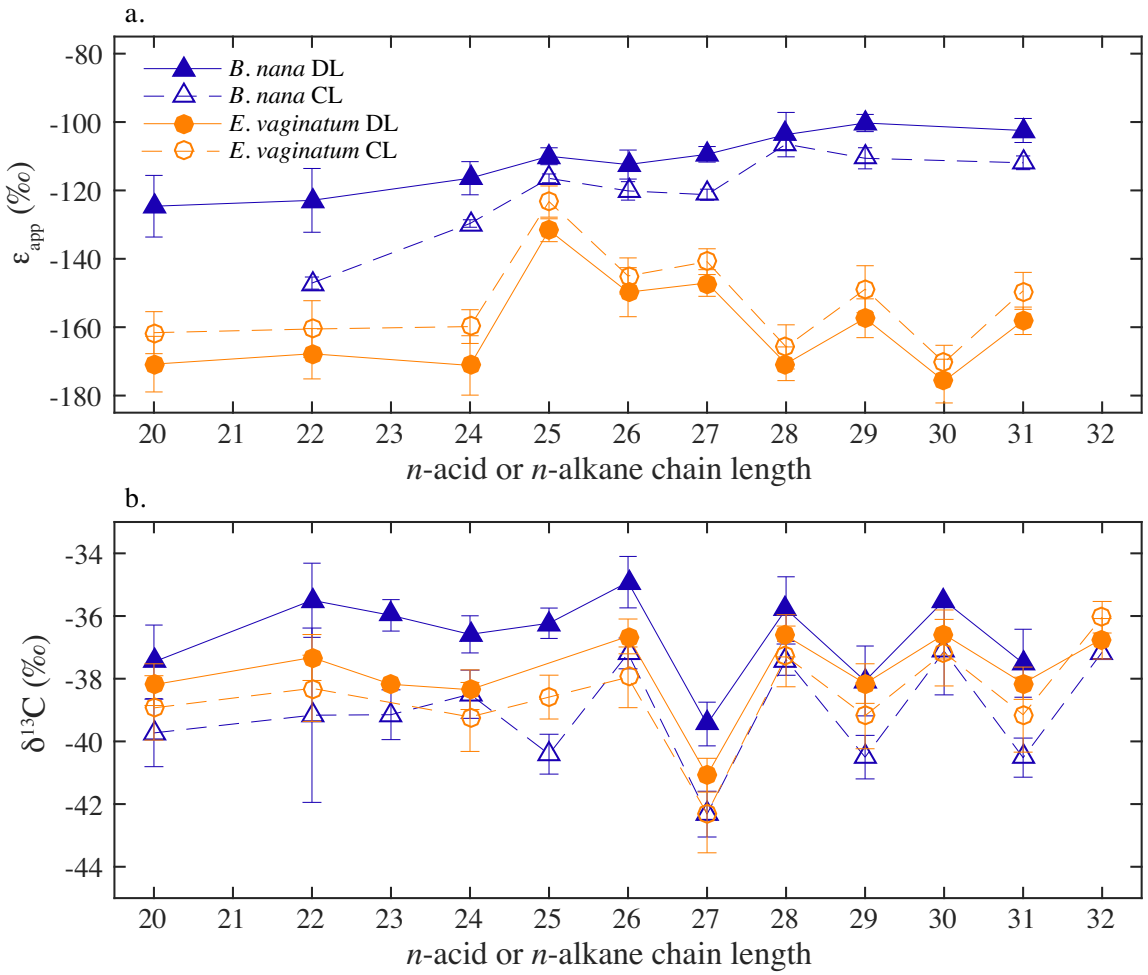

Figure 3. a) Net apparent fractionation between irrigation water (-39.0‰) and leaf waxes under diurnal light (closed symbols) or continuous light (open symbols) for *Betula nana* (blue) and *Eriophorum vaginatum* (orange). Even-numbered chain lengths represent *n*-acids and odd-numbered chain lengths are *n*-alkanes. b) Carbon isotope values of leaf waxes. Symbols and colors are the same as in a. Error bars represent 1σ uncertainty of δD_{wax} or $\delta^{13}C$ based on replicate plant specimens (*n*=5 for *B. nana* and *n*=9 for *E. vaginatum*).

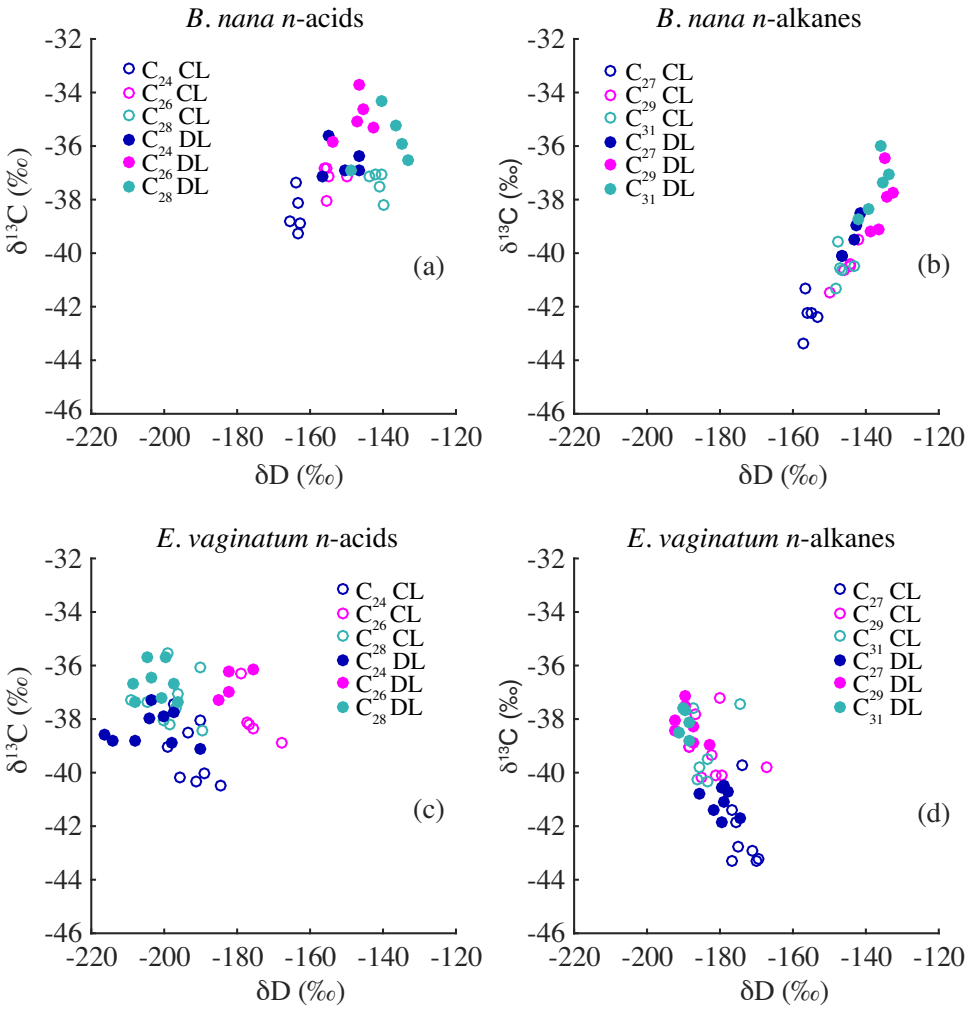

Figure 4. $\delta^{13}C_{wax}$ vs δD_{wax} for *B. nana* (a, b) and *E. vaginatum* (c, d) and for *n*-acids (a, c) and *n*-alkanes (b, d). Filled circles are from the diurnal light treatment (DL), open circles are from the continuous light treatment (CL), and colors represent different chain length homologues.

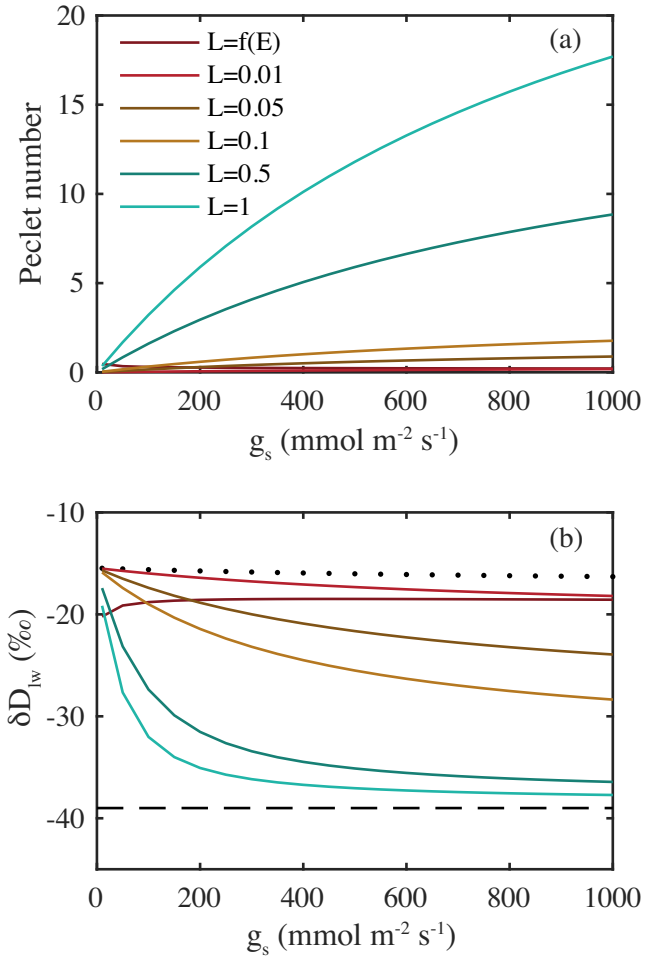

Figure 5. Modeled values of the Péclet number (a) and leaf water D/H ratios (b), as a function of stomatal conductance (g_s) and leaf effective path length (L). L is shown in units of meters, and in the first case, L is calculated as a function of transpiration rate (E, mmol m^{-2} s^{-1}) with the equation $L = 2.36x10^{-2}*E^{-1.2}$ (Song et al. (2013). Colors in panel b are as in panel a, with the dotted line representing the δD_{lw} at the site of evaporation and the dashed line representing the δD of the irrigation water.

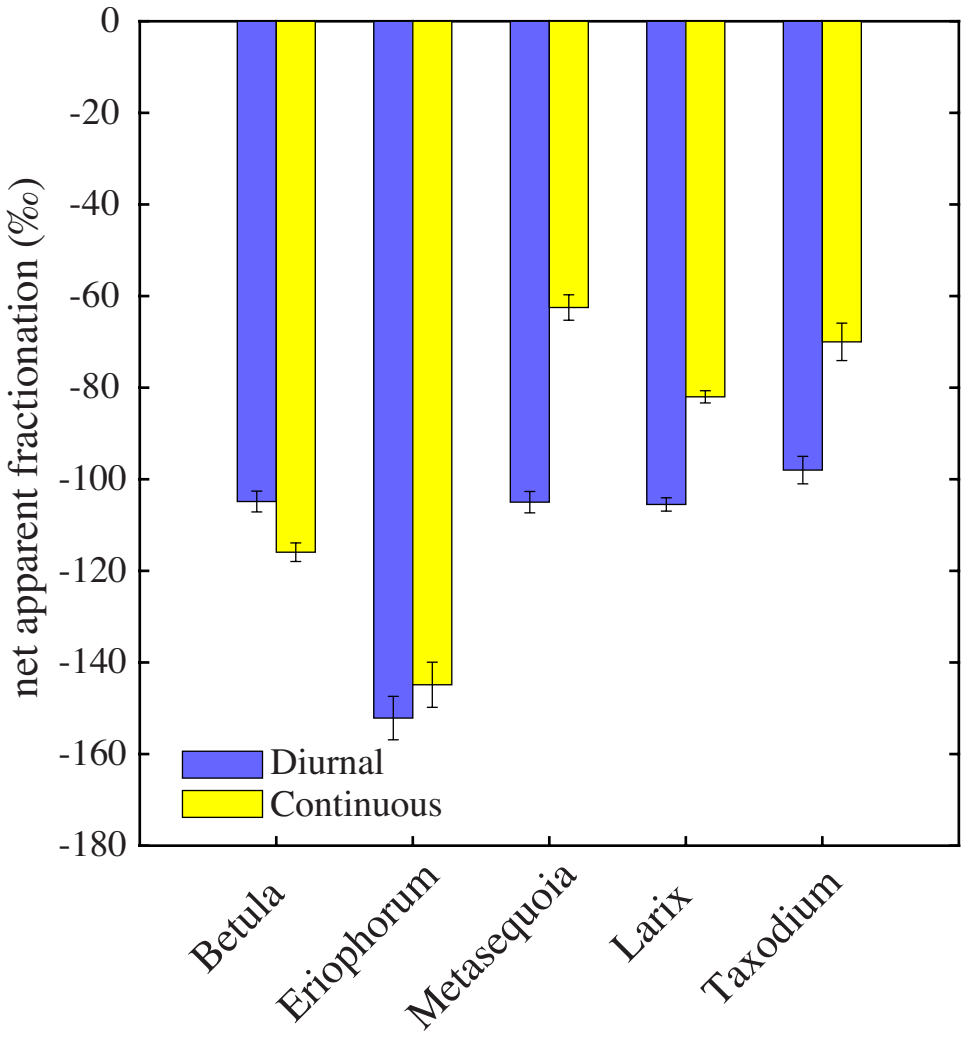

Figure 6. A comparison of net apparent fractionation (ε_{app}) of five plant species grown under diurnal and continuous light conditions. Data for *Metasequoia*, *Larix*, and *Taxodium* are from Yang et al. (2009). Data represent averages of C_{27} and C_{29} *n*-alkanes.