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Inter- and intra-annual C and N isotopic variability of C3 and C4 grasses in a temperate-humid dune environment

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Abstract

Seasonal and annual variations in foliar $\delta^{13}C$ and $\delta^{15}N$ of a C_3 grass, *Ammophila breviligulata*, and a C_4 grass, *Calamovilfa longifolia*, from the sand dunes of Pinery Provincial Park in southern Ontario, Canada were investigated to assess isotopic variability at a single site within a temperate-humid climatic zone. This work quantifies seasonal $\delta^{13}C$ and $\delta^{15}N$ variation, tests for correlation of $\delta^{13}C$ and $\delta^{15}N$ with weather parameters, and evaluates the isotopic responses of these grasses to location within the dune system. Throughout the 2014 growing season, there was ~ 1 to 2 ‰ change in $\delta^{13}C$ and ~ 3 to 4 ‰ change in $\delta^{15}N$ that was related to plant development. Foliar $\delta^{13}C$ and $\delta^{15}N$ are significantly correlated to total summer precipitation amount and, under some conditions, temperature. The foliar isotopic variations are too small, however, to affect paleoclimatic interpretation of such proxies within such a climatic regime.

Keywords

Carbon and nitrogen isotopes, C_3 and C_4 grasses, Pinery Provincial Park, southern Ontario, *Ammophila breviligulata*, *Calamovilfa longifolia*

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Chapter 1

1 Introduction

Carbon and nitrogen isotopic compositions of ancient plant material and organic matter in soil profiles have been studied extensively to understand paleoclimatic conditions (Hatte et al., 2001; Kohn, 2010; Leavitt et al., 2007; Youfeng et al., 2008). The primary factors that govern changes in foliar carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$**,** respectively[1]) have been well established. Environmental conditions, such as precipitation and temperature, have been shown to influence carbon and nitrogen isotopic variability in plant material across environmental gradients (Codron et al., 2013; Craine et al., 2009; Diefendorf et al., 2010; Liu et al., 2014; Murphy and Bowman, 2009; Wang et al., 2010). However, there are few studies that have examined how environmental conditions influence carbon and nitrogen isotopic variability of plant material through time at a single locality (i.e., Codron et al., 2013). More specifically, there are no studies that have attempted to quantify the amount of isotopic variation caused by weather conditions at a single locality on an annual temporal scale in a temperate-humid climate. Here, we (a) quantify seasonal carbon and nitrogen isotopic variations of C_3 and C_4 perennial grasses growing at Pinery Provincial Park (PPP) from April to October 2014, (b) determine whether changes in foliar $\delta^{13}C$ and $\delta^{15}N$ of these grasses are correlated to weather conditions from 2006-2014, and (c) determine whether there are differences in the carbon and nitrogen isotopic responses of C_3 versus C_4 dune grasses and how this relates to our use and interpretation of biological proxies for paleoclimatic reconstruction at Pinery Provincial Park

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[1] Stable isotopic compositions of plant material are reported in *delta*-notation, defined as

$$\delta\,{}^{a}X = \left[\frac{{}^{a}R_x / {}^{a}R_{std}}{{}^{a}R_{std}}\right] * 1000$$

Values are reported in per mil (‰) and are calibrated to the international standard VPDB for $\delta^{13}C$ and AIR for $\delta^{15}N$

1.1 Pinery Provincial Park

Pinery Provincial Park (PPP) (43°15' N, 81°50' W) is located on the southeastern shore of Lake Huron in the Lake Huron-Georgian Bay Climatic Region (Bakowsky, 1990; Morrison and Yarranton, 1974) and is characterized by a more humid and warmer climate relative to the rest of the region (Fig. 1.1) (Bakowsky, 1990). PPP encompasses an area of approximately 2,532 hectares (6,330 acres) and is home to over 757 plant, 325 bird and 60 butterfly species. PPP is the largest protected forest in Southwestern Ontario and consists of ~ 50 % of the world's rare oak savanna ecosystem (the largest in the world) as well as a freshwater coastal dunes system (The Friends of Pinery Park, n.d.). The PPP area has a mean annual temperature of 8.0°C and receives approximately 856 mm of precipitation spread fairly even throughout the year (Bakowsky, 1990). PPP experiences a frost-free period of 150-160 days and has a growing season of 200-210 days (Morrison and Yarranton, 1974).

The PPP area is unique because it is situated on the Lake Huron sand dune system (Baldwin and Maun, 1983). The Lake Huron sand dune system represents a chronosequence that extends from present to at least 5,000 years before present (VandenBygaart and Protz, 1995). The sand dune system formed, and is currently being formed, from sand blown onshore from Lake Huron's beach, which is situated on a gravel bar that was formed at the time of Lake Nipissing, ~ 6,000 years before present (Morrison and Yarranton, 1974). The bedrock in the area consists of grey shale and limestone from the Middle Devonian Hamilton Formation with the dune sands composed of ~ 25 % limestone, 14 % chert, 13 % sandstone, 11 % siltstone 11 % dolostone, 11 % Precambrian mafic fragments, 9 % Precambrian metamorphic fragments and 6 % Precambrian felsic fragments (Cooper, 1979). Grain size analyses characterize the dune sands as 0.1 % gravel, 98.6 % fine or medium-grained, well-sorted, aeolian sand and 1.3 % silt (Steinbach, 1999).

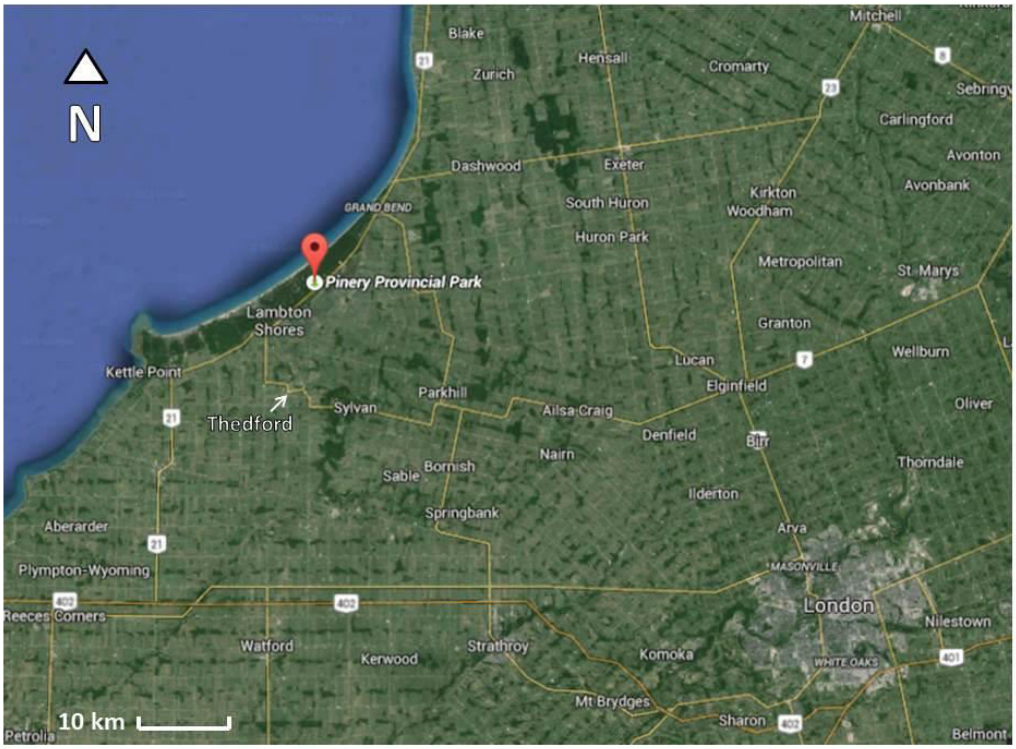

Figure 1.1: Location of Pinery Provincial Park and Thedford, Ontario. Image obtained from Google Maps © 2016.

There are differences in the physical characteristics of the younger and older sand dunes. The sands in the younger dunes are composed of ~ 2.0 % organic matter (VandenBygaart and Protz, 1995) while the organic material content is higher (~ 5.0 %) in soils from older, forested dunes (Ensign et al., 2006). The soil on the foredunes can be classified as an orthic regosol while the soil on the first dunes can be classified as an eluviated eutric brunisol (VandenBygaart and Protz, 1995). These soils developed as a result of historic glaciation and the influx of aeolian material. The younger dunes are also characterized by a lower moisture-retaining capacity, lower concentrations of available K^+ and Mg^+ and higher levels of Ca^{2+} in the surface soil, and increased air-driven turbulence (Baldwin and Maun, 1983; Ensign et al., 2006). The average hydraulic conductivity of the younger dune sands is ~ 2 x 10^{-2} cm/s. The groundwater table in this area is ~ 176-177 m above sea level and does not show a significant fluctuation in depth between months of high and low precipitation due to the proximity to Lake Huron (Steinbach, 1999).

The foredunes and first younger dune (~ 100 years old) are of interest in this study because of the abundant presence of both C_3 and C_4 grasses. The relatively simpler lifecycle of perennial grasses and the fact that grasses can have either a C_3 or C_4 photosynthetic pathway make a grasses a better subject matter compared to complex vegetation such as shrubs and trees. This is further enhanced by the fact that both C_3 and C_4 grasses grow within a similar area of the dunes which enables us to evaluate how the environment influences the observed isotopic trends.

The younger first dune is sparsely vegetated with *Ammophila breviligulata* (*A. breviligulata*) and *Calamovilfa longifolia* (*C. longifolia*). *A. breviligulata* is a cool-season C_3 grass (Maun, 1985) while *C. longifolia* is a warm-season C_4 grass (Barnes and Harrison, 1982). Both *A. breviligulata* and *C. longifolia* are long-lived perennial grasses that initiate growth during the spring. *A. breviligulata* initiates growth ~ 4 weeks earlier than *C. longifolia* and undergoes senescence ~ 4 weeks later than *C. longifolia* (Elfman et al., 1986). Both species have extensive rooting systems and are important for dune stabilization (Lady Bird Johnson Wildflower Center, 2016; Weaver, 1958). *A. breviligulata*, in particular, was introduced in the Pinery in the 1970s in order to promote dune stabilization and the restoration of the natural features (Peach, 2006).

1.2 Perennial Grass Lifecycle

The growth of perennial grasses is characterized by five main stages: germination, vegetative stage, elongation, reproduction (seed-ripening stage) and dormancy (Fig. 1.2). Each stage is characterized by particular developmental and morphological changes in the plant (Moore et al., 1991).

The germination stage is defined as the events that occur after a seed is planted to the emergence of the first leaf from the soil (Moore et al., 1991). Many factors delay germination until conditions are favourable for plant establishment (Maun, 2009). The primary shoot of the perennial grass forms during this stage. There are multiple substages within the germination stage, as defined by the development of specific organs, depending on the species. The germination stage only occurs once – during the

establishment of the plant – in the plant's life history. In subsequent growing seasons in which growth is initiated, tillers[2], rather than shoots, are produced (Moore et al., 1991).

The vegetative stage is the next stage in perennial grass development. It is defined as the period, during each growing season, in which leaf growth and development is initiated by the plant (Moore et al., 1991). Carbohydrate reserves in the stems, roots, rhizomes[3], and stolons[4] are important resources that are utilized to meet the plant demand for energy and carbon during the vegetative stage (Schacht et al., 2005). These reserves are utilized to develop photosynthesizing tissue.

Once a sufficient amount of photosynthesizing tissue has been developed, perennial grasses undergo elongation. This phase is defined by the location of culm[5] elongation (Moore et al., 1991) and can be divided into two distinct phases (Schacht et al., 2005) with multiple substages as defined by the number of nodes developed by the grass (Moore et al., 1991). In the early elongation phase, tissue growth is heavily reliant upon carbon reserves as the energy demand is greater than the energy that can be produced by photosynthesis alone. As elongation and leaf development occurs, the grass transitions into the late elongation phase. At this point, leaves become a source of carbon and energy rather than a sink (Schacht et al., 2005). Also, by this time, carbohydrate reserves become low (Steen and Larsson, 1986; White, 1973). The elongation stage is considered to end when the inflorescence[6] is enclosed in the uppermost leaf sheath[7].

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[2] Tiller is defined as a leafy non-flowering shoot

[3] Rhizome is defined as a horizontal underground plant stems capable of producing roots and shoots

[4] Stolon is defined as a horizontal branch from the base of the plant that is capable of producing roots and aerial nodes

[5] Culm is defined as an aboveground stem

[6] Inflorescence is defined as a cluster of flowered on a branch or system of branches

[7] Sheath is defined as the tubular portion of the leaf which wraps around or encloses the stem

The reproductive stage is the next stage in grass growth and is marked by the emergence of the inflorescence. This period in the grass life cycle continues through to anthesis[8] and fertilization (Moore et al., 1991). During this stage, there is a significant amount of green leaf area and the environment is favorable for photosynthesis. The carbohydrate demand is relatively low as photosynthates are used for seed development, tiller maintenance and rhizome development (Schacht et al., 2005).

The final stage in the life cycle of perennial grasses is dormancy. At this time, the plant begins to prepare to overwinter. A healthy root system has been developed by the plant and carbohydrate reserves in the roots have been replenished. Replenished reserves are important for plant survival overwinter and for the initiation of photosynthesis in the next growing season (Steen and Larsson, 1986; White, 1973).

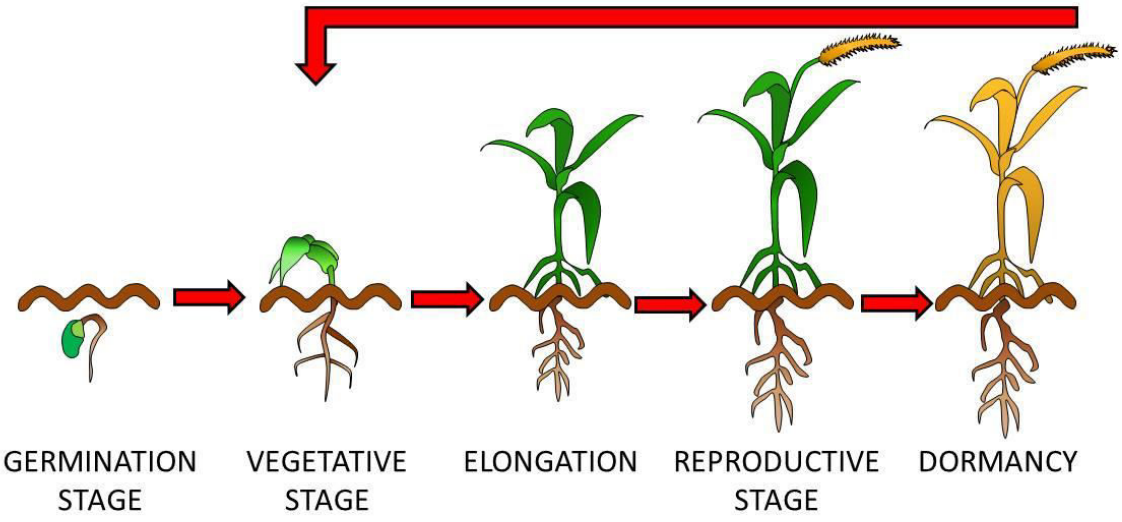

Figure 1.2: Overview of life cycle of a perennial grass.

[8] Anthesis is defined as the time period is which the flower is fully open and functioning

1.3 Plant Physiology

Studies during the 1960s to 1980s have shown that plants can be categorized based on their photosynthetic pathway. These studies established that the photosynthetic pathway primarily governs carbon isotopic discrimination (Bender, 1971; Farquhar, 1983; Farquhar et al., 1989; O'Leary, 1981; Park and Epstein, 1961; Whelan and Sackett, 1973). There are three main photosynthetic pathways – C_3, C_4, and Crassulacean acid metabolism (CAM). C_3 plants have $\delta^{13}C$ ranging from −34 to −24 ‰, while C_4 plants have $\delta^{13}C$ ranging from −19 to −6 ‰ (Smith and Epstein, 1971). CAM plants have intermediate $\delta^{13}C$ ranging from − 20 to −10 ‰ (O'Leary, 1988). Photosynthetic pathways vary amongst plants because they differ in physiology – including leaf structure, and use of metabolites and enzymes (Fig. 1.3) – and their evolutionary history. For example, the first product of CO_2 conversion in the C_3 photosynthetic pathway is 3-phosphoglycerate (PGA), a 3-carbon (3-C) molecule, while in the C_4 photosynthetic pathway, the first product is oxaloacetate, a 4-carbon (4-C) molecule (Gowik and Westhoff, 2011).

Carbon dioxide (CO_2) from the atmosphere is the only carbon source for plants. The diffusion of CO_2 into the internal gas space of the leaf via the stomatal cavity is illustrated in Fig. 1.3. During diffusion, there is a fractionation of ~ 4.4 ‰ against $^{13}CO_2$ in both C_3 and C_4 plants (O'Leary, 1981). Additional carbon isotopic discrimination of the now diffused CO_2 occurs within the leaf and is based on both leaf structure and the physiology of the pathway (Fig. 1.3). Typically, the initial enzymatic process is the rate limiting step and the primary determinant of foliar $\delta^{13}C$ of the plant. There are smaller fractionations associated with CO_2 transformations (Farquhar et al., 1989).

In C_3 plants, most of the carbon isotopic fractionation is attributed to Ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCo) activity (O'Leary, 1988). RuBisCo is involved in the carboxylation of ribulose-1, 5-bisphosphate which produces the biochemical reactants necessary to produce sugars via the Calvin Cycle (Gowik and Westhoff, 2011). RuBisCo is located in the mesophyll cells of C_3 leaves and discriminates against ^{13}C by ~ −29‰ (Fig. 1.3a). RuBisCo can be involved in two distinct, but competing, reactions – one in which oxygen (O_2) is the substrate, and the

other in which CO_2 is the substrate. When O_2 is the substrate of RuBisCo, the reaction is referred to as photorespiration. Photorespiration is the oxidation of sugars during photosynthesis and reduces the net amount of carbon fixed by the plant. When CO_2 is the substrate, two molecules of 3-PGA are produced (Ehleringer and Cerling, 2002; Gowik and Westhoff, 2011). PGA is an intermediate product and the precursor to sugars and starches produced by the plant via photosynthesis (Ehleringer and Cerling, 2002).

As illustrated in Fig. 1.3a, CO_2 initially diffuses from the internal gas space within the leaf into the mesophyll cells, where RuBisCo is located (O'Leary, 1988; von Caemmerer and Furbank, 2003). Carboxylation of CO_2 is an irreversible step. Additional isotopic fractionation also occurs during the production and transport of sugars (Badeck et al., 2005; O'Leary, 1988). Mathematically, the net effect of the primary photosynthetic processes on isotopic discrimination in C_3 photosynthetic plants can be expressed as:

Equation 1.1 $$\Delta = a + (b-a)\, p_i / p_a$$

where Δ is the ^{13}C discrimination between the atmosphere and the plant, a is the fractionation associated with CO_2 diffusion into the plant (~ −4.4 ‰), b is the fractionation associated with carboxylation primarily as a result of discrimination by RuBisCo (~ −29 ‰), and p_a and p_i represent the ambient and intercellular partial pressures of CO_2, respectively (Farquhar et al., 1989).

By comparison, most of the isotopic fractionation in C_4 plants can be attributed to phosphoenolpyruvate carboxylase (PEP carboxylase) activity and the amount of bundle sheath leakage (Φ). Bundle sheath leakage is defined as the ratio of CO_2 released back to the mesophyll cells as a result of decarboxylation of C_4 molecules in the bundle sheath cells (Farquhar, 1983; Fravolini et al., 2002). Like C_3 plants, CO_2 is transported into the leaf by diffusion through the stomatal cavity. However, unlike C_3 plants, there are two simultaneously operating carboxylating mechanisms within the leaf (O'Leary, 1988). This is commonly referred to as the "CO_2 pump" because it ultimately increases CO_2 concentration at the site of decarboxylation as highlighted in Fig. 1.3b (von Caemmerer and Furbank, 2003).

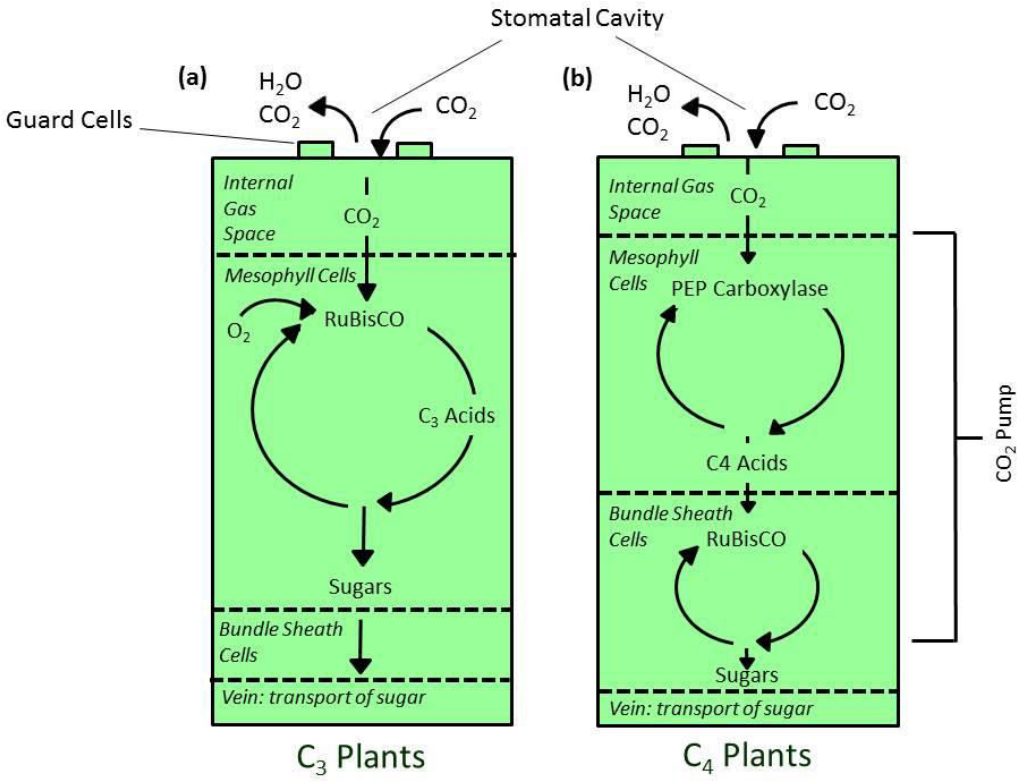

Figure 1.3: Schematic of key differences and key photosynthetic steps in (a) C_3 and (b) C_4 photosynthetic pathways. Adapted from O'Leary (1981).

Carbon dioxide diffuses from the atmosphere into the internal gas space of photosynthetic tissue via the stomatal cavity. The CO_2, once within the internal gas space, then diffuses into mesophyll cells producing bicarbonate (HCO_3^-) ions which are ~ 6 to 9 ‰ enriched in ^{13}C relative to diffused CO_2 (Whelan and Sackett, 1973). The degree of ^{13}C enrichment in HCO_3^- relative to gaseous CO_2 is temperature-dependent: 8.5 ‰ at 20ºC, 7.9 ‰ at 25°C and 7.4‰ at 30°C (Mook et al., 1974). The HCO_3^- ions in the mesophyll cells interact with PEP carboxylase, which discriminates against ^{13}C by 2.2 ‰ (O'Leary, 1981). The product of this interaction is ^{13}C-enriched 4-C acids such as oxaloacetate (Ehleringer and Cerling, 2002). These newly produced 4-C acids subsequently diffuse into the bundle sheath cells where these acids are decarboxylated to produce CO_2 (Ehleringer and Cerling, 2002; O'Leary, 1988). RuBisCo is located in the bundle sheath cells and carboxylates the CO_2 produced from the decarboxylated 4-C to produce 3-PGA molecules. This ultimately leads to the production of sugars via the Calvin Cycle, which

are then stored and/or transported throughout the plant (Fig. 1.3b) (O'Leary, 1988). The primary determinants of $\delta^{13}C$ of C_4 plants can be represented mathematically as:

Equation 1.2 $$\Delta = a + (p_a - p_i)/ p_a + (b_4 + b_3\Phi)\, p_i/ p_a$$

where Δ is defined as the ^{13}C discrimination between the atmosphere and the plant, a is the fractionation associated with CO_2 diffusion (~ −4.4 ‰), b_4 is the fractionation caused by carboxylation as a result PEP carboxylase activity (~ +5.7 ‰), and b_3 is the fractionation caused by carboxylation as a result of RuBisCo activity (~ −29 ‰). The value of Φ represents the amount of leakage from the bundle sheath cells, which limits the extent that fractionation by RuBisCo is expressed, and p_a and p_i represent the ambient and intercellular partial pressures of CO_2 (Farquhar et al., 1989).

Physiological differences between the C_3 and C_4 photosynthetic pathways translate to different competitive abilities, resource use, and optimum growing conditions. C_3 plants discriminate more strongly against ^{13}C during photosynthesis than C_4 plants. This is primarily due to PEP carboxylase activity in the C_4 pathway, which regulates internal CO_2 concentrations more efficiently and significantly reduces the amount of photorespiration (von Caemmerer and Furbank, 2003). Both CO_2 regulation and reduction of photorespiration limits the influence that RuBisCo has on $\delta^{13}C$ by concentrating CO_2 at the site of decarboxylation and reduces the amount of CO_2 recycled in photosynthetic pathway. This also means that C_4 plants have better water use efficiency (WUE) and nitrogen use efficiency (NUE) than C_3 plants. Both WUE and NUE are improved in C_4 plants because the CO_2 concentrating mechanism enables the plant to (a) better regulate the p_i/p_a ratio, and (b) operate at a lower p_i/p_a ratio. This, in turn, reduces the time that the stomata have to remain open, and thereby reduces the amount of transpiration that can occur relative to the amount of carbon fixed by the plant, thus increasing WUE.

There is an energetic tradeoff between the amount of CO_2 that can be fixed and the amount of energy required for photosynthesis. The CO_2 concentrating mechanism in C_4 plants requires more energy than C_3 plants due to the additional carboxylation step (Taylor et al., 2010). As a result, C_4 plants are able to function at lower CO_2

concentrations (Ehleringer and Cerling, 2002) and in warmer, drier climates than C_3 plants (Schulze et al., 1996). It is primarily in these types of environments that the energetic costs are less than the benefits.

1.4 Carbon Isotope Fractionation

1.4.1 Intra-Plant Variation of $\delta^{13}C$

The $\delta^{13}C$ of plants varies among tissues. This intra-plant variation is a result of (a) inherent differences between photosynthetic (autotrophic) and heterotrophic tissues, (b) multiple processes that are simultaneously operating, such as photosynthesis, transport of sugars, and catabolism (i.e. the breakdown of molecules to smaller molecules to release energy), and (c) differences in molecular/biochemical composition of the tissue.

In general, heterotrophic organs (i.e. roots) are enriched in ^{13}C relative to autotrophic organs (i.e. leaves) (Fig. 1.4). For example, tissues such as seeds and tubers are typically enriched in ^{13}C by up to 10 ‰ relative to leaves (O'Leary, 1981). In a meta-analysis of hundreds of isotopic studies, Badeck et al. (2005) demonstrated that belowground biomass was consistently enriched in ^{13}C relative to aboveground biomass on average by 1.2 ‰. Moreover, Badeck et al., (2005) showed that leaves, on average, are 1.3 ‰ depleted of ^{13}C relative to other plant organs and are depleted of ^{13}C relative to roots by 1.1 ‰ in C_3 plants and 0.1 ‰ in C_4 plants. The difference in ^{13}C enrichment among plant parts is significant only in C_3 plants (Badeck et al., 2005; Cernusak et al., 2009). Cernusak et al. (2009) suggest multiple simultaneously operating processes, rather than any one process, are responsible for the observed intra-plant variations in $\delta^{13}C$ between heterotrophic and autotrophic tissues.

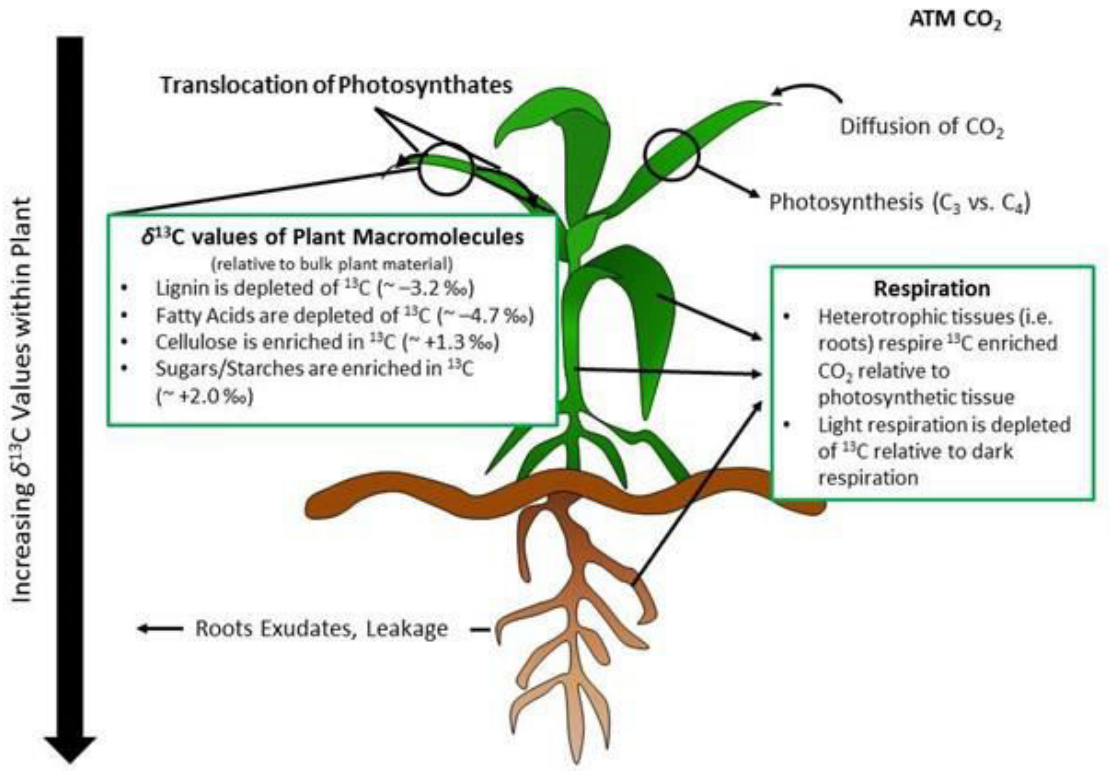

Figure 1.4: Carbon cycle representing the sources and outputs of carbon to a plant and intra-plant variation in $\delta^{13}C$. Values are from Badeck et al. (2005) and O'Leary (1988, 1981).

Post-photosynthetic fractionation and transport of compounds contribute to intra-plant variability in $\delta^{13}C$. Isotopic fractionation associated with enzymatic activity can contribute to some of the observed differences in $\delta^{13}C$ (Tcherkez et al., 2011). For example, invertase is an enzyme that hydrolyses sucrose to fructose and glucose. Invertase is believed to discriminate against ^{13}C, which thereby gradually enriches the remaining sucrose pool within a given organ (i.e. root) in ^{13}C relative to the starting material (Tcherkez et al., 2010). Experimental evidence and mass balance calculations also show that post-photosynthetic transport can contribute to some intra-plant variability (Fig. 1.4) (Terwilliger and Huang, 1996).

Respiration also contributes to intra-plant variability in $\delta^{13}C$. In contrast to photosynthesis, respiration releases CO_2. The $\delta^{13}C$ of respired CO_2 reflects the simultaneous influence of both metabolic pathways and carbon fluxes (Tcherkez et al., 2011). Respired CO_2 from leaves is often depleted of ^{13}C relative to bulk plant material, while CO_2 respired from roots is enriched in ^{13}C relative to bulk plant material (Klumpp et al., 2005; Tcherkez et al., 2011) (Fig. 1.4). As light respiration changes to dark respiration, respired CO_2 becomes more enriched in ^{13}C because different metabolites, with differing $\delta^{13}C$ are utilized by the plant. For example, triose phosphate, which is ^{13}C-depleted relative to bulk plant material, is used by plants during light respiration, while during dark respiration, glucose-6-phosphate, which is enriched in ^{13}C relative to bulk plant material, is utilized (Tcherkez et al., 2010). There is some evidence that carbon isotope fractionation during dark respiration can influence the $\delta^{13}C$ of plant tissue (Wegener et al., 2015). While respiration can cause some intra-plant variation, it does not account for all of the variation that is observed (Cernusak et al., 2009; Wegener et al., 2015).

Individual macromolecules within a plant vary in $\delta^{13}C$, which in turn affects intra-plant carbon isotopic variation (Badeck et al., 2005). Irreversible reactions fractionate against ^{13}C during tissue growth, and thus deplete the products of ^{13}C. Comparatively, reversible reactions favour ^{13}C enrichment, especially when it leads to C-C bond formation (Tcherkez et al., 2011). Lipids tend to be depleted of ^{13}C relative to bulk plant material, while proteins and carbohydrates are enriched in ^{13}C relative to bulk plant material (Badeck et al., 2005; Tcherkez et al., 2011). Cellulose and other carbohydrates are generally ~ 1 to 2 ‰ enriched in ^{13}C than the whole plant tissue, while lignin is ~ 1 to 2 ‰ lighter. Badeck et al. (2005) demonstrated that, on average, lignin and fatty acids are depleted of ^{13}C by 3.2 ‰ and 4.7 ‰ relative to the total organic matter of the organ, respectively. By comparison, both cellulose and sugars/starches are enriched in ^{13}C by 1.3 ‰ and 2.0 ‰ relative to the total organic matter, respectively.

1.4.2 Environmental Controls on $\delta^{13}C$

The $\delta^{13}C$ of plants reflect the balance between photosynthesis, through the ratio of intercellular CO_2 concentrations to ambient CO_2 concentrations (p_i/p_a), and the

environmental conditions under which the plant grows. In particular, the p_i/p_a ratio is influenced by environmental conditions because of the environment's impact on stomatal conductance and the enzymatic activity of RuBisCo (Taylor et al., 2010; Tieszen, 1991). Any environmental factor that affects stomatal conductance will affect ^{13}C discrimination (Farquhar, 1983). Similarly, any environmental factor, such as low nutrient concentrations or temperature that limits enzymatic activity, will limit photosynthesis and thus ^{13}C discrimination. Both stomatal conductance and the enzymatic activity of RuBisCo can be affected simultaneously. Important environmental factors that influence $\delta^{13}C$ are water availability, relative humidity, salinity, elevation, and the canopy effect.

Water availability is negatively correlated to ^{13}C discrimination in leaves. Experimental studies such as those by Sayre et al. (1995) have demonstrated that foliar $\delta^{13}C$ decreases with increasing water availability in several wheat cultivars. Similar trends over precipitation gradients have also been observed in field studies (Diefendorf et al., 2010; Liu et al., 2014; Murphy and Bowman, 2009; Schulze et al., 1996; Wang et al., 2010, 2013). It is hypothesized that this pattern occurs because low water availability promotes stomatal closure in order to reduce water loss through transpiration. This, in turn, decreases the p_i/p_a ratio, and thus reduces discrimination against ^{13}C relative to times when water is more abundant (Farquhar et al., 1989; Taylor et al., 2010). This relationship is stronger in C_3 plants, which are more prone to water loss and are less effective at capturing CO_2 (Taylor et al., 2010). The $\delta^{13}C$ of C_4 plants are less sensitive to water availability because the double carboxylating mechanism concentrates CO_2, even when stomata are closed, thus limiting the amount of transpiration.

Relative humidity is another environmental parameter that contributes to variability in plant $\delta^{13}C$ as it relates to stomatal conductance. Experimental evidence suggests a negative correlation between increasing relative humidity and $\delta^{13}C$ in two C_3 species (Winter et al., 1982). There is some evidence that this negative correlation holds true for most C_3 plants, while the opposite is true for C_4 plants (Madhavan et al., 1991). However, there are conflicting results from field studies about the nature of this relationship with some studies suggesting a relationship between relative humidity and foliar $\delta^{13}C$ and others suggesting no relationship (Li et al., 2007; Zheng and Shimizu, 2005). Likely,

there are differences between species in the $\delta^{13}C$ response to relative humidity that influence the trends observed.

The canopy effect, which refers to re-assimilation of respired CO_2, typically under tree cover, also influences foliar $\delta^{13}C$ (van der Merwe and Medina, 1991). Because CO_2 respired from leaves is depleted of ^{13}C relative to atmospheric CO_2, respiration decreases the $\delta^{13}C$ of ambient CO_2. In turn, this decreases foliar $\delta^{13}C$ of vegetation growing under a dense canopy because isotopically lighter CO_2 is more likely to be used in photosynthesis (Farquhar et al., 1989; Tieszen, 1991; van der Merwe and Medina, 1991).

1.5 Nitrogen Isotope Fractionation

Relative to carbon isotopic fractionation, nitrogen isotopic fractionation in plants is more complex because there are more nitrogen sources and ways that nitrogen can be assimilated into the plant (Fig. 1.5) (Dawson et al., 2002). Additionally, nitrogen, along with phosphorous, is a limiting nutrient for many terrestrial plants and therefore, its availability is an important consideration in isotope fractionation processes. Net nitrogen isotope fractionations tend to be smaller (–10 to +20 ‰) relative to individual fractionating processes such as denitrification (+40 to +60 ‰). The large fractionations commonly associated with nitrification and volatilization are commonly attenuated by other simultaneously occurring processes that fractionate nitrogen isotopes in the opposite direction (Handley and Raven, 1992).

1.5.1 $\delta^{15}N$, the Nitrogen Cycle, and Intra-Plant Variation

For most species, nitrogen is absorbed by plants from the soil as either ammonium (NH_4^+) or nitrate (NO_3^-) (Fig. 1.5) (Evans, 2001). Discrimination against ^{15}N by the plant is generally observed when plant demand for nitrogen is low relative to the supply of nitrogen. Under most field conditions, plant demand for nitrogen tends to exceed nitrogen supply, and therefore plant tissues can be a good indicator of the $\delta^{15}N$ of the nitrogen source (Robinson, 2001).

Ammonium (NH_4^+) is produced in soils as a result of ammonification (mineralization) of organic matter (OM), such as dead plants, and/or animal waste. Microbial activity is

primarily responsible for ammonification and produces ^{15}N rich NH_4^+. N-fixation ($N_{2(atm)} \rightarrow NH_3$) via microbial symbiosis with plants and/or free-living bacteria can also produce NH_4^+ that is enriched in ^{15}N by 0 to 6 ‰ (Evans, 2001). The NH_4^+ produced from ammonification can be absorbed by plants via roots, used by microbes, or used by microbes/fungi and later transferred to the plant (Bernhard, 2010; Robinson, 2001). The $\Delta^{15}N_{plants\text{-}mycorrhiza}$[9] can vary by 8 ‰, with the symbiont being enriched in ^{15}N relative to the plant. This, however, is contingent upon the efficiency of nitrogen transfer, the specific plant-symbiont interaction (Evans, 2001; Hobbie and Högberg, 2012), and the fractionations associated with nitrogen uptake by the plant. When inorganic nitrogen is not limiting, the $\delta^{15}N$ values of the plant will be similar to the $\delta^{15}N$ values of the soil nitrogen because plants typically do not utilize mycorrhizae associations. However, when inorganic nitrogen is limiting, plants will commonly utilize symbiotic microbial associations and therefore the $\delta^{15}N$ of plants will be lower than the $\delta^{15}N$ of the soil nitrogen pool (Evans, 2001).

NH_4^+ can be lost from the soil through either nitrogen volatilization or nitrification. During nitrogen volatilization, N_2 gas is produced and lost to the atmosphere ($NH_4^+ \rightarrow N_2$). This process discriminates against ^{15}N by 8 to 60 ‰ and enriches the remaining NH_4^+ in ^{15}N (Robinson, 2001). The amount of discrimination during volatilization is pH dependent, such that high pH conditions cause ^{15}N discrimination to increase (van Groenigen and van Kessel, 2002). The degree of nitrogen volatilization is also linked to the openness of the nitrogen cycle and water availability. The openness of the nitrogen cycle refers to the amount of nitrogen exchange among the soil, atmosphere and plants. When the nitrogen cycle is more open, there is a significant amount of nitrogen exchange between the soil and atmosphere through nitrogen volatilization. This means that there is less nitrogen (re)cycling between the soil and plants, which enriches the soil in ^{15}N. When the nitrogen cycle is more closed, there is less exchange of nitrogen between the

[9] $\Delta^{15}N_{plants\text{-}mycorrhiza}$ is defined as the difference between in the $\delta^{15}N$ values of plants and mycorrhiza

soil and the atmosphere, nitrogen is cycled more tightly between plants and the soil, and therefore the amount of nitrogen volatilization is reduced.

Ammonium can also be transformed to NO_3^-, another biologically available form of nitrogen, through nitrification ($NH_4^+ \rightarrow NO_3^-$). Nitrification is a multi-step process that is facilitated by microbial activity (Bernhard, 2010). The nitrification process can result in a 40 to 60 ‰ difference in $\delta^{15}N$ between NH_4^+ and NO_3^- (Robinson, 2001) with the product being depleted of ^{15}N (Mariotti et al., 1981). As a result, the $\delta^{15}N$ of NO_3^- can be highly variable.

Like NH_4^+, NO_3^- in the soil is involved in a variety of processes. Free NO_3^- can be utilized by microbes, and/or assimilated into plants through the roots or stem. Assimilation of NO_3^- into organic nitrogen by plants involves nitrogen isotopic fractionation ranging from 0–19 ‰ (Robinson, 2001). This wide range in fractionation reflects different, dynamic factors, such as nitrogen availability, that can influence nitrogen uptake and assimilation in plants. Nitrate can also be lost from the soil through leaching and denitrification ($NO_3^- \rightarrow N_2$) (Bernhard, 2010; Robinson, 2001). Leaching of NO_3^- primarily occurs when the system is saturated with nitrogen. By comparison, denitrification occurs because of microbial activity that reduces NO_3^- to gaseous N_2. There is a fractionation of ~ 28–33 ‰ between N_2O or N_2 and NO_3^- during this process (Robinson, 2001).

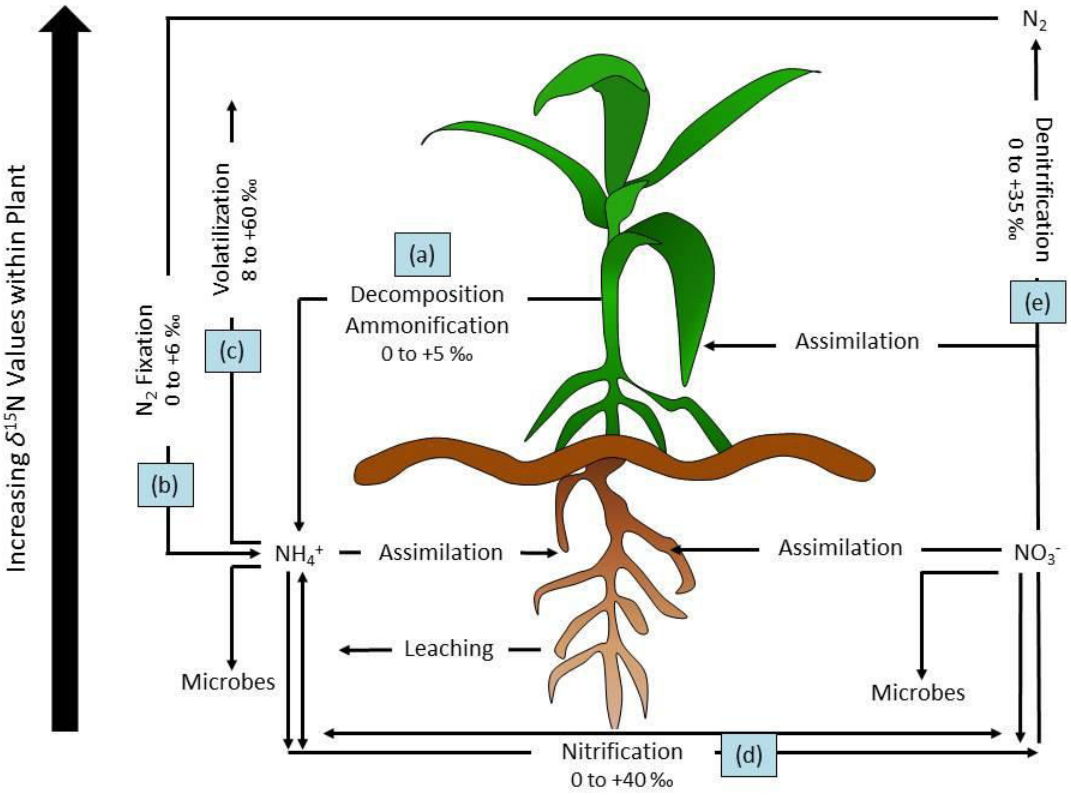

Figure 1.5: Nitrogen cycle representing important sources and outputs of nitrogen to a plant, as well as intra-plant $\delta^{15}N$ variations: (a) ammonification (dead organic material and waste $\rightarrow NH_4^+$); (b) nitrification ($N_2 \rightarrow NH_3$); (c) volatilization ($NH_4^+ \rightarrow N_2$); (d) nitrification ($NH_4^+ \rightarrow NO_3^-$), and (e) denitrification ($NO_3^- \rightarrow N_2$). Fractionations are from Dawson et al. (2002).

There is a significant amount of intra-plant variation in $\delta^{15}N$. A portion of the intra-plant variation results from the $\delta^{15}N$ of the nitrogen source and transport of nitrogen (Dawson et al., 2002; Evans, 2001; Robinson, 2001). When NH_4^+ is the main nitrogen source, nitrogen is assimilated immediately into the plant via the roots. There is only one pathway in which NH_4^+ can be available to plants leading to less intra-plant variation in $\delta^{15}N$. When NO_3^- is the nitrogen source, there is more intra-plant $\delta^{15}N$ variation. This occurs because (a) there are multiple pathways in which NO_3^- can form, and thus more variation in the $\delta^{15}N$ of the nitrogen source, (b) plants can uptake NO_3^- and other derivatives of NO_3^- via the roots and shoots, and there are varying degrees of nitrogen

isotopic fractionation associated with these different nitrogen assimilation pathways, and (c) the system is not as nitrogen-limited, and therefore there is more discrimination against ^{15}N (Evans, 2001). The preferred form of nitrogen for plants is NO_3^-; however other inorganic forms of nitrogen are utilized by plants in nitrogen-limiting environments (Hogberg, 1997).

Nitrogen allocation within a plant changes throughout the growth cycle which also contributes to intra-plant $\delta^{15}N$ variability. Storage of nitrogen, amongst other nutrients and photosynthates, declines with rapid growth and recovers when growth stops or the plant undergoes senescence. Typically, later in the growing season there is the development and/or replenishment of nutrient stores. This competes with growth (Chapin et al., 1990) and therefore can influence foliar $\delta^{15}N$ expressed by the plant based on which phenomenon is more dominant. This is further confounded by the reabsorption of nitrogen which also can influence the $\delta^{15}N$ expressed by the plant. In a meta-analysis, Aerts (1996) demonstrated that ~ 50% of the nitrogen in mature leaves are resorbed during senescence. Nitrogen resorption is controlled by a variety of factors, such as soil moisture and sink strength, and therefore is variable based on the factors that are dominant which in turn contributes to intra-plant $\delta^{15}N$ variation.

1.5.2 Environmental Controls on $\delta^{15}N$

Along with the variable $\delta^{15}N$ of nitrogen sources and the effects of nitrogen uptake, assimilation and translocation in the plant, nitrogen demand and availability also affect ^{15}N discrimination (Evans, 2001). Any environmental factor that affects water availability will ultimately affect the openness of the nitrogen cycle and nitrogen availability, and thus the amount of ^{15}N discrimination (Austin and Vitousek, 1998; Evans, 2001; Murphy and Bowman, 2009). Decreasing water availability increases the amount of nitrogen exchange between the atmosphere and soil, thus effectively increasing the openness of the nitrogen cycle. Because there can only be one limiting resource at a given time, when water becomes more limiting than nitrogen, there is an excess of nitrogen relative to soil water. This, in turn, means that nitrogen in the soil is more prone to volatilization and/or denitrification because it is not being cycled as tightly by plants. Both volatilization and

denitrification discriminate against ^{15}N. This enriches the remaining soil nitrogen pool in ^{15}N, which ultimately is reflected in higher $\delta^{15}N$ of the plant (Evans, 2001).

Typically, $\delta^{15}N$ of plants are higher under more arid conditions and lower under more humid conditions (Aranibar et al., 2004; Austin and Vitousek, 1998; Craine et al., 2009; Murphy and Bowman, 2009; Swap and Aranibar, 2004; Wang et al., 2010). In other words, $\delta^{15}N$ of plants is negatively correlated to water availability. Studies of the Kalahari Desert, which is a sandy and very nutrient-poor ecosystem, are of particular interest (Aranibar et al., 2004; Wang et al., 2010). The results from these studies suggest that water availability may be a more influential factor than nitrogen limitation in controlling $\delta^{15}N$ trends related to environmental conditions.

Temperature is also an important factor controlling the $\delta^{15}N$ of plants. Foliar $\delta^{15}N$ has been shown to be positively correlated with temperature over large temperature gradients (Amundson et al., 2002; Codron et al., 2013; Craine et al., 2009). Craine et al. (2009) demonstrated that on a global scale, plant $\delta^{15}N$ increases with temperature once the spread in the mean annual temperature (MAT) across sites is greater than 0.5°C. Codron et al. (2013) also found that there was a weak, but significant positive correlation between $\delta^{15}N$ and temperature in C_4 grasses on a regional scale in a semi-arid savanna.

1.6 Research Objectives

This study examines the sensitivity of foliar $\delta^{13}C$ and $\delta^{15}N$ of C_3 and C_4 dunes grasses to weather conditions at Pinery Provincial Park (PPP), a temperate humid region located in southwestern Ontario. Two representative grasses, *A. breviligulata*, a C_3 grass, and *C. longifolia*, a C_4 grass, are investigated. The three main objectives of this study are:

(1) To quantify within-plant carbon and nitrogen isotopic variation for *A. breviligulata* and *C. longifolia* growing at a single locality through the growing season (April to October). The isotopic variation observed between different sites will be used to determine whether seasonal isotopic changes are consistent across different areas of PPP.

(2) To determine whether changes in foliar $\delta^{13}C$ and $\delta^{15}N$ of *A. breviligulata* and *C. longifolia* are correlated to weather conditions at a single site within a temperate-humid climatic zone on an inter-annual time scale (2006-2014).

(3) To determine whether or not there are differences in the carbon and nitrogen isotopic responses of C_3 versus C_4 dune grasses from the same temperate humid site, and to explain the observed responses.

1.7 Significance

Previous investigations have attempted to delineate the relationship between both $\delta^{13}C$ and $\delta^{15}N$ and environmental conditions in either experimental (i.e. Ghannoum et al., 2002; Troughton and Card, 1975) or field settings over large environmental gradients (e.g. Amundson et al., 2002; Aranibar et al., 2004; Codron et al., 2013; Craine et al., 2009; Handley and Raven, 1992; Li et al., 2007; Madhavan et al., 1991; Murphy and Bowman, 2009; Swap et al., 2004; van Groenigen and van Kessel, 2002; Wang et al., 2013). There have been few studies that have examined how annual changes in the weather conditions influence carbon and nitrogen isotopic variability at a single site. This is an important consideration because experimental studies do not necessarily capture the dynamic and complex nature of the environment while isotopic relationships determined using environmental gradients do not quantify the amount of isotopic variation associated with site-specific differences.

In addition, there is a lack of diversity in the types of environments studied in the literature. Many field studies have been conducted in tropical and sub-tropical environments (i.e. Aranibar et al., 2004; Austin and Vitousek, 1998; Codron et al., 2013; Liu et al., 2014; Murphy and Bowman, 2009; Swap and Aranibar, 2004; Wang et al., 2013). To date, there are no studies of a temperate humid dune system. Understanding the isotopic response of plants in different climatic regions is beneficial to understanding how climate influences isotopic variability as well as quantifying the range of isotopic variability that can be associated with changing environmental conditions in different climatic zones.

The results of this study are intended to provide an isotopic baseline for dune vegetation at PPP. Such a baseline can be used for (a) paleoclimatic reconstruction of similar

environments, (b) delineating how climate has changed in the area through the use of the dune chronosequence present at PPP, and (c) evaluating future climatic/ecosystem changes. Quantifying the isotopic sensitivity of plants to environmental conditions as a result of annual changes in the growing season has important implications for how isotopic proxy records are interpreted in terms of environmental and ecosystem change. Understanding the isotopic sensitivity of plants to annual changes in environmental conditions and micro-environmental heterogeneity can help constrain interpretation of isotopic results for ancient vegetation in terms of climatic change.

1.8 Outline of Thesis

Chapter Two describes the methods employed in this study to test the isotopic sensitivity of C_3 and C_4 grasses to weather conditions at PPP. Chapter Three reports the carbon and nitrogen elemental and isotopic results on both inter- and intra-annual time scales and spatial scales. Chapter Four interprets these results, discussing the implications of this work. Chapter Five summarizes the major findings and implications of this study, and possible future directions for this work.

Chapter 2

2 Materials and Methods

2.1 Sample Collection

Whole plants of *Ammophila breviligulata* (American Beachgrass), a C_3 grass, and *Calamovilfa longifolia var. magna* (Sandreed), a C_4 grass, were collected from Pinery Provincial Park (PPP) (43°15' N, 81°50' W). PPP is located on the southeastern shore of Lake Huron within the Lake Huron-Georgian Bay Climatic Region (Morrison and Yarranton, 1974; VandenBygaart and Protz, 1995). *A. breviligulata* and *C. longifolia* are abundant along the foredunes and younger first sand dunes of PPP and are key dune-stabilizing species.

Samples were collected annually at the end of the growing season from 2006 to 2014, and monthly from April 2014 to October 2014, from along the first dune ridge near beach 9 (Fig. 2.1 and 2.2). Beach 9 is a conservation area and hence less affected by anthropogenic activity than other areas of PPP. At this site, *A. breviligulata* and *C. longifolia* grow in the open dunes within ~4.5 – 6 m of each other along the foredune and the first sand dune ridge, respectively (Fig. 2.2). Above and belowground biomass was obtained annually, while aboveground biomass of both species was collected monthly from this site.

Aboveground biomass of *C. longifolia* was also collected monthly from July to October 2014 from under canopy cover comprised of primarily coniferous trees. These samples were located behind the first dune ridge (Fig. 2.1). This site is approximately 180 to 200 m inland from the shoreline, and is located close to the parking lot of Beach 9. There is also a portable washroom located ~ 3.0 to 4.5 m east of this collection site. *C. longifolia* samples collected at this site provide a comparison to *C. longifolia* samples collected from the dunes in order to assess isotopic changes within a species arising from environmental conditions, both natural and human-induced.

Atmospheric air samples were collected in septum-sealed vials from June 3, 2014 to December 1, 2014, every month. During collection, the vials were allowed to equilibrate

with the local atmosphere for ~ 5 to 10 minutes. Five air samples were collected behind the first dune ridge, in proximity to the precipitation collector, and five air samples were collected from the dense canopy cover near the Heritage Trail parking lot. These sampling sites were chosen to evaluate the influence of respired CO_2 to the local atmosphere.

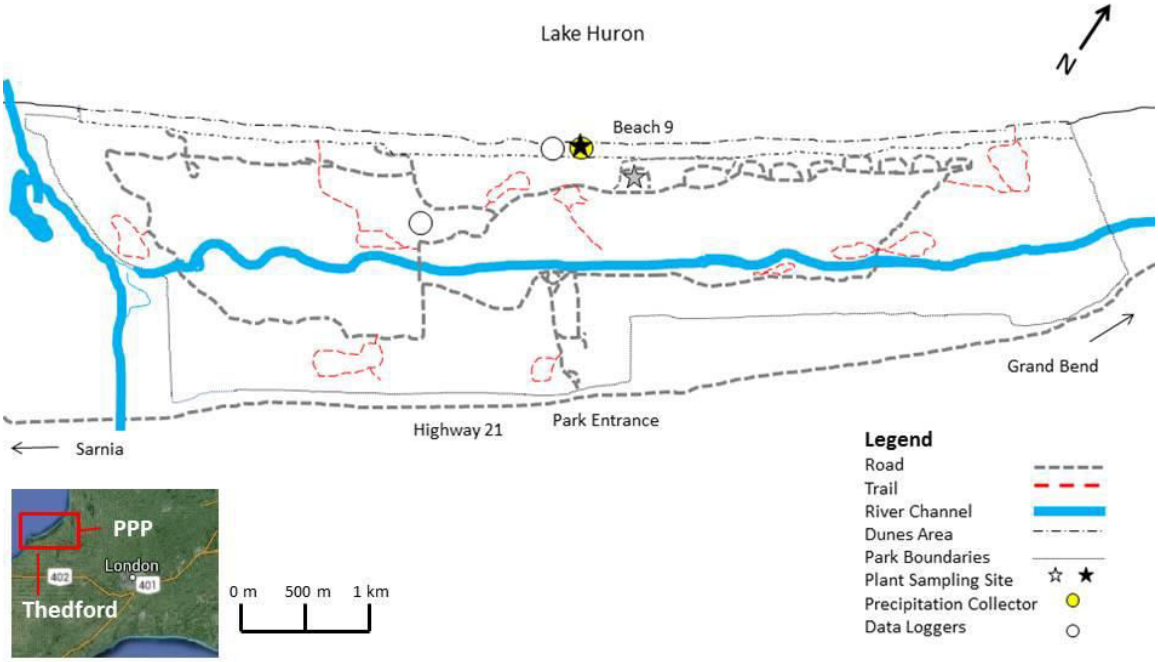

Figure 2.1: Map of Pinery Provincial Park indicating sampling locations. Black star: *A. breviligulata* and *C. longifolia* from sand dunes. Grey star: *C. longifolia* from under canopy cover. Modified from www.mobilemaplets.com.

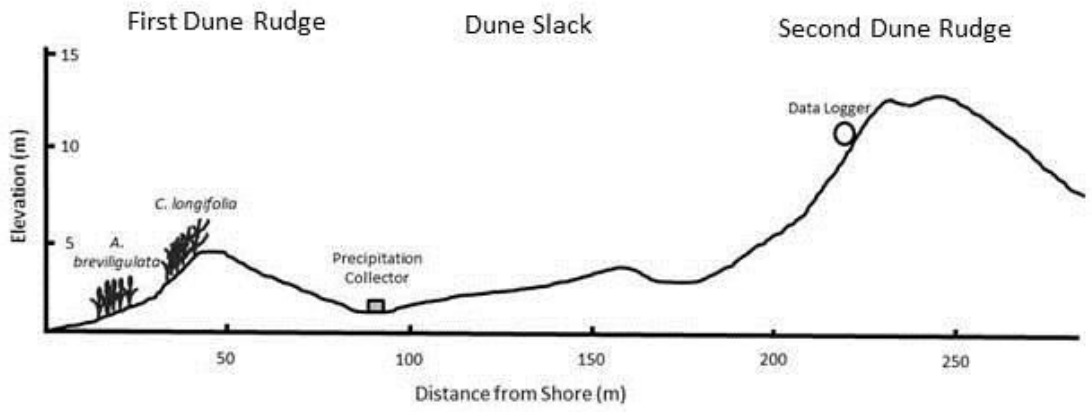

Figure 2.2: Cross-section illustrating the relative locations of *A. breviligulata*, *C. longifolia,* the dune-slack precipitation collector and the data logger at PPP beach 9. Cross-section modified from Baldwin and Maun (1983).

2.2 Weather Information Collection

Monthly precipitation and temperature data for the period 2006 to 2013 were obtained from the Canadian Climate Archives (http://climate.weather.gc.ca/) for the Thedford weather station (43.10°N, 81.51°W), which is located ~15 km southwest of PPP, slightly inland, on the southeastern shore of Lake Huron (Fig. 2.1). For 2014, monthly precipitation and temperature data was collected from both Thedford and PPP. Monthly weather information for Thedford was obtained from the historic weather records for Thedford, Ontario (http://www.theweathernetwork.com/ca/weather/historical-weather/ontario/thedford?intcmp=twn_topnav_fx_historical). For PPP weather data, monthly precipitation amounts were measured using a precipitation collector located in the dune slack situated southwest of the first sand dune near beach 9 (Figs. 2.1 and 2.2). Temperature data was collected using a HOBO Water Temperature Pro v2 Data Logger - U22-001. One data logger was located on the second dune ridge, while the other was located in a Stevenson screen at the PPP Visitor center (Figs. 2.1 and 2.2). Daily weather measurements were averaged to obtain monthly temperature and relative humidity data.

2.3 Sample Preparation

Three individual plants of *A. breviligulata* and *C. longifolia* from each collection year and during 2014, for each collection month, were selected for analysis. Each plant was measured and dissected into individual plant part (i.e. leaf 1, leaf 2). Oldest leaves were labeled as Leaf 1 while the youngest leaf was counted as the highest number (Fig 2.3). Odd-numbered leaves (counted from the bottom of the plant) were washed with distilled water and oven-dried for 20.5 to 21.5 hours at 90°C. Individual leaves were ground into a fine, homogenous powder using a Wig-L-Bug® mechanical grinder.

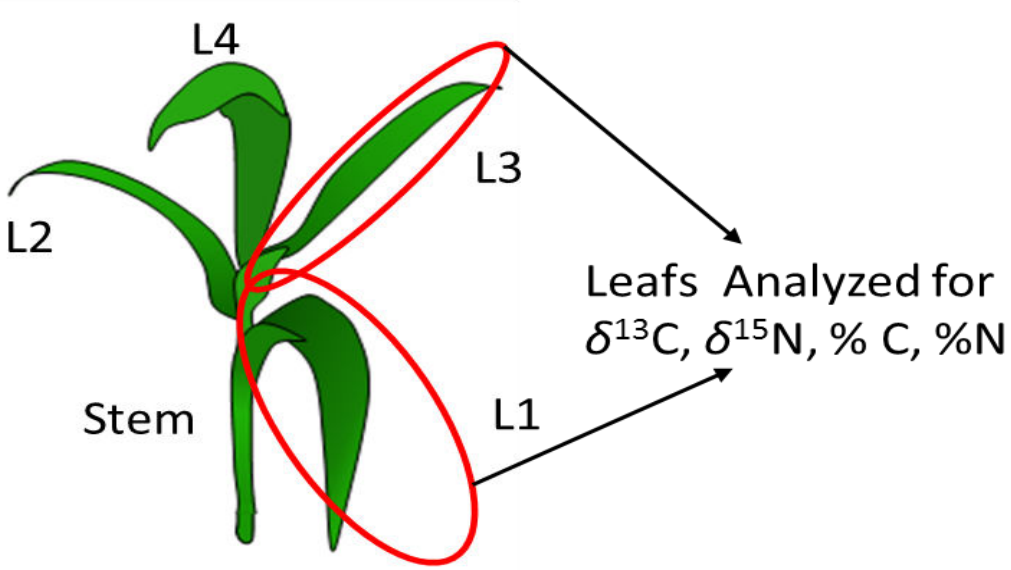

Figure 2.3: Schematic of plant sampling scheme.

2.4 Stable Isotope Analysis

Two to five individual leaves of each plant were analyzed for foliar isotopic ($\delta^{13}C$ and $\delta^{15}N$) and bulk elemental (carbon and nitrogen) compositions using a Costech 4010 Elemental Analyzer (EA) interfaced with a Thermo Scientific Delta^{plus} XL continuous flow stable-isotope-ratio mass-spectrometer (IRMS) at the Laboratory for Stable Isotope Science at the University of Western Ontario (London, ON, Canada). Selected samples were also analyzed for bulk foliar carbon and nitrogen contents using a Fison 1108

Elemental Analyzer, in order to assess the accuracy and precision of carbon and nitrogen contents obtained simultaneously with the isotopic data using the EA-IRMS system. The carbon and nitrogen isotopic analyses were performed in separate analytical sessions, given that nitrogen contents of the plants were too low (< 2.5 wt. %) for simultaneous isotopic measurements. Carbon dioxide produced from samples during the nitrogen isotopic analytical sessions was removed using a Carbo-Sorb trap prior to N isotope analysis.

The $\delta^{13}C$ and $\delta^{15}N$ values are calibrated to VPDB and AIR, respectively, using USGS-40 (accepted values: $\delta^{13}C = -26.39$ ‰, $\delta^{15}N = -4.52$ ‰) and USGS-41 (accepted values: $\delta^{13}C = +37.63$ ‰, $\delta^{15}N = +47.6$ ‰) (www.iaea.org). Additionally, an internal laboratory standard (keratin) and standard reference material (IAEA-CH-6, NIST 1547 (Peach Leaf)) were analyzed to monitor analytical precision and accuracy. The accepted $\delta^{13}C$ of the laboratory keratin is –24.04 ‰, which is comparable to the average $\delta^{13}C$ of −24.06±0.08 ‰ (n = 76) obtained in this study. The accepted $\delta^{13}C$ of IAEA-CH6 is –10.45 ‰ (www.iaea.org), which is comparable to the average –10.48±0.10 ‰; n = 25) obtained in this study. The accepted carbon content of laboratory keratin is 48.2±1.1 wt. ‰, which is comparable to the average (47.1±4.1 wt. %; n = 76) obtained in this study. Carbon elemental data from the seventh analytical EA-IRMS session were not considered because of poor precision and accuracy, as indicated by the large standard deviation of the elemental results obtained for standards (Appendix A). Average sample reproducibility was ±0.06 ‰ (n pairs = 32) for $\delta^{13}C$ and ±0.6 wt. % (n pairs = 32) for carbon content. The accepted $\delta^{15}N$ of laboratory keratin is +6.36 ‰, which is comparable to the average $\delta^{15}N$ (+6.38±0.18 ‰; n = 91) obtained in this study. The accepted $\delta^{15}N$ of NIST 1547 is +1.98 ‰ (www.iaea.org), which is comparable to the average (+1.93±0.12 ‰; n = 73) obtained in this study. The accepted nitrogen content of NIST 1547 is 2.94 wt. %, which is comparable to the average (2.81±0.11%; n = 73) obtained in this study (Appendix B). Average sample reproducibility for duplicates was ±0.05 ‰ for $\delta^{15}N$ (n pairs = 39) and ±0.01 wt. % for nitrogen content (n pairs = 39).

Each month, ten air samples – five samples from the dunes and five samples from under canopy cover – in septum-sealed glass vials were placed on a Thermo Scientific GasBench

block that was coupled to a dual-inlet Delta^{plus} XL-CF-IRMS mass spectrophotometer. The air samples were measured for $\delta^{13}C_{CO2}$. Pure CO_2 from tanks were used as standards and were analyzed for $\delta^{13}C_{CO2}$ using a VG Optima IRMS or VG Prism IRMS. The measured $\delta^{13}C_{CO2}$ of PPP air and standards were calibrated to VPDB using NBS-19 (accepted value: $\delta^{13}C = +1.95$ ‰) and L-SVEC (accepted value*:* $\delta^{13}C = -46.60 \pm 0.2$ ‰).

2.5 Data Treatment and Statistical Analysis

Individual leaves were classified based on species, year (or month) of collection, the plant from which they were collected, leaf position on the plant, and location. Spearman's rank correlation (r) was used to correlate individual foliar $\delta^{13}C$ and $\delta^{15}N$ to precipitation amounts and temperature for various lengths of the growing season. Two-tailed, one-way analysis of variance (ANOVA) was used to compare means of bulk foliar carbon and nitrogen contents, and $\delta^{13}C$ and $\delta^{15}N$ for (a) *A. breviligulata* (or *C. longifolia*) collected during different months or years; (b) *C. longifolia* growing on the dunes versus *C. longifolia* growing under canopy cover; and (c) *A. breviligulata* (or *C. longifolia*.) exposed to varying precipitation amounts or temperatures between years. The $\delta^{13}C$ and $\delta^{15}N$ of individual leaves for each statistical analysis conducted were treated as an individual sample for a given year or month, though average values with standard deviations are plotted in graphs. This was done to capture all intra-plant variation. Levene's test for homogeneity of variance was used to determine the *post-hoc* test that should be used in each ANOVA test. Either Tukey HSD Test (homoscedastic variance) or Dunnet's T3 Test (non-homoscedastic variance) was used to determine which groups in the ANOVA tests were statistically significantly different when significant differences between groups were observed ($p \leq 0.05$). All statistical tests were performed using Statistical Package for Social Sciences (SPSS) with a confidence interval of 95 % ($\alpha = 0.05$).

Chapter 3

3 Results

3.1 Intra-Annual Variation

A total of 189 individual leaves from *A. breviligulata* and *C. longifolia* collected from April to October 2014 were analyzed for carbon and nitrogen content and isotopic composition. These data are presented in Table 3.1 for carbon and Table 3.2 for nitrogen.

There is a large range in the variation between leaves collected from the same plant. For *A. breviligulata*, this variation ranges from 0 to 1.3 ‰ for δ^{13}C, 0.1 to 2.5 wt. % for carbon content, 0 to 1.0 ‰ for δ^{15}N and 0 to 1.1 wt. % for nitrogen content. Comparatively, for *C. longifolia* this variation ranges from 0.1 to 0.8 ‰ for δ^{13}C, 0.1 to 2.6 wt. % for carbon content, 0.1 to 1.2 ‰ for δ^{15}N and 0.1 to 0.5 wt. % for nitrogen content.

3.1.1 Comparison between Species

The δ^{13}C of the leaves of *A. breviligulata* collected monthly in 2014 ranged from –28.0 to –24.4 ‰ with a mean δ^{13}C of –26.1 ± 0.8 ‰ (n = 64) and the δ^{15}N ranged from –2.2 to +1.9 ‰ with a mean δ^{15}N of +0.1 ± 0.9 ‰ (n = 64). The average carbon content of leaves was 46.8 ±1.4 wt. % (n= 47), ranging from 42.1 to 52.5 wt. % and the average nitrogen content of leaves was 0.9 ± 0.6 wt. % (n= 64), ranging from 0.3 to 2.5 wt. %. The δ^{13}C of *C. longifolia* leaves collected on the dunes ranged from –14.5 to –12.6 ‰ with a mean δ^{13}C of –13.7 ± 0.5 ‰ (n = 81) and the δ^{15}N ranged from –6.6 to –2.4 ‰ with a mean δ^{15}N of –4.3 ± 1.0 ‰ (n = 80). The average carbon content was 47.4 ± 1.1 wt. % (n = 77), ranging from 42.3 to 48.8 % and the average nitrogen content was 1.3 ± 0.5 wt. % (n = 80), ranging from 0.4 to 2.4 wt. %. The δ^{13}C of *C. longifolia* leaves collected under canopy cover ranged from –14.8 to –12.9 ‰ with a mean δ^{13}C of –14.1 ± 0.4 ‰ (n = 44) and the δ^{15}N ranged from –4.3 to +0.5 ‰ with a mean δ^{15}N of –1.6 ± 1.2‰ (n = 45). The average carbon content was 47.8 ± 0.8 wt. % (n = 25), ranging from 46.1 to 49.4wt. % and the average nitrogen content was 1.3 ± 0.5 wt. % (n = 45), ranging from 0.6 to 2.0%. Appendix C provides a full list of all samples analyzed.

Table 3.1: Average foliar carbon content and isotopic composition of *A. breviligulata* and *C. longifolia* collected from April to October 2014 at Pinery Provincial Park.

		$\delta^{13}C$							% C					
Julian Calendar Days[1]	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**$\Delta_{oldest-youngest\ leaf}$**	***p*-value[2]**	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**$\Delta_{oldest-youngest\ leaf}$**	***p*-value[2]**
A. breviligulata - Dunes														
122	2	-27.0	0	-27.0	-27.0	-0.1	0.822	2	45.8	1.0	46.5	45.1	1.4	0.527
	2	-27.3	0.8	-27.9	-26.8	-1.1		2	45.4	0.1	45.4	45.3	0.1	
	2	-27.4	0.8	-28.0	-26.8	-1.3		2	43.9	2.5	45.7	42.1	3.6	
154	3	-25.9	0.3	-25.9	-25.7	-0.2	0.430	3	46.0	1.0	46.7	44.7	2.0	0.846
	3	-26.3	0.5	-26.9	-26.0	-1.0		3	46.5	1.1	47.6	46.3	1.4	
	3	-25.5	1.0	-26.7	-24.9	-1.8		3	46.3	1.2	47.2	45.0	2.2	
184	3	-26.4	0.5	-26.5	-25.9	-0.6	0.811	3	47.6	0.6	48.2	47.4	0.8	-
	3	-26.0	1.1	-27.1	-25.0	-2.1		0	-	-	-	-	-	
	3	-25.9	1.1	-27.0	-24.8	-2.2		0	-	-	-	-	-	
213	3	-25.6	1.3	-27.0	-24.4	-2.6	0.902	3	48.1	1.1	46.4	45.3	1.2	0.955
	3	-25.6	0.6	-26.3	-25.2	-1.0		3	48.1	0.2	48.1	48.3	-0.2	
	3	-25.9	0.9	-26.7	-24.9	-1.8		1	47.2	-	47.2	-	47.2	
248	3	-25.7	0.8	-26.6	-25.1	-1.5	0.099	3	46.7	0.6	46.2	46.5	-0.3	0.871
	3	-26.4	0.3	-26.7	-26.3	-0.4		3	46.8	0.5	46.2	46.9	-0.7	
	3	-27.0	0.5	-27.5	-26.5	-1.1		3	46.5	0.9	45.7	47.5	-1.8	
274	4	-26.2	0.3	-26.4	-25.9	-0.4	0.013	4	47.6	0.8	46.5	47.6	-1.1	0.113
	5	-26.2	0.4	-26.9	-25.8	-1.1		5	47.2	0.5	47.0	46.6	0.5	
	4	-25.3	0.5	-25.9	-24.8	-1.1		4	46.7	0.3	46.5	46.5	0	
307	3	-26.0	0.4	-26.5	-25.7	-0.7	0.999	0	-	-	-	-	-	-
	3	-26.0	0.5	-26.6	-25.6	-0.9		0	-	-	-	-	-	

Julian Calendar Days[1]	No. of Samples per Plant	$\delta^{13}C$ Leaf Average	Std. Dev.	Oldest Leaf	Youngest Leaf	$\Delta_{oldest-youngest\ leaf}$	*p*-value[2]	No. of Samples per Plant	% C Leaf Average	Std. Dev.	Oldest Leaf	Youngest Leaf	$\Delta_{oldest-youngest\ leaf}$	*p*-value[2]
	3	-26	0.9	-26.9	-25.1	-1.8		0	-	-	-	-	-	
C. longifolia - Dunes														
154	4	-13.5	0.4	-13.8	-12.9	-0.9	0.480	3	47.2	1.1	47.5	46.2	1.3	0.277
	4	-13.2	0.3	-13.2	-12.8	-0.3		4	47.7	0.4	47.5	47.3	0.2	
	5	-13.3	0.3	-13.2	-12.8	-0.4		5	47.8	0.3	48.0	47.5	0.5	
184	5	-13.4	0.4	-13.7	-12.7	-1.0	0.753	5	46.8	1.1	46.9	45.6	1.4	0.861
	5	-13.2	0.5	-13.6	-12.6	-1.0		5	46.5	1.2	47.1	44.8	2.4	
	5	-13.3	0.3	-13.2	-12.8	-0.5		5	46.9	1.2	46.4	45.2	1.3	
213	5	-13.5	0.3	-13.8	-13.0	-0.7	0.070	5	47.8	0.5	47.0	48.2	-1.2	0.563
	5	-13.6	0.3	-13.8	-13.1	-0.6		5	47.4	0.5	47.5	46.9	0.6	
	5	-13.9	0.1	-13.8	-13.8	0		5	46.7	2.6	48.2	48.5	-0.3	
248	5	-14.2	0.2	-14.3	-13.9	-0.4	0.222	5	47.8	0.1	47.7	47.9	-0.2	0.000
	5	-14.0	0.1	-14.0	-13.8	-0.2		5	47.6	0.3	47.5	47.4	0.1	
	4	-14.1	0.2	-14.0	-14.0	0		4	48.6	0.1	48.5	48.8	-0.3	
274	4	-14.2	0.1	-14.2	-14.1	0	0.923	2	47.4	0.1	47.3	47.4	-0.1	0.287
	4	-14.2	0.2	-14.1	-14.1	0		4	48.1	0.5	48.4	47.4	1.0	
	4	-14.2	0.1	-14.4	-14.1	-0.3		4	46.4	2.1	43.3	47.7	-4.5	
307	4	-13.8	0.2	-14.1	-13.7	-0.4	0.930	3	46.5	0.6	47.3	46.2	1.1	0.010
	4	-13.8	0.3	-14.1	-13.5	-0.7		4	47.6	0.4	47.9	47.1	0.8	
	4	-13.7	0.1	-13.9	-13.7	-0.2		4	48.0	0.4	48.1	47.5	0.6	
C. longifolia – Canopy Cover														
213	5	-13.9	0.3	-14.2	-13.4	-0.8	0.230	5	47.7	0.4	47.8	47.3	0.5	0.963
	5	-14.2	0.2	-14.4	-13.9	-0.5		5	47.8	0.9	47.7	48.4	-0.7	
	3	-14.1	0.2	-14.3	-13.9	-0.4		3	47.7	0.9	46.7	48.5	-1.7	

Julian Calendar Days[1]	No. of Samples per Plant	$\delta^{13}C$ Leaf Average	$\delta^{13}C$ Std. Dev.	$\delta^{13}C$ Oldest Leaf	$\delta^{13}C$ Youngest Leaf	$\Delta_{oldest-youngest\ leaf}$	*p*-value[2]	No. of Samples per Plant	% C Leaf Average	% C Std. Dev.	% C Oldest Leaf	% C Youngest Leaf	$\Delta_{oldest-youngest\ leaf}$	*p*-value[2]
248	5	-13.9	0.7	-14.6	-12.9	-1.8	0.296	5	48.5	0.9	48.5	47.0	1.4	0.025
	3	-13.9	0.8	-14.8	-13.2	-1.5		3	47.6	0.5	48.1	47.2	0.9	
	4	-14.3	0.2	-14.5	-14.1	-0.4		4	46.9	0.7	46.1	47.1	-1.1	
274	3	-14.3	0.2	-14.5	-14.2	-0.3	0.093	0	-	0.7	-	-	-	-
	3	-14.3	0.1	-14.4	-14.3	-0.1		0	-	0.3	-	-	-	
	3	-14.6	0.2	-14.8	-14.4	-0.4		0	-	0.3	-	-	-	
307	4	-13.9	0.1	-14.0	-14.0	0	0.238	0	-	-	-	-	-	-
	3	-14.1	0.2	-14.2	-13.9	-0.2		0	-	-	-	-	-	
	3	-14.1	-	-14.4	-14.0	-0.3		0	-	-	-	-	-	

[1] Julian Calendar Days: date that plants were collected, e.g., 122 represents plants collected on May 2nd, 2014 for April, 154 represents plants collected on June 3rd, 2014 for May, 184 represents plants collected on July 3rd, 2014 for June, 213 represents plants collected on August 1st, 2014 for July, 248 represents plants collected on September 5th, 2014 for August, 274 represents plants collected on October 1st, 2014 for September, 307 represents plants collected on November 3rd, 2014 for October

[2] *p*-value is used to evaluate whether there is a statistically significant difference in the foliar $\delta^{13}C$ and carbon content of plants from the same collection date

Table 3.2: Average foliar nitrogen content and isotopic data for *A. breviligulata* and *C. longifolia* collected from April to October 2014 at Pinery Provincial Park.

Julian Calendar Days[1]	No. of Samples per Plant	δ^{15}N Leaf Average	δ^{15}N Std. Dev.	δ^{15}N Oldest Leaf	δ^{15}N Youngest Leaf	Δ oldest-youngest leaf	*p*-value[2]	No. of Samples per Plant	%N Leaf Leaves	%N Std. Dev.	%N Oldest Leaf	%N Youngest Leaf	Δ oldest-youngest leaf	*p*-value[2]
A. breviligulata - Dunes														
122	2	-0.4	0.0	-0.4	-0.4	0	0.010	2	1.4	0.6	1.0	1.8	-0.9	0.628
	2	0.3	0.1	0.2	0.4	-0.2		2	2.0	0.7	1.5	2.5	-1.0	
	2	0.5	0.1	0.4	0.6	-0.2		2	1.9	0.6	1.5	2.4	-0.9	
154	3	1.3	0.5	1.9	0.9	0.9	0.003	3	1.9	0.4	1.5	2.2	-0.7	0.056
	3	0	0.1	0	0.1	-0.1		3	1.2	0.2	1.1	1.4	-0.3	
	3	1.5	0.3	1.9	1.4	0.5		3	1.7	0.3	1.7	1.9	-0.2	
184	3	0.8	1.0	1.6	-0.3	1.9	0.379	3	0.7	0.4	1.0	0.3	0.7	0.407
	3	0.2	0.1	0.2	0.2	0		3	0.9	0.4	0.5	1.2	-0.7	
	3	0.8	0.2	0.7	1.0	-0.3		3	1.1	0.4	1.1	1.5	-0.4	
213	3	0.3	0.8	1.2	-0.2	1.4	0.010	3	0.8	0.3	0.6	1.1	-0.5	0.969
	3	-0.9	0.0	-0.9	-0.9	0		3	0.8	0.3	0.7	1.1	-0.4	
	3	0.9	0.3	0.9	1.2	-0.3		3	0.8	0.5	0.3	1.3	-0.9	
248	3	-0.3	0.4	0.1	-0.7	0.8	0.013	3	1.0	1.1	0.3	0.4	-0.1	0.519
	3	0.5	0.1	0.5	0.5	0		3	0.4	0.1	0.3	0.4	-0.1	
	3	0.2	0.0	0.2	0.2	0		3	0.5	0.1	0.4	0.6	-0.2	
274	4	-0.3	0.5	-0.9	-0.5	-0.5	0.028	4	0.4	0.2	0.7	0.3	0.4	0.098
	5	-1.5	0.5	-1.7	-0.9	-0.8		5	0.5	0.0	0.6	0.5	0.1	
	4	-1.3	0.7	-0.9	-2.2	1.3		4	0.7	0.1	0.6	0.7	-0.1	
307	3	0.1	0.1	0.1	0	0.2	0.706	3	0.4	0.1	0.3	0.4	-0.1	0.227
	2	-0.2	0.4	0	-0.7	0.7		3	0.4	0.1	0	0.4	-0.4	

Julian Calendar Days[1]	No. of Samples per Plant	δ^{15}N Leaf Average	δ^{15}N Std. Dev.	δ^{15}N Oldest Leaf	δ^{15}N Youngest Leaf	Δ oldest-youngest leaf	δ^{15}N *p*-value[2]	No. of Samples per Plant	%N Leaf Leaves	%N Std. Dev.	%N Oldest Leaf	%N Youngest Leaf	Δ oldest-youngest leaf	%N *p*-value[2]
	3	0.1	0.5	0.2	-0.5	0.7		3	0.5	0.3	0.3	0.5	-0.2	
C. longifolia - Dunes														
154	4	-4.9	***0.2***	-5.2	-4.7	-0.5	0.001	4	2.1	0.2	2.4	1.9	0.5	0.159
	4	-4.5	0.4	-5.0	-4.1	-0.9		4	1.9	0.2	2.0	1.5	0.5	
	5	-3.8	0.3	-4.2	-3.4	-0.8		5	2.1	0.1	2.0	2.0	0	
184	5	-3.1	0.8	-4.2	-2.4	-1.8	0.031	5	1.5	0.2	1.7	1.4	0.3	0.275
	5	-4.4	0.8	-5.6	-3.9	-1.8		5	1.7	0.3	2.0	2.0	0	
	5	-4.6	0.8	-5.7	-3.8	-1.9		5	1.5	0.1	1.5	1.5	0	
213	5	-3.7	0.6	-4.5	-3.0	-1.5	0.063	5	1.3	0.1	1.2	1.5	-0.3	0.486
	5	-3.7	0.5	-4.5	-3.3	-1.1		5	1.2	0.2	1.0	1.0	0	
	5	-4.7	0.9	-5.7	-3.6	-2.0		5	1.3	0.2	0.9	1.4	-0.5	
248	5	-5.3	1.2	-6.6	-4.1	-2.5	0.408	5	1.6	0.5	0.7	1.9	-1.2	0.316
	5	-5.4	0.3	-5.8	-5.1	-0.7		5	1.2	0.4	0.4	1.5	-1.1	
	4	-6.0	0.2	-6.2	-5.7	-0.5		4	1.2	0.5	0.5	1.5	-1.0	
274	4	-2.9	0.6	-3.6	-2.4	-1.2	0.315	4	1.2	0.5	0.7	1.6	-0.9	0.613
	4	-3.6	0.7	-4.6	-2.9	-1.7		4	1.0	0.3	0.6	1.1	-0.6	
	3	-3.5	0.4	-3.3	-4	0.7		3	1.2	0.3	1.1	1.0	0.2	
307	4	-3.8	0.1	-3.9	-3.8	-0.2	0.000	4	0.6	0.1	0.6	0.8	-0.2	0.160
	4	-5.3	0.3	-5.4	-4.9	-0.5		4	0.5	0.1	0.5	0.5	0	
	4	-3.4	0.5	-3.8	-2.8	-1.0		4	0.5	0.1	0.6	0.6	-0.1	
C. longifolia – Canopy Cover														
213	5	-0.5	0.8	-1.6	0.5	-2.2	0.000	5	1.6	0.4	1.1	1.9	-0.8	0.367
	5	-3.0	0.8	-4.1	-2.2	-1.9		5	1.8	0.3	1.3	1.9	-0.6	
	3	-0.2	0.6	-0.8	0.3	-1.1		3	1.9	0.1	1.8	1.9	-0.1	

Julian Calendar Days[1]	No. of Samples per Plant	δ^{15}N Leaf Average	δ^{15}N Std. Dev.	δ^{15}N Oldest Leaf	δ^{15}N Youngest Leaf	Δ oldest-youngest leaf	*p*-value[2]	No. of Samples per Plant	%N Leaf Leaves	%N Std. Dev.	%N Oldest Leaf	%N Youngest Leaf	Δ oldest-youngest leaf	*p*-value[2]
248	5	-1.0	0.8	-1.2	0.2	-1.4	0.244	5	1.3	0.2	1.1	1.4	-0.3	0.008
	4	-2.3	0.4	-2.8	-1.9	-0.9		4	1.4	0.1	1.4	1.4	0	
	4	-0.8	0.2	-1.1	-0.7	-0.4		4	1.6	0.1	1.7	1.7	0	
274	3	-2.9	0.5	-3.4	-2.4	-1.1	0.028	3	0.6	0.1	0.6	0.6	0	0.000
	3	-3.7	0.6	-4.3	-3.1	-1.2		3	0.6	0.0	0.6	0.6	0	
	3	-2.0	0.5	-2.5	-1.5	-1.1		3	1.5	0.1	1.5	1.6	-0.1	
307	4	-0.3	0.2	-0.5	0	-0.5	0.045	4	1.1	0.1	1.0	0.9	0.2	0.220
	3	-1.4	0.8	-2.2	-0.7	-1.5		3	0.8	0.1	0.6	0.8	-0.1	
	3	-1.2	0.5	-1.8	-1.0	-0.8		3	0.8	0.1	0.8	0.7	0.1	

[1] Julian Calendar Days: date that plants were collected, e.g., 122 represents plants collected on May 2nd, 2014 for April, 154 represents plants collected on June 3rd, 2014 for May, 184 represents plants collected on July 3rd, 2014 for June, 213 represents plants collected on August 1st, 2014 for July, 248 represents plants collected on September 5th, 2014 for August, 274 represents plants collected on October 1st, 2014 for September, 307 represents plants collected on November 3rd, 2014 for October

[2] *p*-value is used to evaluate whether there is a statistically significant difference in foliar δ^{15}N and nitrogen content of plants from the same collection date

A. breviligulata and *C. longifolia* are statistically different in foliar carbon content ($F_{[1,147]} = 11.63$, $p = 0.001$), nitrogen content ($F_{[1,187]} = 29.79$, $p = 0.000$), $\delta^{13}C$ ($F_{[1,187]} = 18230.86$, $p = 0.000$), and $\delta^{15}N$ ($F_{[1,187]} = 216.76$, $p = 0.000$). However, because of the large within plant standard deviation, the average difference of ~ 0.7 wt. % in carbon content and ~ 0.4 wt. % in nitrogen content between *C. longifolia* and *A. breviligulata* are negligible. On average, *A. breviligulata* is depleted of ^{13}C by 12.3 ‰ relative to *C. longifolia*, as expected due to differences in the C_3 and C_4 photosynthetic pathway, and enriched in ^{15}N by 3.5 ‰ compared to *C. longifolia.* For grasses collected only from the dunes, *A. breviligulata* is depleted of ^{13}C by 12.4 ‰ and enriched in ^{15}N by 4.3 ‰, on average, compared to *C. longifolia* (Fig. 3.1). *C. longifolia* collected from the dunes versus under canopy cover had statistically significant differences in foliar $\delta^{13}C$ ($F_{[1, 123]} = 27.14$, $p = 0.000$) and foliar $\delta^{15}N$ ($F_{[1,123]} = 176.67$, $p = 0.000$). On average, *C. longifolia* collected from under canopy cover was depleted of ^{13}C by 0.4 ‰ but enriched in ^{15}N by 2.7 ‰ relative to *C. longifolia* collected from the dunes (Fig. 3.1). However, there were no significant differences in carbon content ($F_{[1,110]} = 2.21$, $p = 0.140$) or nitrogen content ($F_{[1,123]} = 0.08$, $p = 0.773$) of *C. longifolia* between the two sites.

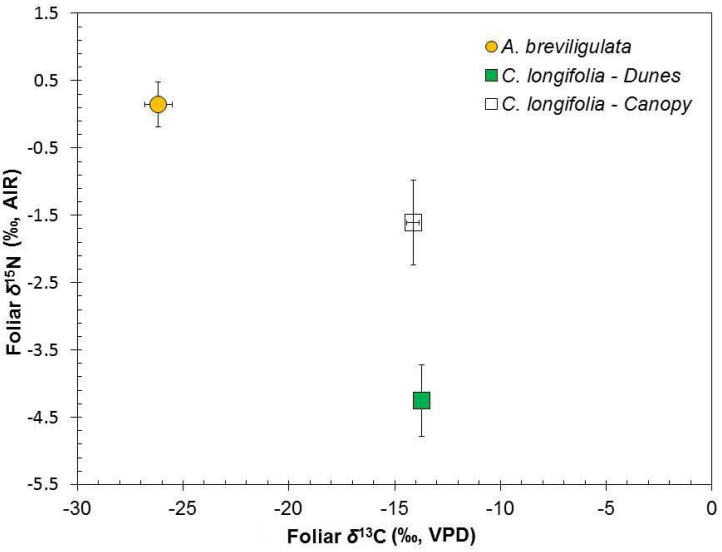

Figure 3.1: Comparison of average foliar $\delta^{13}C$ and $\delta^{15}N$ o*f A. breviligulata* and *C. longifolia* collected from different sites from April to October 2014.

3.1.2 Comparison of *A. breviligulata* Among Months

The foliar carbon content ($F_{[5,41]} = 4.97$, $p = 0.001$), nitrogen content ($F_{[6,57]} = 15.59$, $p = 0.000$), foliar $\delta^{13}C$ ($F_{[6,57]} = 3.66$, $p = 0.000$), and foliar $\delta^{15}N$ ($F_{[6,57]} = 10.50$, $p = 0.000$) of *A. breviligulata* were significantly different between months (Fig. 3.2). The carbon content of April leaves were significantly lower than the carbon content of leaves collected in May, June, July, and September (Fig. 3.2a). The foliar $\delta^{13}C$ of April leaves was also significantly lower than leaves collected in other months (Fig. 3b). Leaves from September *A. breviligulata* plants were significantly lower in foliar $\delta^{15}N$ than leaves collected in other months, with the exception of plants collected in July (Fig. 3.2e). Furthermore, plants collected in April and May had high nitrogen contents relative to plants collected in other months (Fig. 3.2d).

3.1.3 Comparison of *C. longifolia* Among Months

The foliar nitrogen content ($F_{[5,74]} = 39.65$, $p = 0.000$), $\delta^{13}C$ ($F_{[5,75]} = 26.16$, $p = 0.000$), and $\delta^{15}N$ ($F_{[5,74]} = 10.83$, $p = 0.000$) of *C. longifolia* collected from the dunes significantly differed between months (Fig. 3.2c, d, e). Leaves of *C. longifolia* plants collected in May, June, and July were significantly higher in $\delta^{13}C$ compared to *C. longifolia* plants collected in August, September, and October. Plants collected in August, September, and October were statistically different from each other in $\delta^{13}C$ with the lowest foliar $\delta^{13}C$ values associated with September *C. longifolia* plants and higher foliar $\delta^{13}C$ associated with October plants. The foliar $\delta^{15}N$ of August *C. longifolia* was significantly lower compared to plants collected in other months. Additionally, the foliar $\delta^{15}N$ of *C. longifolia* collected in May (average $\delta^{15}N$ of -4.3 ± 0.6 ‰ (n = 13)) was significantly lower than foliar $\delta^{15}N$ of *C. longifolia* collected in September (average $\delta^{15}N$ of -3.3 ± 0.6 ‰ (n = 12)). *C. longifolia* collected in May and October was statistically different in nitrogen content than *C. longifolia* collected in other collection months.

C. longifolia plants growing under canopy cover collected in all months significantly differed from each other in nitrogen content ($F_{[3,40]} = 22.33$, $p = 0.000$), while *C. longifolia* plants collected in September differed from other months in foliar $\delta^{15}N$ ($F_{[3,40]} = 6.26$, $p = 0.001$) (Fig 3.1, 3.2d,e). There were no statistically significant differences

between months in foliar bulk carbon content ($F_{[1,23]} = 0.00$, $p = 0.956$) and $\delta^{13}C$ ($F_{[3,40]} = 2.74$, $p = 0.056$).

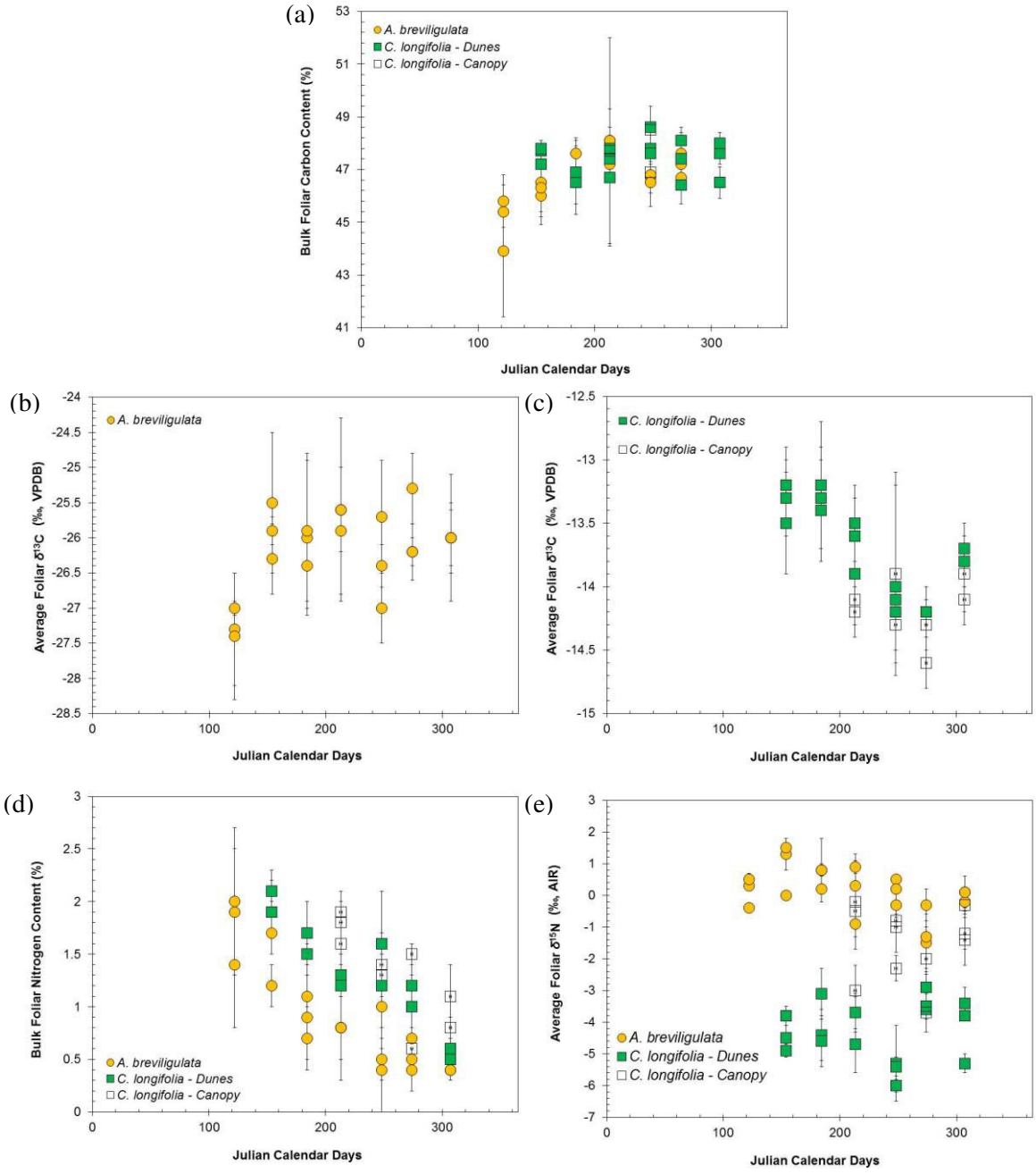

Figure 3.2: Seasonal variation (April to October 2014) of foliar (a) carbon content, (b) $\delta^{13}C$ of *A. breviligulata*, (c) $\delta^{13}C$ of *C. longifolia*, (d) nitrogen content and (e) $\delta^{15}N$ of *A. breviligulata* and *C. longifolia*.

3.2 Inter-Annual Variation

C. longifolia and *A. breviligulat*a leaves collected from the dunes at the end of each growing season from 2006 to 2014 were analyzed for carbon and nitrogen content and isotopic composition (Fig. 3.3, Tables 3.3, 3.4). Over this time period *A. breviligulata* and *C. longifolia* were statistically significantly different from each other in foliar nitrogen content ($F_{[1,178]} = 102.06$, $p = 0.000$), $\delta^{13}C$ ($F_{[1,181]} = 24544.31$, $p = 0.000$), and $\delta^{15}N$ ($F_{[1,177]} = 504.31$, $p = 0.000$). The statistical difference in $\delta^{13}C$ was expected due to differences in the C_3 and C_4 photosynthetic pathway. When only end of season plants are considered, there were no statistically significant differences in the foliar carbon content between *A. breviligulata* and *C. longifolia* ($F_{[1,167]} = 0.71$, $p = 0.402$). The average $\delta^{13}C$ for *A. breviligulata* leaves was –26.2 ± 0.7‰ (n = 70) with $\delta^{13}C$ ranging from –28.2 to –25.0 ‰, while the average $\delta^{15}N$ for *A. breviligulata* leaves was –0.6 ± 1.4 ‰ (n = 69) with $\delta^{15}N$ ranging from –5.7 to +1.8 ‰. The average carbon content was 46.4 ± 1.2 wt. % (n = 59), ranging from 43.2 to 48.6 wt. %, while the average nitrogen content was 0.4 ± 0.1 wt. % (n = 69), ranging from 0.3 to 0.9 wt. %. The $\delta^{13}C$ of *C. longifolia* ranged from –15.0 to –13.1 ‰ with a mean $\delta^{13}C$ of –14.0 ± 0.4 ‰ (n = 113), while the $\delta^{15}N$ ranged from –7.1 ‰ to –1.9 ‰ with a mean $\delta^{15}N$ of –4.6 ± 1.1 ‰ (n = 110). The average carbon content was 46.6 ± 1.1 wt. % (n = 110), ranging from 43.9 to 48.7 wt. % while the average nitrogen content was 0.7 ± 0.2 wt. % (n = 110), ranging from 0.3 to 1.3 wt. %. Appendix D provides a full list of all samples analyzed.

Foliar $\delta^{13}C$ ($F_{[6,63]} = 4.66$, $p = 0.001$) and $\delta^{15}N$ ($F_{[6,62]} = 10.77$, $p = 0.000$) of *A. breviligulata* differed between years (Fig. 3.3b,e). Foliar $\delta^{13}C$ of *A. breviligulata* plants collected 2006 vs. 2008, 2006 vs. 2013, and 2008 vs. 2009 were significantly different with plants grown in years of higher precipitation (2008, 2013) exhibiting lower $\delta^{13}C$ compared to plants grown in years of lower precipitation (2006, 2009). The $\delta^{15}N$ of *A. breviligulata* collected in 2007 was also statistically lower than plants collected from other years. *A. breviligulata* plants from 2008 vs. 2009 and 2008 vs. 2014 were also significantly different with 2008 plants exhibiting lower $\delta^{15}N$.

C. longifolia varied significantly between years in foliar $\delta^{13}C$ ($F_{[1,105]} = 8.75$, $p = 0.000$), and $\delta^{15}N$ ($F_{[7,102]} = 16.13$, $p = 0.000$) (Figs. 3.3 c, e). Leaves of *C. longifolia* plants collected in 2007 and 2012 exhibited significantly lower $\delta^{13}C$ compared to plants collected other years, with the exception of 2011 and 2014. The $\delta^{15}N$ of *C. longifolia* from 2008 and 2012 were significantly lower than plants collected in all other years. Additionally, there were significant differences in $\delta^{15}N$ between the following years: 2006 vs. 2013, 2007 vs. 2010, 2007 vs. 2014, 2010 vs. 2013, and 2013 vs. 2014.

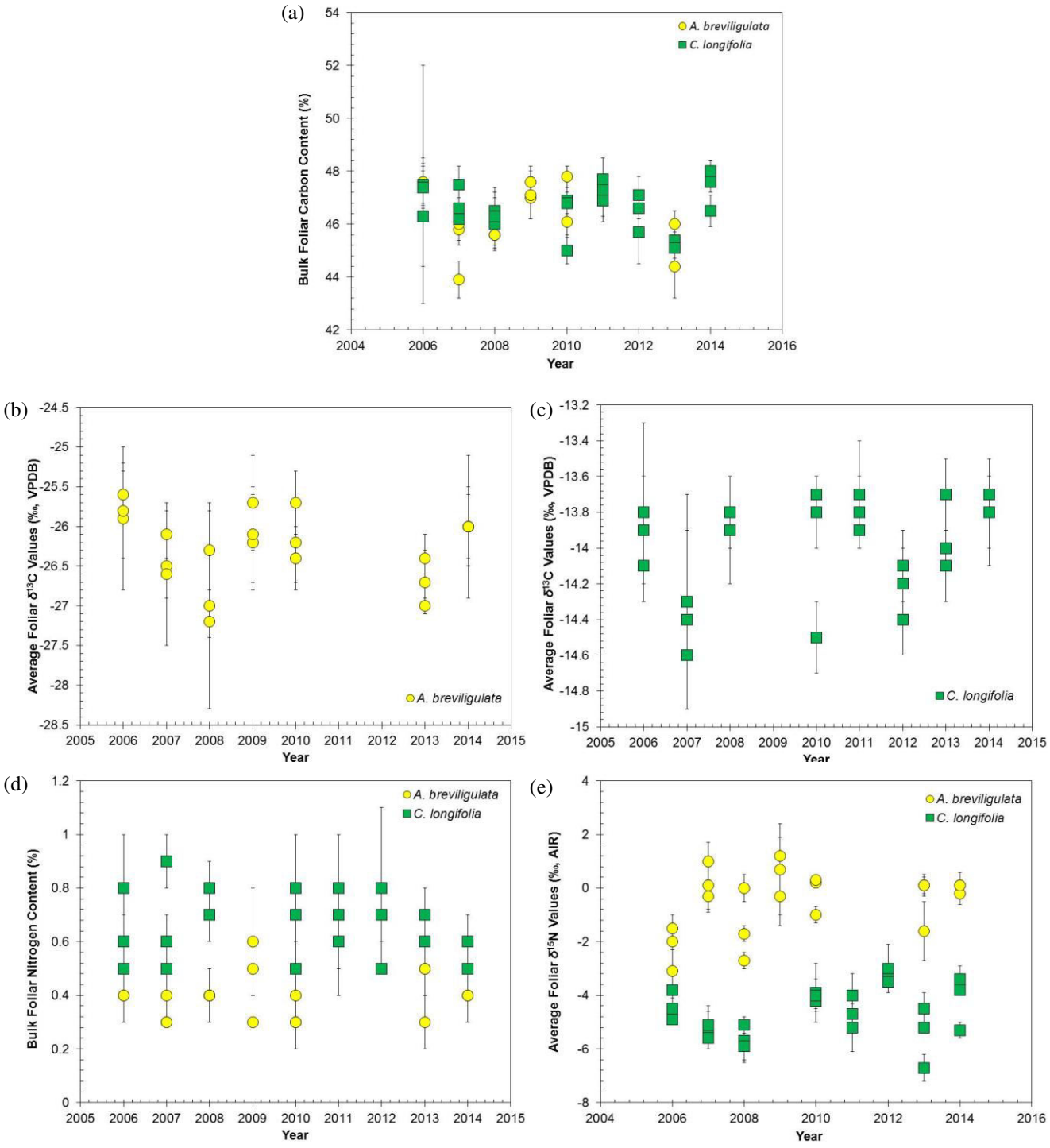

Figure 3.3: Annual variation (2006-2014) of foliar (a) carbon content, (b) δ^{13}C of *A. breviligulata*, (c) δ^{13}C of *C. longifolia*, (d) nitrogen content, and (e) δ^{15}N of *A. breviligulata* and *C. longifolia*.

Table 3.3: Average carbon elemental and isotopic data for *A. breviligulata* and *C. longifolia* plants collected annually from 2006 to 2014 at Pinery Provincial Park.

			$\delta^{13}C$							% C					
Year	**Collection Date**	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**
A. breviligulata															
2006	07/01/2007	4	-25.9	0.9	-25.6	-25.1	-0.5	0.901	4	47.5	0.8	46.6	48.0	-1.3	0.947
		4	-25.6	0.3	-26.1	-25.7	-0.4		4	47.6	0.9	47.6	46.4	1.2	
		5	-25.8	0.6	-25.0	-25.5	0.5		5	47.4	0.8	46.7	47.3	-0.6	
2007	27/09/2007	3	-26.1	0.3	-26.4	-25.8	-0.6	0.658	2	45.8	0.2	45.6	45.9	-0.3	0.029
		3	-26.5	0.4	-26.9	-26.1	-0.8		3	46.0	0.8	45.6	45.4	0.2	
		3	-26.6	0.9	-27.5	-25.7	-1.8		3	43.9	0.7	43.7	44.7	-1.0	
2008	23/03/2009	3	-27.2	0.2	-27.1	-27.4	0.3	0.393	3	45.6	0.5	45.3	46.1	-0.8	0.651
		3	-27.0	1.3	-28.2	-25.6	-2.6		3	46.1	1.1	44.8	46.9	-2.1	
		3	-26.3	0.5	-26.9	-25.8	-1.0		3	45.6	0.1	45.6	45.6	0	
2009	06/05/2010	3	-26.2	0.6	-26.7	-25.6	-1.1	0.476	3	47.6	0.6	47.2	47.4	-0.1	0.552
		4	-25.7	0.6	-25	-25.7	0.7		4	47.0	0.8	46.0	47.7	-1.7	
		4	-26.1	0.6	-26.9	-25.8	-1.1		4	47.1	0.9	47.1	47.8	-0.8	
2010	04/10/2010	4	-26.2	0.5	-26.4	-26.9	0.5	0.188	4	46.1	0.6	46.3	45.3	1.0	0.008
		3	-26.4	0.4	-26.9	-26.3	-0.6		3	47.8	0.4	47.5	48.2	-0.6	
		4	-25.7	0.4	-25.5	-26.3	0.9		3	46.8	0.4	47.0	46.3	0.7	
2013	03/04/2014	3	-26.7	0.4	-27.2	-26.3	-0.9	0.222	3	45.1	0.2	45.2	44.9	0.3	0.108
		3	-27.0	0.1	-27	-26.8	-0.1		3	46.0	0.5	45.8	45.5	0.3	
		2	-26.4	0.3	-26.6	-26.2	-0.4		2	44.4	1.2	45.3	43.6	1.7	
2014	04/11/2014	3	-26.0	0.4	-26.5	-25.7	-0.7	0.999	0	-	-	0	0	0	-

Year	Collection Date	No. of Samples per Plant	$\delta^{13}C$ Leaf Average	$\delta^{13}C$ Std. Dev.	$\delta^{13}C$ Oldest Leaf	$\delta^{13}C$ Youngest Leaf	Δ oldest-youngest leaf	*p*-value[1]	No. of Samples per Plant	% C Leaf Average	% C Std. Dev.	% C Oldest Leaf	% C Youngest Leaf	Δ oldest-youngest leaf	*p*-value[1]
		3	-26.0	0.5	-26.6	-25.6	-0.9		0	-	-	0	0	0	
		3	-26.0	0.9	-26.9	-25.1	-1.8		0	-	-	0	0	0	
C. longifolia															
2006	07/01/2007	4	-14.1	0.2	-13.8	-14.1	0.3	0.503	3	47.5	4.5	47.7	46.0	1.7	0.408
		4	-13.8	0.5	-13.2	-14.2	1.0		4	46.3	1.9	44.0	47.5	-3.5	
		5	-13.9	0.3	-13.4	-13.9	0.5		5	47.4	0.6	47.5	48.0	-0.4	
2007	27/09/2007	5	-14.6	0.3	-14.4	-14.2	-0.1	0.584	5	46.2	0.8	47.0	45.3	1.7	0.011
		6	-14.4	0.5	-13.6	-14.1	0.5		6	46.6	0.4	46.1	46.6	-0.6	
		7	-14.3	0.6	-13.1	-14.0	0.9		7	47.5	0.7	46.8	47.7	-0.9	
2008	21/03/2009	5	-13.9	0.3	-14.2	-13.6	-0.6	0.584	5	46.0	0.5	46.5	45.2	1.3	0.629
		5	-13.8	0.2	-13.5	-13.8	0.3		5	46.5	0.5	46.7	46.5	0.2	
		6	-13.8	0.2	-14.2	-14.0	-0.2		6	46.3	1.1	46.4	48.4	-2.0	
2010	04/10/2010	4	-14.5	0.2	-14.8	-14.3	-0.5	0.079	4	45.0	0.5	44.2	45.4	-1.2	0.158
		4	-13.7	0.1	-13.5	-13.6	0.1		4	46.9	1.3	47.0	45.6	1.4	
		4	-13.8	0.2	-14.1	-13.8	-0.3		4	46.8	0.9	46.7	45.6	1.1	
2011	06/01/2012	6	-13.7	0.3	-13.2	-13.6	0.4	0.616	6	47.3	1.2	48.3	45.9	2.4	0.515
		5	-13.8	0.2	-14.0	-13.6	-0.5		5	46.9	0.6	47.4	46.8	0.6	
		3	-13.9	0.1	-14.0	-13.7	-0.2		3	47.7	0.8	47.6	46.9	0.7	
2012	11/11/2012	5	-14.2	0.2	-14.5	-13.9	-0.6	0.074	5	46.6	0.4	47.1	46.4	0.7	0.093
		5	-14.1	0.2	-14.4	-14.1	-0.3		5	45.7	1.2	46.7	45.2	1.5	
		5	-14.4	0.2	-14.6	-14.2	-0.4		4	47.1	0.7	47.2	46.1	1.2	
2013	03/04/2014	5	-14.0	0.1	-14.0	-14.0	-0.1	0.019	5	45.2	0.5	45.4	46.0	-0.6	0.811
		4	-13.7	0.2	-13.8	-13.5	-0.4		4	45.4	0.7	46.3	44.7	1.6	
		4	-14.1	0.2	-14.4	-13.9	-0.6		4	45.1	0.8	45.5	43.9	1.6	

			δ^{13}C						% C					
Year	**Collection Date**	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**	**No. of Samples per Plant**	**Leaf Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**
2014	04/11/2014	4	-13.8	0.2	-14.1	-13.7	-0.4	0.93	3	46.5	0.6	47.3	46.2	1.1	0.01
		4	-13.8	0.3	-14.1	-13.5	-0.7		4	47.6	0.4	47.9	47.1	0.8	
		4	-13.7	0.1	-13.9	-13.7	-0.2		4	48.0	0.4	48.1	47.5	0.6	

[1] *p*-value indicates whether there is a statistically significant difference in δ^{13}C and carbon content of plants from the same collection date

Table 3.4: Average nitrogen elemental and isotopic data for *A. breviligulata* and *C. longifolia* plants collected annually from 2006 to 2014 at Pinery Provincial Park.

			δ^{15}N						% N					
Year	**Collection Date**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**
A. breviligulata															
2006	07/01/2007	4	-1.5	0.5	-2.1	-1.1	-1.0	0.125	4	0.5	0.0	0.6	0.5	0.1	0.401
		4	-3.1	1.7	-2.1	-5.7	3.6		4	0.4	0.1	0.5	0.4	0.1	
		5	-2.0	0.3	-2.3	-1.8	-0.5		5	0.4	0.1	0.5	0.3	0.2	
2007	27/09/2007	3	0.1	1.0	-0.9	0.2	-1.0	0.195	3	0.3	0.0	0.3	0.4	-0.1	0.228
		3	1.0	0.7	0.2	1.5	-1.2		3	0.3	0.0	0.4	0.3	0.1	
		3	-0.3	0.5	-0.8	0.3	-1.1		3	0.4	0.1	0.3	0.4	-0.1	
2008	23/03/2009	3	-1.7	0.3	-2.0	-1.8	-0.2	0.000	3	0.4	0.0	0.3	0.4	-0.1	0.465
		3	0.0	0.5	-0.5	0.2	-0.7		3	0.4	0.0	0.5	0.4	0.1	
		3	-2.7	0.3	-2.9	-2.9	0.0		3	0.4	0.1	0.4	0.5	-0.1	
2009	06/05/2010	3	1.2	0.7	1.5	1.7	-0.2	0.355	3	0.5	0.1	0.4	0.4	0.0	0.083
		4	0.7	1.7	-1.8	1.8	-3.6		4	0.6	0.2	0.4	0.9	-0.5	
		4	-0.3	1.1	-2.0	0.3	-2.2		4	0.3	0.0	0.3	0.3	0.0	
2010	04/10//2010	4	-1.0	0.3	-0.7	-1.3	0.6	0.000	4	0.4	0.1	0.3	0.6	-0.3	0.324
		3	0.2	0.2	0.2	0.0	0.3		3	0.3	0.0	0.3	0.3	0.1	
		4	0.3	0.1	0.5	0.2	0.3		4	0.3	0.1	0.3	0.4	-0.2	
2013	03/04/2014	3	0.1	0.4	0.3	-0.4	0.7	0.078	3	0.3	0.1	0.3	0.4	-0.1	0.201
		2	-1.6	1.1	-0.8	-2.4	1.5		2	0.5	0.1	0.4	0.5	-0.1	
		2	0.1	0.3	0.4	-0.1	0.4		2	0.5	0.1	0.4	0.6	-0.1	
2014	04/11/2014	3	0.1	0.1	0.1	0.0	0.2	0.706	3	0.4	0.1	0.3	0.4	-0.1	0.227
		2	-0.2	0.4	0.0	-0.7	0.7		3	0.4	0.0	0.3	0.4	-0.1	
		2	0.1	0.5	0.2	-0.5	0.7		3	0.5	0.2	0.3	0.5	-0.2	

			δ^{15}N						% N					
Year	**Collection Date**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**
C. longifolia															
2006	07/01/2007	4	-4.5	0.4	-5.2	-4.2	-1.0	0.012	4	0.8	0.2	0.6	1.0	-0.4	0.032
		4	-3.8	0.7	-4.7	-3.3	-1.4		4	0.6	0.1	0.6	0.7	0.0	
		5	-4.9	0.2	-5.2	-4.9	-0.3		5	0.5	0.1	0.5	0.5	0.0	
2007	27/09/2007	5	-5.2	0.6	-6.2	-4.6	-1.5	0.221	5	0.5	0.1	0.5	0.5	0.0	0.000
		6	-5.6	0.4	-6.0	-5.0	-1.0		6	0.6	0.1	0.5	0.6	-0.1	
		6	-5.1	0.7	-4.8	-6.0	1.2		6	0.9	0.1	0.9	0.9	0.0	
2008	21/03/2009	5	-5.7	0.7	-6.9	-5.1	-1.8	0.099	5	0.8	0.1	0.6	0.9	-0.2	0.279
		5	-5.9	0.6	-6.9	-5.3	-1.6		5	0.7	0.1	0.8	0.7	0.1	
		5	-5.1	0.3	-5.1	-4.7	-0.4		5	0.7	0.1	0.6	0.8	-0.1	
2010	04/10/2010	4	-4.2	0.3	-4.5	-3.8	-0.7	0.981	4	0.7	0.1	0.7	0.6	0.1	0.239
		4	-3.9	1.1	-3.6	-2.6	-1.0		4	0.5	0.1	0.4	0.5	0.0	
		4	-4.0	0.6	-4.1	-3.2	-0.9		4	0.8	0.2	0.9	0.9	0.0	
2011	06/01/2012	6	-5.2	0.9	-6.1	-4.6	-1.4	0.086	6	0.7	0.3	0.9	0.3	0.6	0.667
		5	-4.0	0.8	-4.4	-2.6	-1.8		5	0.8	0.2	0.7	1.2	-0.5	
		3	-4.7	0.2	-4.9	-4.8	-0.2		3	0.6	0.1	0.7	0.5	0.2	
2012	11/11/2012	5	-3.0	0.9	-4.3	-1.9	-2.3	0.419	5	0.8	0.3	0.6	1.3	-0.7	0.069
		5	-3.4	0.3	-3.8	-2.9	-0.9		5	0.5	0.0	0.6	0.5	0.1	
		5	-3.5	0.4	-4.0	-3.3	-0.7		5	0.7	0.1	0.6	0.6	-0.1	
2013	03/04/2014	4	-5.2	0.2	-5.4	-5.0	-0.4	0.000	4	-	-	0.6	0.5	0.1	0.147
		4	-6.7	0.5	-7.1	-6.0	-1.1		4	0.6	0.1	0.6	0.5	0.1	
		4	-4.5	0.6	-4.9	-4.8	-0.1		4	0.7	0.1	0.9	0.6	0.3	
2014	04/11/2014	4	-3.8	0.1	-3.9	-3.8	-0.2	0.000	4	0.6	0.1	0.6	0.8	-0.2	0.160
		4	-5.3	0.3	-5.4	-4.9	-0.5		4	0.5	0.0	0.5	0.5	0.0	
		4	-3.4	0.5	-3.8	-2.8	-1.0		4	0.5	0.1	0.6	0.6	-0.1	

			δ^{15}N						% N					
Year	**Collection Date**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value**	**No. of Samples**	**Plant Average**	**Std. Dev.**	**Oldest Leaf**	**Youngest Leaf**	**Δ oldest-youngest leaf**	***p*-value[1]**

[1]*p*-value is used to evaluate whether there is a statistically significant difference in δ^{13}C and carbon content of plants from the same collection date.

3.3 Weather Variables

Table 3.5 summarizes the precipitation and temperature data available for the growing season at Pinery Provincial Park from 2006 to 2014. Appendix E contains a full list of all measurements made to calculate annual weather parameters. Total summer precipitation (April to September) from 2006 to 2014 ranged from 288 mm (2007) to 624 mm (2008) with an average summer precipitation of 473 mm. Mean summer temperature (April to September) ranged from 10.1°C (2009) to 21.8°C (2011) with a mean summer temperature of 16.6°C. The mean maximum and mean minimum temperatures for both June and July were also considered. The mean maximum temperature for June was 25.2°C (23.4°C to 30.3°C) while the mean maximum temperature for July was 27.1°C (24.1°C to 29.9°C). In comparison, the mean minimum temperature for June was 13.2°C (11.5°C to 14.1°C) while the mean minimum temperature for July was 15.9°C (13.8°C to 17.4°C).

Table 3.5: Weather data measured at Thedford, Ontario from 2006 to 2014. Summer precipitation and mean summer temperatures are calculated from April to September for a given year.

Year	Summer Precipitation (mm)	Mean Summer T (°C)	June Mean Max T (°C)	June Mean Min T (°C)	July Mean Max T (°C)	July Mean Min T (°C)
2006	451	16.6	23.7	12.4	27.9	17.1
2007	288	17	26.9	12.9	26.6	14.3
2008	624	16.7	25.4	14.8	27.1	15.8
2009	460	10.1	22.1	11.5	24.1	13.8
2010	436	18.2	24.7	13.9	28.5	17.2
2011	572	21.8	23.4	13.1	28.9	17.4
2012	359	17.4	26.4	14.1	29.9	16.6
2013	612	16.3	23.7	13.7	26.6	16.3
2014	453	15.5	30.3	12.8	24.7	14.2
AVG	**473**	**16.6**	**25.2**	**13.2**	**27.1**	**15.9**

Total monthly precipitation, mean monthly temperatures, mean monthly maximum temperatures and mean monthly minimum temperatures from 2006 to 2014 were compared between years to determine if there were any statistically significant differences among years. Weather parameters from April to September for each year were considered. No statistically significant differences in the total summer precipitation ($F_{[8,45]} = 2.000$, $p = 0.068$), mean summer temperature ($F_{[8,45]} = 0.129$, $p = 0.998$), mean maximum summer temperature ($F_{[8,45]} = 0.137$, $p = 0.997$), and mean summer minimum temperature ($F_{[8,45]} = 0.112$, $p = 0.99$) were observed. Furthermore, there was no correlation between total summer precipitation and mean summer temperature.

Monthly weather conditions for 2014 were assessed using data collected at both Pinery Provincial Park and Thedford, Ontario. Appendix F lists the measurements used to calculate the monthly weather parameters. Weather data for Thedford, Ontario are summarized in Table 3.6. The total summer precipitation from April to October 2014 was 502 mm, while the mean temperature was 14.8°C. The mean daily maximum temperature for this period was 21.0°C while the mean daily minimum temperature was 9.4°C. There are significant differences in mean temperature ($F_{[6,207]} = 55.89$, $p = 0.000$), maximum temperature ($F_{[6,207]} = 7.75$, $p = 0.000$) and minimum temperature ($F_{[6,208]} = 63.05$, $p = 0.000$) between months. There were no statistically significant differences in precipitation amounts between months ($F_{[6,209]} = 0.635$, $p = 0.702$). There is a positive correlation between precipitation amount and mean temperature ($r_s = 0.201$, $n = 214$, $p < 0.01$), precipitation amount and minimum temperature ($r_s = 0.239$, $n = 214$, $p < 0.01$) and mean daily maximum temperature and mean daily minimum temperature ($r_s = 0.422$, $n = 214$, $p < 0.01$).

Table 3.6: Average monthly weather data for 2014 at Thedford, Ontario.

Period Beginning	Period End	Total Precipitation (mm)	Mean Temperature (°C)	Mean Daily Maximum Temperature[1] (°C)	Mean Daily Minimum Temperature[2] (°C)
April 1	May 2	69	6.7	12.4	0.9
May 3	June 3	74	14.0	20.3	7.9
June 4	July 3	38	18.6	30.0	13.1
July 5	August 1	77	19.7	24.8	13.9
August 2	September 5	121	20.1	25.1	15.1
September 6	October 1	79	14.7	20.4	9.0
October 2	November 3	50	9.9	14.4	5.5

[1]Means are calculated from the absolute daily maximum temperature
[2] Means are calculated from the absolute daily minimum temperatures

Monthly weather conditions for 2014 at Pinery Provincial Park are summarized in Table 3.7. There were no statistically significant differences between weather data collected from the logger located on the second dune ridge and the Stevenson screen. The mean temperature was 14.8°C. The mean daily maximum temperature from April to October was 14.15 – 14.29°C while the mean relative humidity for this period was 78.37 – 76.49 %. The temperatures measured are comparable to temperatures obtained from Thedford, Ontario (Table 3.6).

Table 3.7: Average monthly weather data for 2014 at Pinery Provincial Park, Ontario.

		Dune		Stevenson Screen	
Period Beginning	Period End	Mean Temperature (°C)	Relative Humidity (%)	Mean Temperature (°C)	Relative Humidity (%)
01-Apr [1]	02-May	5.47	72.50	5.87	69.82
03-May	03-Jun	12.21	78.82	12.39	76.12
04-Jun	03-Jul	18.72	78.41	18.98	76.01
05-Jul	01-Aug	19.48	78.06	19.46	76.88
02-Aug	05-Sep	N/A	N/A	N/A	N/A
06-Sep	01-Oct	14.88	84.03	14.74	83.64
02-Oct	03-Nov	N/A	N/A	N/A	N/A

[1] incomplete data set

Chapter 4

4 Discussion

4.1 Comparison of Elemental and Isotopic Composition of *A. breviligulata* and *C. longifolia*

Through the 2014 growing season *A. breviligulata* and *C. longifolia* are significantly different in their respective carbon and nitrogen contents with *C. longifolia* plants having ~ 0.7 wt. % more carbon and ~ 0.4 wt. % more nitrogen than *A. breviligulata*. The statistically significant difference in the foliar carbon content between *A. breviligulata* and *C. longifolia*, however, is similar to analytical reproducibility for carbon (~ ±0.6 wt. %), and therefore not considered further. Furthermore, because these is a large spread in the within foliar carbon content of *A. breviligulata* (0.1 to 2.5 wt. %) and *C. longifolia* (0.1 to 2.6 wt. %) and there is no consistent difference between the two species throughout the growing season (Fig. 3.2a), the significant difference was not considered.

By comparison, the statistically significant difference in foliar nitrogen content between *A. breviligulata* and *C. longifolia* likely represents a true biological difference between the species, despite the large variation in the range of nitrogen content (*A. breviligulata*: 0 to 1.1 wt. % and *C. longifolia*: 0.1 to 0.5 wt. %). The average reported difference in foliar nitrogen content of ~ 0.4 wt. % is greater than the analytical reproducibility of ±0.01 wt. %. Also, the foliar nitrogen content of *C. longifolia* (~ 1.3±0.5 wt. %) is consistently 0 to 0.8 wt. % higher than the foliar nitrogen content of *A. breviligulata* (~ 0.9±0.6 wt. %) each month throughout the growing season. The largest differences in foliar nitrogen content between the two species occurred during June to September (Fig. 3.2d) while the smallest difference occurred earlier (May) and later (October) in the growing season. This suggests that species-specific growth patterns influence foliar nitrogen content in these grasses.

On average, *C. longifolia* plants expressed higher average foliar nitrogen content than *A. breviligulata*. In other studies, the crude protein content, a measure of the approximate amount of protein calculated from the nitrogen content, has been reported

to be higher in *A. breviligulata* than *C. longifolia*: 10.4 to 16.9 % in *A. breviligulata* (Goldberg et al., 1980) versus 1 to 11 % (Burzlaff, 1971) and ~ 5 to 15 % (Hendrickson et al., 1997) in *C. longifolia.* This difference is consistent with the idea that C_3 plants generally have a higher nitrogen content than C_4 plants due to better NUE exhibited by C_4 plants (Taylor et al., 2010).

Why *C. longifolia* at PPP has higher foliar nitrogen content relative to *A. breviligulata* at the Pinery is not known. One possibility is that this difference is related to mycorrhizae associations. Hetrick et al. (1990) suggested that the phenology and rooting structure of C_3 and C_4 species can determine the dependence of a given species on mycorrhizae associations. Many C_3 species have facultative mycorrhizae associations which means that they only use mycorrhizae associations when there is inadequate supply of nitrogen (Hetrick et al., 1988). Access to nitrogen by *A. breviligulata* may be affected by the species richness and composition of its facultative mycorrhizae associations as well as how often *A. breviligulata* depends on these associations. This, in turn, influences the efficiency of nitrogen transfer and the specific plant-symbiont interaction which has been shown to influence $\delta^{15}N$ (Evans, 2001; Hobbie and Högberg, 2012) and therefore may potentially explain why *C. longifolia* at PPP has higher foliar nitrogen content relative to *A. breviligulata*.

Differences in foliar nitrogen content of these grasses from April to October 2014 are mirrored in differences in the foliar C:N ratio (Appendices C and D). On average, *A. breviligulata* has a higher C:N ratio than *C. longifolia*, consistent with previous reports for these species (United States Department of Agriculture Natural Resources Conservation Service, n.d.). Early in the growing season, the foliar C:N ratio of both *A. breviligulata* and *C. longifolia* is ~ 20-30 (Fig. 4.1). The lower C:N ratio of grasses early in the season is associated with lower lignin and cellulose content, higher nitrogen content (Fig. 3.2d), and higher palatability and nutritional quality of leaves when plants are initiating photosynthesis and undergoing vegetative growth (Burzlaff, 1971; Goldberg et al., 1980; Hendrickson et al., 1997; Kamstra, 1973; Northup and Nichols, 1998). As the season progresses, the C:N ratio of both *A. breviligulata* and *C. longifolia* increases, but at a faster rate for the former than the latter (Fig. 4.1). This increase is

associated with multiple, simultaneously operating factors including seasonal decreases in foliar nitrogen content (Fig. 3.2d) associated with decreasing protein concentration, translocation of nitrogen-rich macromolecules from the leaves to organs such as the seeds and roots, increasing lignification of the plant with maturity, and decline in plant growth and demand for fixed carbon (Burzlaff, 1971; Goldberg et al., 1980; Hendrickson et al., 1997; Kamstra, 1973; Northup and Nichols, 1998). The difference in the rate of seasonal increase in the C:N ratio of *A. breviligulata* and *C. longifolia* suggests differences in plant phenology and plant growth stages at a given time.

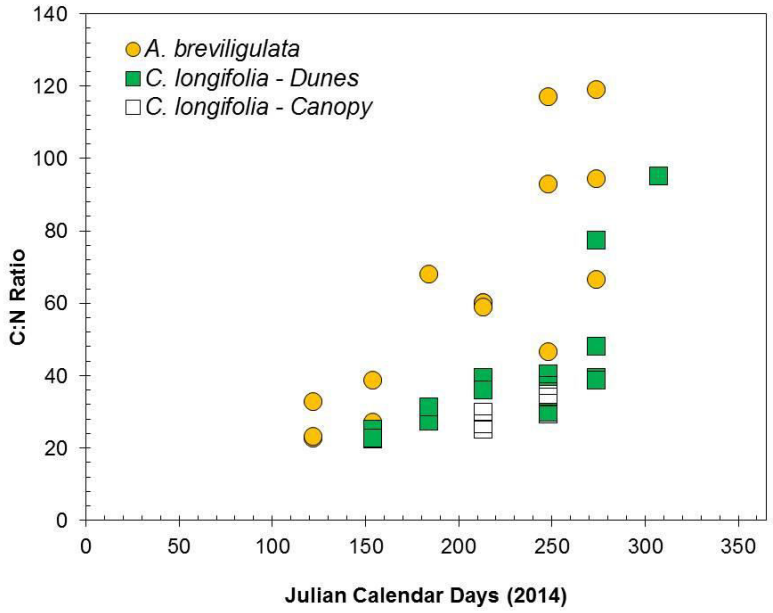

Figure 4.1: Seasonal changes in the C:N ratio of *A. breviligulata* and *C. longifolia* in 2014.

The carbon isotopic compositions of the two species reflect differences in their photosynthetic pathways and physiology. On average, from May to October 2014 *A. breviligulata* is depleted of ^{13}C by 12.3 ‰ compared to *C. longifolia. A. breviligulata* is a cool season C_3 grass (Maun, 1985) while *C. longifolia* is a warm season C_4 grass (Barnes and Harrison, 1982). As discussed in Chapter 1, the C_4 photosynthetic pathway is a biochemical and morphological modification of the C_3 photosynthetic pathway such that it concentrates CO_2 at the site of carboxylation and reduces the amount of

photorespiration within the leaf (Ehleringer and Cerling, 2002; Farquhar et al., 1989; O'Leary, 1988; von Caemmerer and Furbank, 2003). This modification translates into higher foliar $\delta^{13}C$ in C_4 plants.

Factors such as age (Distel et al., 2005), changing environmental conditions (Codron et al., 2013; Liu et al., 2014; Wang et al., 2005, 2010, 2013) and varying macromolecular compositions (Badeck et al., 2005) may increase or reduce foliar $\delta^{13}C$ in both C_3 and C_4 species. In both *A. breviligulata* and *C. longifolia*, younger leaves tend to be more enriched in ^{13}C relative to older leaves (Tables 3.3, 3.4), indicating that the age and stage of development of each individual leaf influences the $\delta^{13}C$ measured. This is further confounded by the fact that sampled tissues may be older than one month and therefore foliar isotopic composition also reflects differences in the weather conditions throughout various months. For example, the low $\delta^{13}C$ of tissues sampled from September *C. longifolia* plants reflects the fact that the tissues most likely grew in both September and August. Similarly, the relatively lower foliar $\delta^{13}C$ of August *A. breviligulata* (average of ~ –26.3 ‰) is related to that month's high precipitation (121.4 mm) while the seasonal decline in foliar $\delta^{13}C$ of *C. longifolia* (Fig. 3.2c) is related, at least in part, to increasing lignin contents, which increases with maturity (Distel et al., 2005; Kamstra, 1973).

Modifications of the local nitrogen cycle by plant cover, rooting depth and mycorrhizae association may contribute to the average 3.5 ‰ difference in foliar $\delta^{15}N$ between *A. breviligulata* and *C. longifolia*. Each possibility is considered below.

The type of vegetative cover influences soil properties and fertility by indirectly creating negative and positive feedbacks in the nutrient cycle and by directly affecting the soil nitrogen pool through uptake, nutrient utilization and loss of nutrients (Hobbie, 1992; Wedin and Tilman, 1990). Wedin and Tilman (1990) showed that net *in-situ* nitrogen mineralization diverged by ~ 10-fold in identical soils covered by monocultures of differing species during a three-year period. These differences were attributed to variation in the quantity and quality of belowground litter, which drives the extent of mineralization. Several studies have shown that litter nitrogen content, C:N ratio, and

lignin:N ratio are important predictors of decomposition rates and the ability of litter to immobilize nitrogen (Flanagan and Cleve, 1983; Meentemeyer, 1978; Pastor and Post, 1986). Decomposition and nutrient release is often positively correlated to the nitrogen contents of litter and negatively correlated to the C:N ratio (Aerts, 1996). These factors influence nitrogen availability and therefore the amount of nitrogen mineralization. Increasing net mineralization is positively correlated with increasing soil and foliar $\delta^{15}N$ (Kahmen et al., 2008). Relative to *C. longifolia*, the higher $\delta^{15}N$ of *A. breviligulata* may therefore reflect lower mineralization rates and be related to the quality of belowground litter that is produced.

Denitrification may also be affected by vegetation cover based on a species' preference for NH_4^+ versus NO_3^-. Ammonium (NH_4^+) is typically more enriched in ^{15}N than NO_3^- (Makarov, 2009). Therefore, as the proportion and abundance of NH_4^+ and NO_3^- in the soil nitrogen pool changes, based on the ratio of NH_4^+:NO_3^- the plant uptakes, the isotopic composition of bulk nitrogen soil pool changes. This, in turn, is reflected in the foliar $\delta^{15}N$ of the plant (Evans, 2001).

Rooting depth of *A. breviligulata* and *C. longifolia* also exerts an important control on these species' nitrogen sources. Both *A. breviligulata* and *C. longifolia* are known to have extensive rooting systems with the rooting system of *A. breviligulata* extending ~ 3 to 4 m below the surface (Lady Bird Johnson Wildflower Center, 2016) and the rooting system of *C. longifolia* reaching depths of 3.0 m (Weaver, 1958). Soil $\delta^{15}N$ increases with depth, with maximum enrichments in ^{15}N of 4.6±0.5 ‰ in AM dominated systems (Hobbie and Ouimette, 2009; Makarov, 2009). Therefore, species with deeper roots are expected to have higher $\delta^{15}N$ because of the higher $\delta^{15}N$ of the nitrogen source that is utilized by the plant. This is further exacerbated by the locations of *A. breviligulata* and *C. longifolia* on the dunes. *A. breviligulata* grows on the foredune while *C. longfolia* grows higher on the first dune ridge (Fig. 2.2). This means that *A. breviligulata* roots have access to nitrogen sources with higher $\delta^{15}N$ as well as additional groundwater nitrate sources due to the proximity of the roots to the Lake Huron water table, which is ~ 176.9 to 177.4 m above sea level and does not show large seasonal fluctuations in the groundwater table depth (Steinbach, 1999). Groundwater nitrate at PPP has average

annual $\delta^{15}N$ of ~ +2.5 ±1.0 ‰ (Russell, 2015) and therefore is likely reflected in the higher foliar $\delta^{15}N$ expressed by *A. breviligulata*.

Lastly, differences in $\delta^{15}N$ between *A. breviligulata* and *C. longifolia* may also be related, in part, to mycorrhizae associations. Many dune species rely upon mycorrhizae association to obtain limiting nutrients. Both *A. breviligulata* and *C. longifolia* have arbuscular mycorrhizal (AM) associations (Maun, 2009). Globally, AM associations have been shown to deplete plants of ^{15}N by ~ 2.9 ‰ relative to non-mycorrhizal plants (Craine et al., 2009). Due to the taxonomic, physiological and morphological diversity of AM, however, there is likely variation in the discrimination against ^{15}N depending on AM species composition and inter-species competition. The difference in $\delta^{15}N$ between arbuscular mycorrhizal associated and non-mycorrhizal plants would be accentuated depending on whether the AM are facultative or obligatory. Plants with obligatory AM, such as C_4 species (Hetrick et al., 1988), would likely have lower $\delta^{15}N$ than plants with facultative AM, such as C_3 species (Hetrick et al., 1988), due the constant influx of ^{15}N-depleted nitrogen from the former (Craine et al., 2009). Hence, it is plausible that the obligatory AM of C_4 plants in part has contributed to the lower $\delta^{15}N$ of *C. longifolia* relative to *A. breviligulata.*

4.2 Variation in $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia* Between Sites

Environmental processes on a very local scale also influence the isotopic composition of the carbon and nitrogen sources in the environment as well as carbon and nitrogen cycling within the plant. There were statistically significant differences in the $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia* collected from the dunes versus under canopy cover. *C. longifolia* collected from under canopy cover was on average depleted of ^{13}C by ~ 0.4 ‰ and enriched in ^{15}N by ~ 2.7 ‰ relative to *C. longifolia* plants collected from the dunes. The largest difference in $\delta^{13}C$ was observed in July *C. longifolia* while the largest difference in $\delta^{15}N$ was observed in August.

The difference in foliar $\delta^{13}C$ between *C. longifolia* plants collected from the different sites is related to the canopy effect. Plants have only one source of carbon – atmospheric CO_2 – and therefore changes in the $\delta^{13}C$ of the carbon source (atmospheric CO_2) as a

result of respired CO_2 from the canopy and soil would be reflected in foliar $\delta^{13}C$ of *C. longifolia* collected from under canopy cover. Respired CO_2 has a lower $\delta^{13}C$ than bulk plant material and atmospheric CO_2 (Klumpp et al., 2005; Tcherkez et al., 2011). This depletion of ^{13}C in respired CO_2 is reflected in lower $\delta^{13}C$ of understory plants that recycle this CO_2 (Murphy and Bowman, 2009; van der Merwe and Medina, 1989; van der Merwe, 1982). These observations are further supported by the $\delta^{13}C$ of atmospheric CO_2 collected from the forest near Heritage trail during the 2014 growing season (Fig. 4.2, Appendix G). On average, the atmospheric CO_2 from under canopy cover is depleted of ^{13}C by ~ 0.2 ‰ relative to atmospheric CO_2 from the dunes during the same time period (Fig. 4.2). Factors such as low $\delta^{13}C_{CO2}$ from fossil fuels also contribute to some of the variability in the foliar $\delta^{13}C$ values of plants collected from under canopy cover, as these plants grew in proximity to a parking lot and are close to anthropogenic activity which influence atmospheric $\delta^{13}C_{CO2}$.

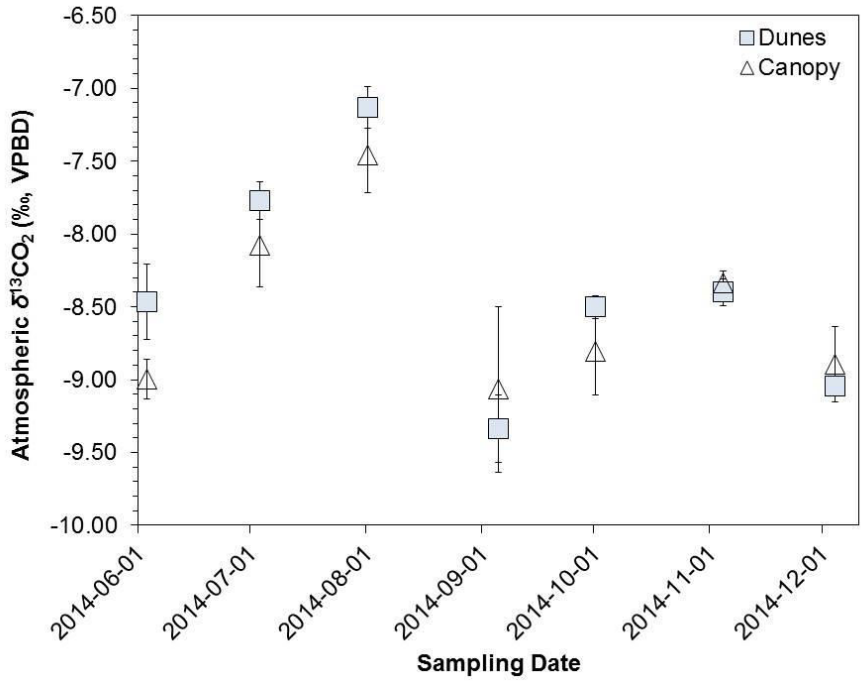

Figure 4.2: Seasonal changes in the $\delta^{13}C$ of atmospheric CO_2 collected from PPP.

In comparison, the enrichment in^{15}N exhibited by *C. longifolia* growing under canopy cover reflects variations in the local nitrogen cycle (Fig. 3.2e). There is gradual ~3 %

increase in organic content of soils from the dunes to soils in the forested areas because of greater litter accumulation from the canopy and understory in the older dunes (Ensign et al., 2006; VandenBygaart and Protz, 1995). Higher organic matter content increases microbial decomposition, which in turn increases the biologically available nitrogen to *C. longifolia* growing under canopy cover. Nitrogen is therefore less limiting for *C. longifolia* under the canopy than on the dunes. If either the supply of nitrogen is greater than demand or there is a more limiting resource, such as water, the soil nitrogen pool becomes relatively larger for plants growing those conditions. As a result, the nitrogen pool for plants growing under canopy cover would be more prone to losses through denitrification and volatilization, which discriminates against ^{15}N (Robinson, 2001). This ultimately enriches the soil nitrogen pool in ^{15}N and therefore the nitrogen source utilized by the plant, which in turn is reflected in foliar $\delta^{15}N$ (Evans, 2001).

4.3 Seasonal Variation

4.3.1 Carbon Isotope Variation

A. breviligulata and *C. longifolia* showed divergent seasonal changes in the foliar $\delta^{13}C$. Foliar $\delta^{13}C$ of *A. breviligulata* increased by ~ 1.2 ‰ from April to May 2014 and then remained relatively unchanged from May to October 2014 (Fig. 3.2b). By comparison, foliar $\delta^{13}C$ of *C. longifolia* from the dunes decreased negligibly (by ~ 0.4 ‰) from May to October 2014 (Fig. 3.2c), though May, June and July *C. longifolia* plants were statistically different from August, September and October plants. These divergent patterns between the species arise from a variety of factors including the use of photosynthates from the previous growing season, increasing WUE and changes in plant macromolecular contents, each of which are discussed next.

The lower foliar $\delta^{13}C$ of *A. breviligulata* collected in April reflects the isotopic compositions of macromolecules and/or photosynthates, such as fructan, glucose and fructose, produced in the previous growing season (2013) and stored in the roots for early growth in 2014. Fructan, glucose and fructose are the primary components of the non-structural carbohydrate reserve in the roots (Steen and Larsson, 1986). The 2013 growing season was characterized by high precipitation (~ 600 mm from April to September

2013) relative to the 2014 growing season (~ 450 mm from April to September 2014) and this greater water availability resulted in 2013 plant tissues that were ~ 1 ‰ depleted of ^{13}C relative to 2014 plants. If the foliage of April 2014 plants was developed from the root reserves produced during the 2013 growing season, this would explain why April 2014 leaves have lower $\delta^{13}C$ than leaves formed late in the growing season. The increase in the bulk foliar carbon content as the plant matures reflects the initiation and increase of photosynthetic activity to meet increasing demands of fixed carbon for plant growth (Chapin, 1980).

Other explanations, such as increasing WUE and changing macromolecular composition, particularly increasing cellulose content, as the growing season progresses, may contribute and somewhat exacerbate the seasonal $\delta^{13}C$ patterns observed in *A. breviligulata*. However, the caveat with these explanations is the fact that they predict increasing $\delta^{13}C$ throughout the growing season. These explanations do not explain why the foliar $\delta^{13}C$ remain relatively unchanged from May to October. For example, if WUE was a potential explanation for the observed patterns, then a continual increase in the foliar $\delta^{13}C$ of *A. breviligulata* would be predicted because of increasing temperature and evaporative demand, especially during the warmer part of the growing season (Table 3.6). This pattern was not observed, however, because *A. breviligulata* has deep roots in proximity to the water table which is ~ 176.9 to 177.4 m above sea level. The deep root system provides the plant access to water and therefore, it is unlikely that WUE would change significantly, especially from April to May where there is lower evaporative demand relative to the warmer part of the growing season. Similarly, the foliar macromolecular content of the leaves changes as the plant matures throughout the growing season(Goldberg et al., 1980). It is unlikely that the macromolecular content would only change in significant ways from April to May and not from May onwards as it is known that there is continual lignification during growth of the plant (Distel et al., 2005; Goldberg et al., 1980; Kamstra, 1973). With this, it may also be possible that the macromolecular changes that occur throughout the rest of the growing season may not be significantly affecting $\delta^{13}C$.

The seasonal foliar $\delta^{13}C$ patterns observed for *C. longifolia* are most likely related to the life-cycle growth stage. Foliar $\delta^{13}C$ of *C. longifolia* collected in May, June and July were statistically different from *C. longifolia* collected in August, September and October. In May, June and July, *C. longifolia* plants are in the vegetative and early elongation growth stage (Hendrickson et al., 1998; Moore et al., 1991), while in August and September, plants are in the late elongation and reproductive stages (Duckwitz and Wynia, 2006) and in October, the plants are beginning to undergo senescence. Different physiological processes occur during these stages, which in turn are reflected in varying foliar $\delta^{13}C$ expressed. For example, from May, June and July, the plant is initiating growth which reflects usage of stored photosynthates, increasing cellulose and lignin content while the higher foliar $\delta^{13}C$ in October is consistent with reallocation of carbon molecules to sinks such as roots, given the discrimination against ^{13}C during translocation (Cyr et al., 1990).

Factors such as the use of photosynthates from the previous growing season, changing WUE and changing macromolecular contents may also contribute to the seasonal foliar $\delta^{13}C$ patterns observed for *C. longifolia*. However, these explanations do not fully account for the observed patterns. For example, changes in the macromolecular make up may influence foliar $\delta^{13}C$. Lignin and cellulose content increases with plant maturity and have been associated with a decrease in $\delta^{13}C$ (Burzlaff, 1971; Hendrickson et al., 1997; Northup and Nichols, 1998). Cellulose is enriched in ^{13}C (by ~ +1‰) relative to bulk plant material while lignin is depleted of ^{13}C (by ~ –3‰) relative to bulk plant material (Badeck et al., 2005). An increase in cellulose content should therefore cause an increase in foliar $\delta^{13}C$ whereas an increase in lignin content should cause a decrease; an equal increase in the abundance of both would attenuate any overall change in carbon isotopic composition. For *C. longifolia*, there is only ~ 1 to 1.5 % increase in lignin content of *C. longifolia* tillers (stems) from June to October (Northup and Nichols, 1998), which is insufficient to explain the measured decrease of 1.5 ‰ decrease in foliar $\delta^{13}C$.

4.3.2 Nitrogen Isotope Variation

There were variations in foliar $\delta^{15}N$ (by ~ 1.9 to 2.2 ‰) of *A. breviligulata* and *C. longifolia* from April-May to October 2014. Foliar $\delta^{15}N$ for *A. breviligulata* were highest in May and lowest in September whereas foliar $\delta^{15}N$ of *C. longifolia* from the dunes were

the highest in September and the lowest in August. Foliar δ^{15}N of *C. longifolia* collected under the canopy cover did not vary significantly among the months of July, August and October but were significantly lower for plants collected in September (Fig. 3.2e).

Seasonal variations in foliar δ^{15}N are related to plant life cycle and the dynamic nature of the local nitrogen cycle. These processes compete with one an another during plant growth and senescence (Chapin et al., 1990), which causes the variability in foliar δ^{15}N. From April-May to October 2014, there was a decrease of ~ 0.9 to 1.5 wt. % in the foliar nitrogen content of both *A. breviligulata* and *C. longifolia* (Fig. 3.2d). Higher foliar nitrogen content early in the season is associated with initiation of photosynthesis and plant growth. Nitrogen is translocated from the roots to leaves in order to facilitate plant growth (Cyr et al., 1990). As the plants mature, foliar demand for nitrogen decreases. Nitrogen and nitrogen-products are then translocated and resorbed from the leaves to sinks such roots, to support root growth and replenish the nitrogen stores to ensure plant survival over winter (Chapin, 1980; Cyr et al., 1990). Translocation of nitrogen and nitrogen-products between leaves and other organs, such as roots and seeds, changes the relative size of the nitrogen pool within the leaf, which in turn affects the δ^{15}N of the nitrogen pool. This is further exacerbated by fractionations against ^{15}N during translocation and re-allocation of these compounds within the plant.

The local nitrogen cycle is another important factor that contributes to the seasonal variation observed in the foliar δ^{15}N. The nitrogen cycle is dynamic and considerably influenced by species-specific effects, weather patterns and dunes topography. Wedin and Tilman (1990) have shown that the type of vegetation cover influences the amount of mineralization that occurs in the soil. The amount of nitrogen mineralization that occurs varies through time and therefore, as the vegetation grows throughout the season, there likely is a change in the amount of mineralization that occurs in the soil as a result of plant growth. This, in turn, influences the δ^{15}N of the nitrogen source, which ultimately contributes to seasonal variation in foliar δ^{15}N.

The local nitrogen cycle also changes throughout the season because the openness of the nitrogen cycle is linked to water availability. Several studies have shown that higher

precipitation is correlated to lower $\delta^{15}N$, while lower precipitation is correlated to higher $\delta^{15}N$ (Aranibar et al., 2004; Austin and Vitousek, 1998; Craine et al., 2009; Murphy and Bowman, 2009; Swap and Aranibar, 2004). Monthly changes in the amount of precipitation influences the $\delta^{15}N$ of the nitrogen source, which is reflected in the foliar $\delta^{15}N$ of plants collected in the following month. For example, there is a slight, but not significant, increase in foliar $\delta^{15}N$ of *A. breviligulata* from July to August (Fig. 3.2e). Higher foliar $\delta^{15}N$ of August plants is likely a result of lower precipitation amounts in July relative to August. This is further supported by the fact that the average volumetric water content of PPP dune soils is positively correlated with the precipitation amount during the preceding ~ 30 days (Ensign et al., 2006). A similar pattern is observed with *A. breviligulata* and *C. longifolia* collected from under canopy cover in September. September plants had the lowest $\delta^{15}N$ during September, which is consistent with a 30-day lag between precipitation and soil moisture contents (Ensign et al., 2006). Conversely, the statistically lower foliar $\delta^{15}N$ of *C. longifolia* collected from the dunes in August may be linked to high monthly precipitation in August (~ 121.4 mm). The immediate response to high precipitation could indicate use of shallow roots at the dune crest to capture water. In all cases, the changes in $\delta^{15}N$ are undoubtedly confounded by maximum rooting depth, dune topography and the AM species composition, which can buffer changes in water availability.

4.4 $\delta^{13}C$ and $\delta^{15}N$ Correlation with Weather Variables among Years

The foliar carbon and nitrogen isotopic compositions of *A. breviligulata* and *C. longifolia* were tested for correlations with weather conditions for each growing season from 2006 to 2014. Weather variables considered were total summer precipitation (April to September), mean summer temperature (April to September), and June (July) mean maximum and minimum temperatures. The correlation coefficient (r) and p-values of the correlations between these weather parameters and foliar $\delta^{13}C$ and $\delta^{15}N$ for *A. breviligulata* and *C. longifolia* are presented in Table 4.1 and 4.2, respectively.

4.4.1 Precipitation

Total precipitation is a proxy for water availability and is a factor known to influence both carbon and nitrogen isotopic variation in plants. There is a possible significant negative correlation (r_s = -0.260, p-value = 0.030) between total summer precipitation and foliar δ^{13}C of *A. breviligulata* but a significant positive correlation (r_s = 0.485, p-value = 0.000; r_s = 0.532, p-value = 0.007) between total summer precipitation and foliar δ^{13}C of the individual and youngest leaves of *C. longifolia*. Additionally, there is a significant negative correlation between total summer precipitation and foliar δ^{15}N in the individual and oldest leaves of *C. longifolia* (r_s = - 0.312, p-value = 0.001; r_s = - 0.408, p-value = 0.048).

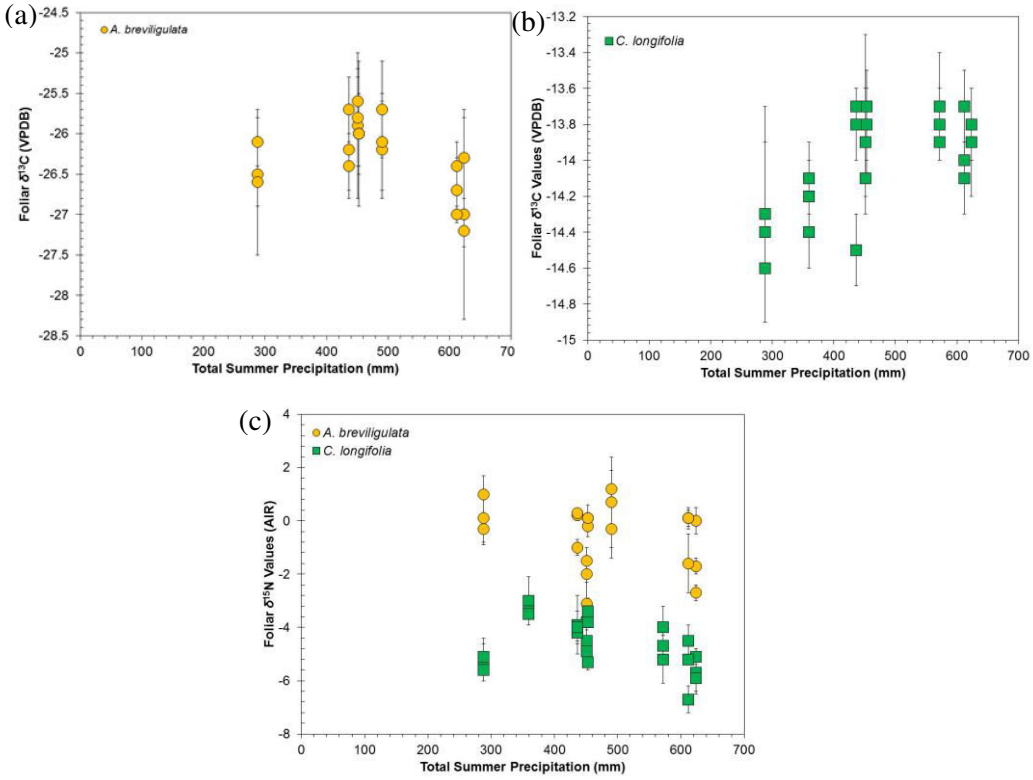

Figure 4.3: Relationship between total summer precipitation and (a) δ^{13}C of *A. breviligulata*, (b) δ^{13}C of *C. longifolia* and (c) δ^{15}N values of *A. breviligulata* and *C. longifolia* plants collected from 20016 to 2014.

4.4.1.1 Foliar $\delta^{13}C$

The general negative correlation between precipitation and foliar $\delta^{13}C$ of C_3 plants is well known (Codron et al., 2013; Diefendorf et al., 2010; Liu et al., 2014; Murphy and Bowman, 2009; Schulze et al., 1996; Wang et al., 2013) and is supported by physiological theory (Farquhar et al., 1989). The amount of discrimination against ^{13}C is controlled by the c_i/c_a ratio (Equation 1.1), which is a measure of stomatal conductivity. When less water is available, plants conserve water by increasing stomatal closure to reduce the amount of transpiration during photosynthesis. This results in a lower c_i/c_a ratio in the internal gas space of the leaf and reduces the amount of discrimination against ^{13}C by RuBisCo. Because of the limited amount of CO_2 available, this situation results in higher foliar $\delta^{13}C$. When more water is available, the amount of transpiration does not limit stomatal conductivity and photosynthetic activity. Hence, the c_i/c_a ratio in the internal gas space is higher than under drier conditions because of higher stomatal conductance. This situation allows RuBisCO to discriminate more strongly against ^{13}C, and results in lower foliar $\delta^{13}C$ during times of higher water availability. As this theory predicts, there are significant differences in the $\delta^{13}C$ of *A. breviligulata* plants collected in 2006 vs. 2008, 2006 vs. 2013, and 2008 vs. 2009. *A. breviligulata* exhibited higher foliar $\delta^{13}C$ in years that had lower water availability (2006 and 2009 each had ~ 450 mm precipitation) and lower $\delta^{13}C$ in years that had higher water availability (2008 and 2013 each had ~600 mm precipitation).

A positive correlation between $\delta^{13}C$ and water availability is less commonly reported (Murphy and Bowman, 2009). Typically, it is assumed that C_4 plants do not show a strong change in $\delta^{13}C$ arising from water stress because of the double carboxylating system that concentrates CO_2 at the site of carboxylation (Codron et al., 2013; Ehleringer and Cerling, 2002; von Caemmerer and Furbank, 2003). Nonetheless, the c_i/c_a ratio has a similar effect on ^{13}C discrimination by RuBisCO in C_4 plants during photosynthesis but these effects are confounded by bundle sheath leakiness (Φ), which influences the relationship between ^{13}C discrimination and c_i/c_a ratio (Fig. 1.3, Equation 1.2). The value of Φ affects the slope of the linear relationship between c_i/c_a ratio and ^{13}C discrimination, making the relationship either positive, negative or neutral (Farquhar, 1983). When Φ is

< 0.37 (c_i/c_a is 0), there is a negative relationship between ^{13}C discrimination and c_i/c_a ratio. This produces a positive relationship between $\delta^{13}C$ and c_i/c_a ratio.

Henderson et al. (1992) have shown that several C_4 plants are able to maintain $\Phi < 0.37$ under a range of environmental conditions and moderate stress. Therefore, this may explain the positive correlation observed here between foliar $\delta^{13}C$ of the individual and youngest leaves of *C. longifolia* and precipitation amount. In particular, the $\delta^{13}C$ of *C. longifolia* plants collected in 2007 and 2012 were significantly lower than other years in the study period. Incidentally, these years were years of the lowest and second lowest total summer precipitation throughout the study period. *C. longifolia* plants grown in 2011 and 2014 did not appear to follow this trend as plants from these years had the highest foliar $\delta^{13}C$ though there was only 572 and 453 mm of precipitation, respectively. It is unknown why plants from these years do not follow the predicted trends.

4.4.1.2 Foliar $\delta^{15}N$

A negative correlation between water availability (precipitation) and plant $\delta^{15}N$ has been reported previously (Aranibar et al., 2004; Austin and Vitousek, 1998; Craine et al., 2009; Murphy and Bowman, 2009; Swap and Aranibar, 2004). When water availability is more limiting to plant growth than nitrogen availability, the nitrogen cycle becomes more open. In this situation, soil nitrogen is more prone to losses via volatilization and denitrification (Robinson, 2001), both of which discriminate against ^{15}N. Consequently, this enriches in ^{15}N the remaining nitrogen pool, and the plants that utilize it (Evans, 2001). The negative correlation between foliar $\delta^{15}N$ of individual and oldest leaves of *C. longifolia* and precipitation is consistent with what has been reported in the literature. However, the lack of an statistically significant relationship between $\delta^{15}N$ of *A. breviligulata* and total summer precipitation contradicts the literature and suggests another factor that influences the observed relationship.

Foliar $\delta^{15}N$ of *A. breviligulata* collected in 2007 was statistically different from other analyzed years. *A. breviligulata* collected in 2007 experienced low water availability as 2007 was characterized by the lowest total summer precipitation, ~ 288 mm (Table 3.5). Only plants collected in 2009 had higher $\delta^{15}N$ than those collected in 2007. It is unknown

why 2009 *A. breviligulata* had higher foliar $\delta^{15}N$ but it is thought that while 2009 plants had higher water availability, they were exposed to lower average growing season temperatures than the plants in 2007, which may have led to foliar enrichment in ^{15}N.

The significantly lower foliar $\delta^{15}N$ of *A. breviligulata* in 2008 versus 2009 can also be linked to the higher water availability in 2008. Only plants from 2006 had lower $\delta^{15}N$, even though they received less (~ 450 mm) precipitation. Why this occurred is unknown, though it may reflect microsite heterogeneity in nitrogen sources and/or differences in rooting depth among individual *A. breviligulata* plants.

The foliar $\delta^{15}N$ of *C. longifolia* plants collected in 2008 and 2012 were significantly different from other years analyzed. Plants collected in 2008, which were exposed to high water availability (624 mm), had the lowest $\delta^{15}N$, while those collected in 2012, which were exposed to low water availability (359 mm), had the highest $\delta^{15}N$, consistent with predicted behavior. Significant differences in foliar $\delta^{15}N$ between 2006 and 2013, 2010 and 2013, and 2014 and 2013 further support this observation as 2013 was characterized by a higher amount of precipitation (~ 600 mm) and lower foliar $\delta^{15}N$ than the other years.

The statistically significant differences in foliar $\delta^{15}N$ of *C. longifolia* between 2007 and 2010, and 2007 and 2014, however, do not support a negative correlation with water availability. These observations once again point to greater complexity in the control of foliar $\delta^{15}N$ than simply water availability.

4.4.2 Mean Summer Temperature

No significant correlations were observed between mean summer temperature and foliar $\delta^{13}C$ of *A. breviligulata* and *C. longifolia*. There are very few studies that have examined the relationship between temperature and plant $\delta^{13}C$. Troughton and Card (1975) demonstrated experimentally that foliar $\delta^{13}C$ does not change significantly from 14 to 40°C. A field study by Codron et al. (2013) reported a weak, but significant relationship between temperature and foliar $\delta^{13}C$ for both C_3 and C_4 vegetation with a negative relationship for seasonal data (ranged from 35 to 40°C) and positive correlation for

annual data (ranged from 25 to 40°C). In Codron et al. (2013), however, seasonal temperature was calculated using mean daily maximum temperatures. The present study suggests that mean summer temperature (temperature from April to September 2014, Table 4.1, 4.2) does not correlate significantly with foliar $\delta^{13}C$, consistent with the results of Troughton and Card (1975).

While a significant negative correlation between foliar $\delta^{15}N$ of the youngest *A. breviligulata* leaves and mean summer temperature was observed, this relationship disappears when 2009 data, which are relatively lower, are excluded (r_s = 0.442, *p-value* = 0.045). This suggests that a non-linear trend or threshold point may exist for *A. breviligulata* beyond which a relationship between temperature and foliar $\delta^{15}N$ becomes apparent. No significant correlation between temperature and foliar $\delta^{15}N$ was observed for *C. longifolia*. Similar findings were reported by Codron et al. (2013) and Murphy and Bowman (2009) who demonstrated that there is no correlation between foliar $\delta^{15}N$ and temperature for plants that grew over a limited temperature range (Codron et al., 2013: 25 to 40°C, Bowman and Murphy (2009): 6 to 26°C) . This contradicts the results of studies that have reported a relationship between $\delta^{15}N$ and temperature over larger temperature and environmental ranges (i.e. Aranibar et al., 2004; Craine et al., 2009) .

4.4.3 Mean Maximum and Minimum June and July Temperatures

The foliar $\delta^{13}C$ and $\delta^{15}N$ of both *A. breviligulata* and *C. longifolia* were correlated to mean June (July) maximum and minimum temperatures. Mean June (July) maximum and minimum temperatures were chosen to test for correlations in order to evaluate whether the extreme temperature ranges of a given month best predict foliar carbon and nitrogen isotopic variation. June and July maximum and minimum temperatures were used because temperatures tend to be higher during these months and this is the peak of the growing season. This rationale is similar to that used by Teeri and Stowe (1976) who demonstrated that the abundance of C_4 grasses is best predicted by daily minimum June temperatures.

A significant negative correlation between foliar $\delta^{13}C$ and mean maximum June temperatures was observed for individual leaves of *C. longifolia* (r_s = - 0.267, *p*-value =

0.004). This suggests that increasing temperatures does not limit growth of C_4 vegetation and supports the idea that C_4 plant thrive in warmer, drier climates than C_3 plants (Schulze et al., 1996). In comparison, a significant negative correlation between foliar $\delta^{13}C$ and mean minimum June temperatures was observed for individual (r_s = - 0.371, *p*-value = 0.002) and the oldest leaves (r_s = - 0.616, *p*-value = 0.003) of *A. breviligulata* and oldest leaves of *C. longifolia* (r_s = - 0.484, *p*-value = 0.017) (Tables 4.1, 4.2). A negative correlation suggests that minimum temperatures, most likely night temperatures, may not be as limiting or deleterious to photosynthesis as previously suggested (Teeri and Stowe, 1976) as there is more discrimination against ^{13}C with higher temperatures. Lower foliar $\delta^{13}C$ suggest more discrimination against ^{13}C as a result of RuBisCo, which in turn, reflects the removal of limitations associated with enzymatic fractionations in C_4 plants.

There were no significant correlations between mean minimum June temperature and foliar $\delta^{15}N$. No significant correlations were also observed between mean maximum June temperatures and foliar $\delta^{13}C$ or $\delta^{15}N$ for either *A. breviligulata* or *C. longifolia* when the isotopic compositions of each of individual leaves, oldest leaves and youngest leaves were considered.

There was a significant negative correlation between foliar $\delta^{15}N$ of *A. brevilugulata* for both individual leaves and youngest leaves and (a) mean maximum July temperature, and (b) mean minimum July temperature (Table 4.1). By comparison, there was a significant positive correlation between foliar $\delta^{15}N$ of *C. longifolia* and (a) mean maximum July temperatures (individual and youngest leaves), and (b) mean minimum July temperatures (individual leaves) (Table 4.2). The divergent correlations are related to species-specific factors. *A. breviligulata* is less limited by water than *C. longifolia* because of the former grows on the foredunes (Fig. 2.2) and has deep roots that allow the plant to have access to the water table. As a result, nitrogen is more limiting than water. This means that there is a less open nitrogen cycle, which is reflected in the negative correlation between July temperature and foliar $\delta^{15}N$. Comparatively, *C. longifolia* grows on the crest of the first dune ridge (Fig. 2.2). Though *C. longifolia* has a deep root system, the location in which *C. longifolia* grows on the dunes prevents the roots from accessing groundwater table

which is located ~ 176.9 to 177.4 m above sea level and does not significantly fluctuate in the depth seasonally (Steinbach, 1999). This means that water is more limiting to plant growth than nitrogen, which results in a more open nitrogen cycle and thus is reflected in the positive correlation between foliar $\delta^{15}N$ and increasing temperature (and hence evaporative demand).

Table 4.1: Spearman's rank correlation between weather variables and carbon and nitrogen isotopic compositions of individual leaves, oldest leaves and youngest leaves of *A. breviligulata* collected from 2006 to 2014.

	No. of Samples	Total Summer Precipitation (mm)[1]		Mean Summer Temperature (˚C)[2]		Mean Maximum June Temperature (˚C)[3]		Mean Minimum June Temperature (˚C)[4]		Mean Maximum July Temperature (˚C)[5]		Mean Minimum July Temperature (˚C)[6]	
		r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
δ[13]C													
Individual Leaves	70	-0.260*	0.030	-0.082	0.502	-0.145	0.232	-0.371**	0.002	0.092	0.449	0.72	0.556
Oldest Leaf	21	-0.386	0.084	-0.065	0.778	-0.260	0.255	-0.473*	0.030	0.164	0478	0.182	0.429
Youngest Leaf	21	-0.067	0.772	-0.442*	0.045	0.082	0.724	-0.616**	0.003	-0.307	0.176	-0.388	0.082
δ[15]N													
Individual Leaves	69	-0.096	0.432	-0.137	0.260	0.022	0.857	-0.095	0.437	-0.411**	0.000	-0.0381**	0.001
Oldest Leaf	21	-0.085	0.715	-0.026	0.912	0.122	0.597	0.136	0.557	-0.195	0.397	-0.057	0.806
Youngest Leaf	21	-0.248	0.278	-0.132	0.568	-0.069	0.768	-0.294	0.196	-0.498*	0.22	-0.538*	0.012

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
[1] Total summer precipitation from April to September
[2] Mean summer temperature from April to September
[3] Mean daily maximum June temperature
[4] Mean daily minimum June temperature
[5] Mean daily maximum July temperature
[6] Mean daily minimum July temperature

Table 4.2: Spearman's rank correlation between weather variables and carbon and nitrogen isotopic compositions of individual leaves, oldest leaves and youngest leaves of *C. longifolia* collected from 2006 to 2014.

	No. of Samples	Total Summer Precipitation (mm)[1]		Mean Summer Temperature (˚C)[2]		Mean Maximum June Temperature (˚C)[3]		Mean Minimum June Temperature (˚C)[4]		Mean Maximum July Temperature (˚C)[5]		Mean Minimum July Temperature (˚C)[6]	
		r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
$\delta^{13}C$													
Individual Leaves	113	0.485**	0.000	-0.103	0.275	-0.267**	0.004	-0.029	0.764	-0.051	0.592	0.141	0.135
Oldest Leaf	24	0.084	0.694	-0.083	0.700	-0.0270	0.202	-0.484*	0.017	-0.188	0.380	0.084	0.695
Youngest Leaf	24	0.532**	0.007	-0.086	0.690	-0.139	0.517	0.025	0.907	-0.174	0.417	0.001	0.995
$\delta^{15}N$													
Individual Leaves	110	-0.312**	0.001	0.0240*	0.012	0.060	0.534	0.002	0.0984	0.451**	0.000	0.291**	0.002
Oldest Leaf	24	-0.408*	0.048	0.213	0.318	0.204	0.340	0.017	0.937	0.336	0108	0.201	0.346
Youngest Leaf	24	-0.353	0.091	0.362	0.082	0.023	0.914	0.033	0.879	0.520**	0.009	0.393	0.057

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
[1] Total summer precipitation from April to September
[2] Mean summer temperature from April to September
[3] Mean daily maximum June temperature
[4] Mean daily minimum June temperature
[5] Mean daily maximum July temperature
[6] Mean daily minimum July temperature

4.5 Isotopic Response of C_3 and C_4 Plants

The results of this study indicate that there are different and often divergent, isotopic responses of temperate dune C_3 and C_4 grasses to environmental conditions. In the literature it is commonly reported that C_3 plants respond more closely to environmental conditions than C_4 plants due to the C_3 photosynthetic pathway, which does not moderate the effects of environmental conditions (Codron et al., 2013; Farquhar et al., 1989). The present study, however, determined that C_4 plants respond to environmental conditions to the same or greater extent than C_3 plants in environments such as PPP.

Both $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia*, a C_4 dune grass, correlated more strongly to precipitation and temperature compared to *A. breviligulata*, a C_3 grass (Table 4.1, 4.2). The correlations in the present study are significant and comparable to those reported by Codron et al. (2013), but are weaker than correlations observed in studies that examined plants growing across larger environmental gradients (Table 4.3) (i.e. Craine et al., 2009; Diefendorf et al., 2010).This suggests that weather conditions cause foliar carbon and nitrogen isotopic variability in plants growing at a single locality on monthly and annual time scales in a temperate-humid climatic zone such as PPP. The amount of isotopic variation associated with environmental conditions at a single site, however, is less than the variation associated with changing conditions over a large environmental gradient.

Table 4.3: Comparison of reported correlations (*r*) between $\delta^{13}C$ and $\delta^{15}N$ and environmental conditions in various studies.

Study	Environmental Variable	Climatic Zone	Scale	Range	$\delta^{13}C$	$\delta^{15}N$	Correlation	*r**
Aranibar et al. (2004)	Mean Annual Precipitation	Semi-Arid Savanna	Spatial	230 to 978 mm		X	Negative	0.70
Codron et al. (2013)	Annual Rainfall	Semi-Arid Savanna	Temporal	Max. 800 mm	X		Positive (C_4)	0.25
	Seasonal Temperature	Semi-Arid Savanna	Temporal	35 to 40°C	X		Negative (C_4)	0.23
	Annual Temperature	Semi-Arid Savanna	Temporal	25 to 40°C	X		Positive (C_4)	0.12
	Seasonal Temperature	Semi-Arid Savanna	Temporal	35 to 40°C		X	Positive (C_4)	0.27
	Seasonal Rainfall	Semi-Arid Savanna	Temporal	Max. 800 mm		X	Positive (C_4)	0.23
Diefendorf et al. (2010)	Mean Annual Precipitation	Global	Spatial	147 to 3700 mm	X		Positive	0.74
Liu et al. (2014)	Humidity Index	Arid, Semi-Arid and Semi-Humid	Spatial	0.08 to 0.28	X		Negative	0.80
Murphy and Bowman (2009)	Water Availability Index	Temperate, Arid, Tropical, Mediterranean	Spatial	0.14 to 0.73	X		Negative (C_3); Positive (C_4)	0.41 - 0.46
	Water Availability Index	Temperate, Arid, Tropical, Mediterranean	Spatial	0.14 to 0.73		X	Negative	0.63
Wang et al. (2010)	Mean Annual Precipitation	Arid	Spatial	365 to 698 mm		X	Negative	0.52 - 0.81
	Mean Annual Precipitation	Arid	Spatial	365 to 698 mm	X		Negative (C_3); Non-Linear (C_4)	0.78 - 0.98

* reported r^2 values were converted to *r* values

There was ~ 1 to 2 ‰ variation in $\delta^{13}C$ and ~ 3 to 4 ‰ variation in $\delta^{15}N$ of grass leaves throughout the study period. Though a portion of this variation can be attributed to environmental conditions, variability caused by intra-species differences and factors, such as life cycle, dune topography and species-specific differences, are an important consideration. These factors either confound or accentuate isotopic variability associated with environmental conditions. For example, water availability is known to play a crucial role in determining foliar $\delta^{13}C$ and $\delta^{15}N$ of grasses. This study determined that water availability affects foliar $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia* more strongly than *A. breviligulata*. This observation reflects the location of each species on the dunes and rooting depth of each species (*C. longifolia*, dune ridge, shorter roots; *A. breviligulata*, foredune, longer roots). Dune topography influences soil moisture content and the flow of water (Ensign et al., 2006) while the plant's location on the dune and rooting depths determine whether a grass has access to groundwater as shown in Fig. 4.4.

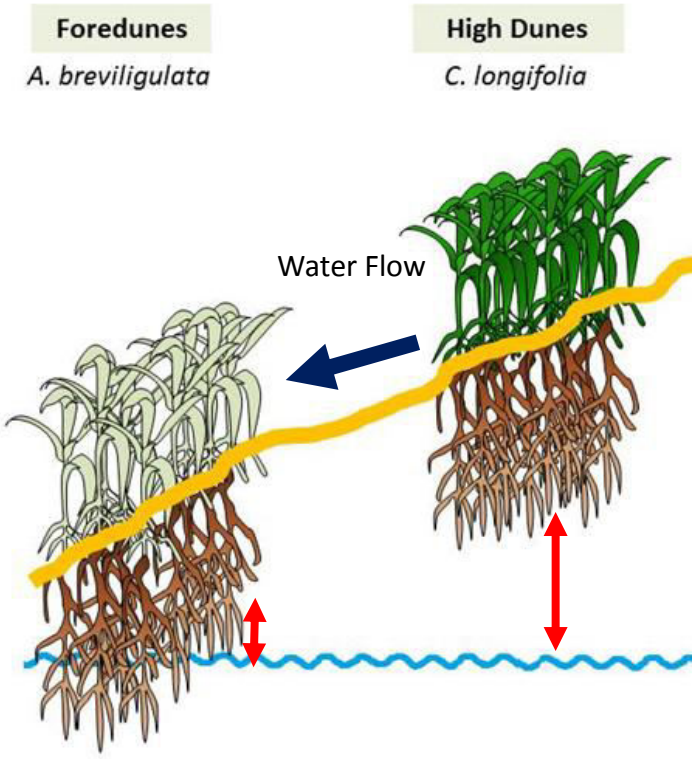

Figure 4.4: Schematic demonstrating how dune topography, rooting depth and hydrological flow influences water availability to *A. breviligulata* and *C. longifolia.*

Likewise, a large part of the foliar isotopic variation between *A. breviligulata* and *C.* longifolia at PPP is species-specific. *C. longifolia* growing in two different sites (dunes vs. under canopy) showed similar seasonal trends in foliar $\delta^{13}C$ and $\delta^{15}N$ as the season progress, supporting the notion that these patterns are species-specific rather than location-specific. That being said, the statistically significant differences in both foliar $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia* between the two sites demonstrate that micro-environmental conditions related to location (e.g., topography, canopy cover, soil development) are also important factors affecting isotopic composition.

Chapter 5

5 Conclusions

This study examined the temporal sensitivity of foliar $\delta^{13}C$ and $\delta^{15}N$ of temperate C_3 and C_4 dune grasses to weather conditions at PPP, a temperate humid climatic region located in southwestern Ontario. Multiple studies have shown that environmental conditions influence foliar $\delta^{13}C$ and $\delta^{15}N$ of vegetation on large spatial scales. To date, however, there have been no studies that have examined how environmental conditions influence foliar $\delta^{13}C$ and $\delta^{15}N$ of vegetation on a seasonal and annual scale in a temperate dune system such as PPP.

The first objective of this study was to quantify carbon and nitrogen isotopic variation for *A. breviligulata* and *C. longifolia* growing at a single locality through the season (April to October). Throughout the 2014 growing season, there was a ~ 1 to 2 ‰ difference in $\delta^{13}C$ and ~3 to 4 ‰ difference in $\delta^{15}N$ in both *A. breviligulata* and *C. longifolia*. These seasonal changes are related to changes in the macromolecule contents, growth patterns and to some extent, changes in the weather conditions, all of which influence local nutrient cycling and hence foliar isotopic compositions. *C. longifolia* from two different sites (dunes versus under canopy cover) showed similar seasonal changes in $\delta^{13}C$ and $\delta^{15}N$, though there were statistically significant differences in the carbon and nitrogen isotopic compositions at each site. In short, the differences in isotopic patterns observed throughout the season are most strongly related to grass species (i.e., C_3 versus C_4), though environmental and site conditions also influence the isotopic compositions of these plants.

The second objective of this study was to determine whether changes in foliar $\delta^{13}C$ and $\delta^{15}N$ of *A. breviligulata* and *C. longifolia* are correlated to weather conditions at a single site within a temperate-humid climatic zone on an inter-annual time scale (2006-2014). There was a ~ 1 to 2 ‰ variation in $\delta^{13}C$ and ~ 4 ‰ variation in $\delta^{15}N$ for each of *A. breviligulata* and *C. longifolia* over a range of 288 - 624 mm of precipitation and 6.7 – 20.1 °C in growing season temperature

(April to September) between the 2006 to 2014 period. These variations in isotopic composition were significantly correlated with changes in the environmental conditions, namely total summer precipitation and mean maximum and minimum June and July temperatures. Total summer precipitation did not significantly correlate to the $\delta^{15}N$ of *A. breviligulata*. Average growing season temperature did not correlate with foliar $\delta^{13}C$ and $\delta^{15}N$ for either *A. breviligulata* or *C. longifolia*. These correlations were significant and are comparable to correlations reported by Codron et al. (2013) but are not comparable to correlations made in studies conducted in tropical environments where there was a larger gradient of change in weather conditions. This suggests that environmental conditions do influence carbon and nitrogen isotopic variability on small scales, though this variation may be confounded and/or accentuated by other external factors, such as plant development.

The third objective of this study was to determine whether there are differences in the carbon and nitrogen isotopic responses of C_3 and C_4 dune grasses from the same temperate humid site, and to explain any observed responses. The study has demonstrated that *A. breviligulata,* a C_3 perennial grass, and *C. longifolia*, a C_4 perennial grass, have divergent isotopic responses to environmental conditions. Foliar $\delta^{13}C$ and $\delta^{15}N$ of *C. longifolia* were more strongly correlated to environmental conditions than *A. breviligulata.* It is likely that location within the PPP dune system and species-specific characteristics, such as rooting depth, most strongly influence the observed isotopic trends. For example, because *C. longifolia* grows on the dune ridge, water availability likely causes its foliar $\delta^{13}C$ and $\delta^{15}N$ to be more strongly correlated to precipitation amount than is the case for *A. breviligulata,* which grows on the foredunes.

The present study has shown that environmental conditions cause inter-annual variability in foliar $\delta^{13}C$ and $\delta^{15}N$ of grasses at a single site. The amount of change, however, is small over the 9 years investigated. These changes can be significantly correlated to water availability and mean maximum and minimum June / July temperatures, but are weaker than correlations observed for tropical

environments (i.e. Aranibar et al., 2004; Murphy and Bowman, 2009; Swap and Aranibar, 2004; Wang et al., 2005). As a result, inter-annual variation in $\delta^{13}C$ and $\delta^{15}N$ arising from inter-annual fluctuations in climatic conditions (i.e., response to normal variations in weather) does not appear to greatly confound interpretation of vegetation proxies used in paleoclimatic reconstruction of environments like that of the PPP. Conditions well outside of the normal range of weather conditions are likely required to produce significant shifts in isotopic composition. This also suggests that isotopic variability measured for organic matter in the soil profile is likely to be representative of larger environmental changes over time rather than small annual fluctuations in the environmental conditions, provided that isotopic effects arising from degradation are also understood. Based on the findings of this study, $\delta^{13}C$ and $\delta^{15}N$ from biological proxies can be used to a reliable degree in climatic models to estimate paleoclimatic conditions without being confounded by intra- and inter-annual variation of weather conditions.

Though this study determined that intra- and inter-annual weather does not cause for large amounts of variation in the foliar isotopic values, this study leaves many unanswered questions about the isotopic sensitivity of grasses to climatic conditions in environments like the PPP. For example:

- Are nine years of isotopic and weather data sufficient to capture the natural variability of the climate regime?
- Are the seasonal variations in the isotopic compositions determined for *A. breviligulata* and *C. longifolia* unique to the 2014 growing season?
- Are the findings of this study applicable to all vegetation types or functional groups at PPP?
- Are there isotopic responses to large scale-weather patterns such as El Niño and La Niña events in the PPP vegetation records?

It would be useful: (a) to extend the present study over a longer time scale (i.e. ~20 years), (b) to test for seasonal variations in isotopic composition over several more seasons, and (c) to add different species of grasses as well as other vegetation types (i.e. forbs, sedges). These additional studies would: (a)

strengthen the current findings by capturing more variability in the climatic regime in the PPP, (b) demonstrate whether the seasonal trends observed in 2014 are representative over multiple growing seasons, and (c) test whether the trends observed for *A. breviligulata* and *C. longifolia* can be applied more generally to other life forms and species of vegetation. Determining whether cyclic large-scale weather patterns, such as El Niño and La Niña events, can measurably influence the foliar isotopic composition of plants as well as understanding how water supply to *A. breviligulata* and *C. longifolia* influences the isotopic variability via an integrated plant-water, soil water and groundwater study would also help to refine our ability to recognize such phenomena in paleoclimatic records.

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Appendices

Appendix A: Analytical accuracy and precision for all carbon standards utilized during carbon content and carbon isotopic analysis**.**

Standard	Analytical Session	$\delta^{13}C$ (‰, VPDB)			C (wt. %)	
		Mean of Analysis	Std. Dev. of Analysis	Accepted Value	Mean of Analysis	Std. Dev. of Analysis
ANU Sucrose	1	-10.46	0.04	-10.45	41.32	0.29
	2	-10.48	0.06	-10.45	41.45	1.76
	3	-10.52	0.05	-10.45	42.76	2.51
	4	-10.35	0.08	-10.45	10.46	0.96
	5	-10.43	0.08	-10.45	40.36	0.99
	6	-10.58	0.08	-10.45	41.00	0.40
	7	-10.53	0.19	-10.45	35.36	8.62
	8	-10.55	0.18	-10.45	40.78	0.48
Keratin	1	24.02	0.06	-24.04	44.59	5.79
	2	-24.06	0.05	-24.04	45.84	0.99
	3	-24.07	0.12	-24.04	45.31	2.61
	4	-24.04	0.04	-24.04	46.58	0.46
	5	-24.06	0.05	-24.04	44.41	5.25
	6	-24.07	0.11	-24.04	46.87	0.51
	7	-24.08	0.08	-24.04	44.97	6.61
	8	-24.06	0.06	-24.04	46.29	5.60
USGS-40	1	-26.40	0.09	-26.39	38.78	1.09
	2	-26.40	0.06	-26.39	39.90	0.46
	3	-26.40	0.02	-26.39	38.91	1.38
	4	-26.39	0.08	-26.39	40.17	0.80
	5	-26.39	0.05	-26.39	38.06	3.58
	6	-26.39	0.15	-26.39	39.48	0.39
	7	-26.39	0.16	-26.39	35.26	7.79
	8	-26.39	0.03	-26.39	39.50	0.94
USGS-41	1	37.60	0.07	37.63	40.70	0.23
	2	37.60	0.08	37.63	40.70	0.49
	3	37.60	0.11	37.63	40.72	1.42
	4	37.63	0.12	37.63	40.70	1.36
	5	37.63	0.07	37.63	40.70	0.35
	6	37.63	0.15	37.63	40.70	0.52
	7	37.63	0.18	37.63	40.74	2.93
	8	37.63	0.10	37.63	40.70	0.65

Appendix B: Analytical accuracy and precision for all nitrogen standards utilized during nitrogen content and nitrogen isotopic analysis

Standard	Analysis Session	δ^{15}N (‰, AIR)			N (wt. %)	
		Mean of Analysis	Std. Dev of Analysis	Accepted Value	Mean of Analysis	Std. Dev of Analysis
Keratin	1	6.39	0.03	6.36	14.51	0.53
	2	6.40	0.09	6.36	14.11	1.00
	3	6.39	0.14	6.36	13.71	0.47
	4	6.35	0.09	6.36	14.13	0.44
	5	6.45	0.13	6.36	14.65	0.27
	6	6.34	0.13	6.36	15.09	0.28
	7	6.44	0.09	6.36	14.87	0.26
	8	6.33	0.11	6.36	14.85	0.32
	9	6.36	0.16	6.36	14.35	1.02
	10	6.40	0.12	6.36	14.67	0.36
	11	6.43	0.12	6.36	14.73	0.19
	12	6.35	0.14	6.36	14.45	0.66
	13	6.40	0.08	6.36	14.78	0.27
	14	6.18	0.61	6.36	14.70	0.15
USGS-40	1	-4.52	0.05	-4.52	8.83	0.68
	2	-4.52	0.13	-4.52	9.23	0.15
	3	-4.52	0.02	-4.52	9.10	0.07
	4	-4.52	0.10	-4.52	9.23	0.15
	5	-4.52	0.10	-4.52	8.98	0.10
	6	-4.52	0.04	-4.52	9.37	0.13
	7	-4.52	0.03	-4.52	9.16	0.46
	8	-4.52	0.07	-4.52	9.03	0.30
	9	-4.52	0.10	-4.52	9.29	0.17
	10	-4.52	0.08	-4.52	9.13	0.26
	11	-4.52	0.04	-4.52	9.21	0.14
	12	-4.52	0.05	-4.52	9.07	0.29
	13	-4.52	0.05	-4.52	9.02	0.18
	14	-4.52	0.04	-4.52	9.25	0.07
USGS-41	1	47.57	0.19	47.57	9.50	0.15
	2	47.57	0.04	47.57	9.50	0.24
	3	47.57	0.15	47.57	9.50	0.16
	4	47.57	0.40	47.57	9.50	0.10
	5	47.57	0.05	47.57	9.50	0.06
	6	47.57	0.12	47.57	9.51	0.37
	7	47.57	0.27	47.57	9.50	0.30
	8	47.57	0.20	47.57	9.50	0.12
	9	47.57	0.30	47.57	9.53	0.19

Standard	Analysis Session	δ^{15}N (‰, AIR)			N (wt. %)	
		Mean of Analysis	Std. Dev of Analysis	Accepted Value	Mean of Analysis	Std. Dev of Analysis
Peach Leaves	10	47.57	0.09	47.57	9.50	0.29
	11	47.57	0.52	47.57	9.49	0.08
	12	47.57	0.15	47.57	9.50	0.38
	13	47.57	0.09	47.57	9.50	0.13
	14	47.57	0.10	47.57	9.50	0.05
	1	1.88	0.06	1.98	2.73	0.06
	2	1.89	0.06	1.98	2.72	0.05
	3	1.88	0.03	1.98	2.66	0.03
	4	1.95	0.19	1.98	2.68	0.21
	5	2.00	0.14	1.98	2.82	0.02
	6	1.99	0.09	1.98	2.92	0.03
	7	2.04	0.04	1.98	2.84	0.04
	8	1.95	0.12	1.98	2.67	0.12
	9	1.87	0.19	1.98	2.78	0.04
	10	1.89	0.14	1.98	2.90	0.14
	11	2.01	0.04	1.98	2.85	0.05
	12	1.81	0.11	1.98	2.78	0.08
	13	1.94	0.08	1.98	2.84	0.03
	14	1.85	0.17	1.98	1.85	0.17

Appendix C: All sample data from *A. breviligulata* and *C. longifolia* collected from April to October 2014 at Pinery Provincial Park.

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
A. breviligulata												
122	Dunes	1	3	G14-AB1-04-L3	-27.0		45.0		-0.4	0.1	0.9	0.0
122	Dunes	1	4	G14-AB1-04-L4	-		-		-0.4		1.8	
122	Dunes	1	5	G14-AB1-04-L5	-27.0		43.6		-		-	
122	Dunes	2	3	G14-AB2-04-L3	-27.9		44.0		0.2		1.5	
122	Dunes	2	4	G14-AB2-04-L4	-26.8		43.9		0.4		2.5	
122	Dunes	3	4	G14-AB3-04-L4	-28.0		44.2		0.4		1.4	
122	Dunes	3	5	G14-AB3-04-L5	-26.8		40.7		0.6		2.3	0.0
154	Dunes	1	1	G14-05-AB1-L1	-25.9		45.2		1.9	0.2	1.4	
154	Dunes	1	3	G14-05-AB1-L3	-26.2		45.0		1.2		1.9	
154	Dunes	1	4	G14-05-AB1-L4	-25.7		43.3		0.9		2.2	0.0
154	Dunes	2	1	G14-05-AB2-L1	-26.9		46.2		0.0	0.1	1.1	0.0
154	Dunes	2	2	G14-05-AB2-L2	-		-		-0.1	0.2	1.0	
154	Dunes	2	3	G14-05-AB2-L3	-26.0		44.0		0.1		1.4	
154	Dunes	2	5	G14-05-AB2-L5	-26.0		44.8		-		-	
154	Dunes	3	1	G14-05-AB3-L1	-26.7		45.8		1.9		1.7	
154	Dunes	3	2	G14-05-AB3-L2	-		-		1.3		1.4	
154	Dunes	3	3	G14-05-AB3-L3	-25.0		45.3		1.4		1.9	
154	Dunes	3	5	G14-05-AB3-L5	-24.9		43.6		-		-	
184	Dunes	1	1	G14-06-AB1-L1	-26.5		46.7		1.6		1.0	
184	Dunes	1	3	G14-06-AB1-L3	-26.8	0.0	45.6	0.7	1.1		0.7	
184	Dunes	1	5	G14-06-AB1-L5	-25.9		46.0		-0.3		0.3	
184	Dunes	2	1	G14-06-AB2-L1	-27.1		-		0.2		0.4	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
184	Dunes	2	3	G14-06-AB2-L3	-26.1		-		0.1		1.0	
184	Dunes	2	5	G14-06-AB2-L5	-25.0		-		0.2		1.1	0.0
184	Dunes	3	1	G14-06-AB3-L1	-27.0		-		0.7	0.0	1.1	
184	Dunes	3	3	G14-06-AB3-L3	-26.0		-		0.7		0.7	
184	Dunes	3	5	G14--06-AB3-L5	-24.8		-		1.0		1.4	0.1
213	Dunes	1	1	G14-07-AB1-L1	-27.0	0.1	42.3	3.9	1.2	0.0	0.6	
213	Dunes	1	3	G14-07-AB1-L3	-25.3		-		-0.1		0.7	
213	Dunes	1	5	G14-07-AB1-L5	-24.4		43.8		-0.2		1.1	
213	Dunes	2	2	G14-07-AB2-L2	-26.3		46.6		-0.9		0.6	
213	Dunes	2	3	G14-07-AB2-L3	-25.3		46.5		-0.9		0.5	0.0
213	Dunes	2	4	G14-07-AB2-L4	-25.2		46.8		-0.9	0.0	1.0	
213	Dunes	3	1	G14-07-AB3-L1	-26.7		45.7		0.9		0.3	
213	Dunes	3	3	G14-07-AB3-L3	-26.2		-		0.7		0.7	
213	Dunes	3	5	G14-07-AB3-L5	-24.9		-		1.2		1.2	0.0
248	Dunes	1	1	G14-08-AB1-L1	-26.6		44.7		0.1	0.0	0.3	
248	Dunes	1	3	G14-08-AB1-L3	-25.3		45.9		-0.3		2.2	
248	Dunes	1	5	G14-08-AB1-L5	-25.1		45.1		-0.7		0.3	
248	Dunes	2	1	G14-08-AB2-L1	-26.7		44.7		0.5		0.3	
248	Dunes	2	3	G14-08-AB2-L3	-26.2	0.0	45.7	0.4	0.6		0.3	
248	Dunes	2	5	G14-08-AB2-L5	-26.3		45.5		0.5		0.4	
248	Dunes	3	1	G14-03-AB3-L1	-27.5		44.2		0.2		0.4	
248	Dunes	3	3	G14-08-AB3-L3	-26.8		44.8		0.2		0.5	
248	Dunes	3	5	G14-08-AB3-L5	-26.5		46.0		0.2		0.5	0.0
274	Dunes	1	1	G14-09-AB1-L1	-26.4		45.1		-0.9	0.0	0.7	0.0
274	Dunes	1	2	G14-09-AB1-L2	-26.5		46.5		-0.1	0.0	0.3	
274	Dunes	1	3	G14-09-AB1-L3	-26.2		46.9		0.3		0.3	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
274	Dunes	1	4	G14-09-AB1-L4	-25.9		46.2		-0.5		0.3	0.0
274	Dunes	2	1	G14-09-AB2-L1	-26.9		45.5		-1.7	0.1	0.3	0.0
274	Dunes	2	2	G14-09-AB2-L2	-26.4		46.1		-2.2	0.0	0.3	0.0
274	Dunes	2	3	G14-09-AB2-L3	-26.1	0.1	46.4	0.2	-1.2	0.1	0.3	
274	Dunes	2	4	G14-09-AB2-L4	-26.0		45.9		-1.3	0.0	0.3	
274	Dunes	2	5	G14-09-AB2-L5	-25.8		45.1		-0.9		0.4	
274	Dunes	3	1	G14-09-AB3-L1	-25.9		45.0		-0.9		0.6	
274	Dunes	3	2	G14-09-AB3-L2	-25.6		45.5		-1.4		0.6	
274	Dunes	3	3	G14-09-AB3-L3	-25.1		45.5		-0.6		0.6	
274	Dunes	3	4	G14-09-AB3-L4	-24.8		45.0		-2.2		0.7	
307	Dunes	1	1	G14-10-AB1-L1	-26.5		-		0.1		0.3	
307	Dunes	1	3	G14-10-AB1-L3	-25.9		-		0.2		0.3	
307	Dunes	1	4	G14-10-AB1-L4	-25.7		-		0.0		0.4	
307	Dunes	2	1	G14-10-AB2-L1	-26.6		-		0.0		0.3	
307	Dunes	2	3	G14-10-AB2-L3	-25.9		-		0.1		0.3	
307	Dunes	2	5	G14-10-AB2-L5	-25.6		-		-0.7		0.4	
307	Dunes	3	1	G14-10-AB3-L1	-26.9		-		0.2		0.3	
307	Dunes	3	3	G14-10-AB3-L3	-26.2	0.0	-		0.5		0.8	
307	Dunes	3	5	G14-10-AB3-L5	-25.1		-		-0.5		0.5	
C. longifolia												
154	Dunes	1	1	G14-05-CL1-L1	-13.8	0.1	47.4	0.4	-5.2		2.4	
154	Dunes	1	2	G14-05-CL1-L2	-13.7		46.0		-5.0		2.4	
154	Dunes	1	3	G14-05-CL1-L3	-13.5		46.5		-4.7		2.1	
154	Dunes	1	4	G14-05-CL1-L4	-12.9		44.8		-4.7		2.1	
154	Dunes	2	1	G14-05-CL2-L1	-13.2		46.0		-5.0		2.0	
154	Dunes	2	2	G14-05-CL2-L2	-13.4		46.7		-4.7		1.9	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
154	Dunes	2	3	G14-05-CL2-L3	-13.3		46.6		-4.3		2.0	
154	Dunes	2	4	G14-05-CL2-L4	-12.8		45.8		-4.1		1.5	
154	Dunes	3	1	G14-05-CL3-L1	-13.2		46.5		-4.2		2.0	
154	Dunes	3	2	G14-05-CL3-L2	-13.5		46.3		-4.0		2.2	0.0
154	Dunes	3	3	G14-05-CL3-L3	-13.5	0.0	46.8	0.1	-3.6		2.0	
154	Dunes	3	4	G14-05-CL3-L4	-13.4		46.3		-3.6	0.1	2.1	0.0
154	Dunes	3	5	G14-05-CL3-L5	-12.8		46.0		-3.4		2.0	
184	Dunes	1	1	G14-06-CL1-L1	-13.7		45.5		-4.2	0.0	1.7	
184	Dunes	1	3	G14-06-CL1-L3	-13.7		45.8		-3.8		1.6	
184	Dunes	1	5	G14-06-CL1-L5	-13.6		46.8		-3.0		1.6	
184	Dunes	1	7	G14-06-CL1-L7	-13.2		44.5		-2.4		1.3	
184	Dunes	1	9	G14-06-CL1-L9	-12.7		44.1		-2.4		1.4	
184	Dunes	2	1	G14-06-CL2-L1	-13.6		45.7		-5.6		2.0	
184	Dunes	2	3	G14-06-CL2-L3	-13.8		46.5		-4.8		1.7	
184	Dunes	2	5	G14-06-CL2-L5	-13.1		45.2		-3.9		1.5	
184	Dunes	2	7	G14-06-CL2-L7	-12.7		44.7		-3.7		1.3	0.0
184	Dunes	2	8	G14-06-CL2-L8	-12.6		43.3		-3.9		1.9	
184	Dunes	3	1	G14-06-CL3-L1	-13.2	0.0	45.0	0.4	-5.7	0.1	1.5	
184	Dunes	3	3	G14-06-CL3-L3	-13.7	0.0	45.8	0.1	-5.3		1.6	
184	Dunes	3	5	G14-06-CL3-L5	-13.4	0.0	47.0	0.3	-4.0		1.3	
184	Dunes	3	7	G14-06-CL3-L7	-13.3		45.9		-4.4		1.5	
184	Dunes	3	9	G14-06-CL3-L9	-12.8		43.7		-3.8		1.4	
213	Dunes	1	1	G14-07-CL1-L1	-13.8		45.6		-4.5		1.2	
213	Dunes	1	3	G14-07-CL1-L3	-13.6		46.8		-4.0		1.2	
213	Dunes	1	5	G14-07-CL1-L5	-13.6		46.0		-3.6		1.2	
213	Dunes	1	7	G14-07-CL1-L7	-13.3		46.6		-3.3		1.4	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
213	Dunes	1	9	G14-07-CL1-L9	-13.0		46.8		-3.0		1.5	
213	Dunes	2	1	G14-07-CL2-L1	-13.8		46.0		-4.5		1.0	
213	Dunes	2	3	G14-07-CL2-L3	-13.9		45.5		-3.8		1.3	
213	Dunes	2	5	G14-07-CL2-L5	-13.7		46.6		-3.6		1.3	
213	Dunes	2	7	G14-07-CL2-L7	-13.5	0.0	46.2	0.1	-3.4		1.2	
213	Dunes	2	9	G14-07-CL2-L9	-13.1		45.4		-3.3		1.0	
213	Dunes	3	1	G14-07-CL3-L1	-13.8		46.7		-5.7		0.9	
213	Dunes	3	3	G14-07-CL3-L3	-14.0		45.4		-5.1		1.3	
213	Dunes	3	5	G14-07-CL3-L5	-13.8		46.5		-5.0		1.3	
213	Dunes	3	7	G14-07-CL3-L7	-13.8		40.8		-3.9		1.3	
213	Dunes	3	9	G14-07-CL3-L9	-13.8		47.0		-3.6		1.4	
248	Dunes	1	1	G14-08-CL1-L1	-14.3		46.2		-6.6		0.7	
248	Dunes	1	3	G14-08-CL1-L3	-14.5		46.5		-6.4		1.6	
248	Dunes	1	5	G14-08-CL1-L5	-14.4		46.2		-5.2		1.8	
248	Dunes	1	7	G14-08-CL1-L7	-14.1		46.2		-4.3		1.8	
248	Dunes	1	9	G14-08-CL1-L9	-13.9		46.4		-4.1		1.9	
248	Dunes	2	1	G14-08-CL2-L1	-14.0		46.0		-5.8		0.4	
248	Dunes	2	3	G14-08-CL2-L3	-14.1		46.4		-5.6		1.3	
248	Dunes	2	5	G14-08-CL2-L5	-14.1		46.4		-5.4		1.3	
248	Dunes	2	7	G14-08-CL2-L7	-13.9	0.1	45.9	0.5	-5.2		1.4	
248	Dunes	2	9	G14-08-CL2-L9	-13.8		45.9		-5.1		1.5	
248	Dunes	3	1	G14-08-CL3-L1	-14.0		47.0		-6.2		0.4	0.0
248	Dunes	3	3	G14-08-CL3-L3	-14.2		47.0		-6.1		1.3	
248	Dunes	3	5	G14-08-CL3-L5	-14.3		47.2		-5.9	0.1	1.3	
248	Dunes	3	7	G14-08-CL3-L7	-14.0		47.3		-5.7		1.4	
274	Dunes	1	1	G14-09-CL1-L1	-14.2		45.8		-3.6		0.6	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
274	Dunes	1	3	G14-09-CL1-L3	-14.2		-		-3.2		1.0	
274	Dunes	1	5	G14-09-CL1-L5	-14.3		-		-2.6		1.5	
274	Dunes	1	7	G14-09-CL1-L7	-14.1		46.0		-2.4		1.6	
274	Dunes	2	1	G14-09-CL2-L1	-14.1		46.9		-4.6		0.5	
274	Dunes	2	3	G14-09-CL2-L3	-14.4		47.1		-3.6		1.1	
274	Dunes	2	5	G14-09-CL2-L5	-14.4		46.8		-3.2		1.1	
274	Dunes	2	7	G14-09-CL2-L7	-14.1		45.9		-2.9		1.1	
274	Dunes	3	1	G14-09-CL3-L1	-14.4	0.0	41.8	4.6				0.0
274	Dunes	3	3	G14-09-CL3-L3	-14.2		46.3		-3.3		1.1	
274	Dunes	3	5	G14-09-CL3-L5	-14.2		45.3		-3.3	0.0	1.4	
274	Dunes	3	7	G14-09-CL3-L7	-14.1		46.3		-4.0		0.9	
307	Dunes	1	1	G14-10-CL1-L1	-14.1		45.8		-3.9		0.5	
307	Dunes	1	3	G14-10-CL1-L3	-13.7		44.7		-3.8		0.6	
307	Dunes	1	5	G14-10-CL1-L5	-13.7	0.1	44.7	1.1	-3.8		0.5	
307	Dunes	1	7	G14-10-CL1-L7	-13.7		-		-3.8		0.7	
307	Dunes	2	1	G14-10-CL2-L1	-14.1		46.4		-5.4		0.5	
307	Dunes	2	3	G14-10-CL2-L3	-13.9		46.1		-5.5		0.5	
307	Dunes	2	5	G14-10-CL2-L5	-13.7		46.5		-5.5		0.5	
307	Dunes	2	7	G14-10-CL2-L7	-13.5		45.6		-4.9		0.4	
307	Dunes	3	1	G14-10-CL3-L1	-13.9		46.7		-3.8		0.5	
307	Dunes	3	3	G14-10-CL3-L3	-13.8		47.0		-3.9		0.4	
307	Dunes	3	5	G14-10-CL3-L5	-13.6		46.6		-3.2		0.5	
307	Dunes	3	7	G14-10-CL3-L7	-13.7		46.0		-2.8		0.6	
213	Canopy Cover	4	1	G14-07-CL4-S2-L1	-14.2	0.0	46.3	0.2	-1.6		1.0	0.0
213	Canopy Cover	4	3	G14-07-CL4-S1-L3	-14.2		46.9		-0.9		1.7	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
213	Canopy Cover	4	5	G14-07-CL4-S1-L5	-13.9		46.0		-0.3	0.1	1.7	
213	Canopy Cover	4	7	G14-07-CL4-S1-L7	-13.7		46.0		0.0		1.4	
213	Canopy Cover	4	9	G14-07-CL4-S1-L9	-13.4		45.9		0.5		1.9	
213	Canopy Cover	5	1	G14-07-CL5-S1-L1	-14.4		46.3		-4.1		1.3	
213	Canopy Cover	5	3	G14-07-CL5-S1-L3	-14.4		46.8		-3.3		1.9	
213	Canopy Cover	5	5	G14-07-CL5-S1-L5	-14.3		44.9		-2.8		2.0	
213	Canopy Cover	5	7	G14-07-CL5-S1-L7	-14.1		46.8		-2.4		1.9	
213	Canopy Cover	5	9	G14-07-CL5-S1-L9	-13.9		47.0		-2.2		1.9	
213	Canopy Cover	6	1	G14-07-CL6-S1-L1	-14.3		45.3		-0.8		1.8	
213	Canopy Cover	6	3	G14-07-CL6-S2-L3	-14.1	0.0	46.6	0.5	-0.1		1.8	
213	Canopy Cover	6	5	G14-07-CL6-L5	-13.9		47.0		0.3		1.9	
248	Canopy Cover	4	1	G14-08-CL4-L1	-14.6		47.0		-1.2		1.0	
248	Canopy Cover	4	3	G14-08-CL4-L3	-14.3		47.2		-0.8		1.2	
248	Canopy Cover	4	5	G14-08-CL4-L5	-14.0		47.5		-1.1		1.3	
248	Canopy Cover	4	7	G14-08-CL4-L7	-13.7		47.9		-2.0		1.4	
248	Canopy Cover	4	9	G14-08-CL4-L9	-12.9		45.6		0.2		1.3	
248	Canopy Cover	5	1	G14-08-CL5-L1	-14.8	0.2	46.6	0.6	-2.8		1.4	0.0
248	Canopy Cover	5	3	G14-08-CL5-L3	-		-		-2.5		1.5	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
248	Canopy Cover	5	5	G14-08-CL5-L5	-13.8		46.1		-2.2	0.1	1.3	
248	Canopy Cover	5	7	G14-08-CL5-L7	-13.2		45.7		-1.9		1.4	
248	Canopy Cover	6	1	G14-08-CL6-L1	-14.5		44.6		-1.1		1.6	
248	Canopy Cover	6	3	G14-08-CL6-L3	-14.4	0.0	46.2	0.2	-0.9		1.5	
248	Canopy Cover	6	5	G14-08-CL6-L5	-14.2		45.1		-0.6		1.6	
248	Canopy Cover	6	6	G14-08-CL6-L6	-14.1		45.7		-0.7		1.7	0.0
274	Canopy Cover	4	1	G14-09-CL4-L1	-14.5		49.2		-3.4		0.5	
274	Canopy Cover	4	3	G14-09-CL4-L3	-14.4		49.6		-3.0	0.0	0.6	
274	Canopy Cover	4	5	G14-09-CL4-L5	-14.2		48.3		-2.4		0.6	
274	Canopy Cover	5	1	G14-09-CL5-L1	-14.4		48.7		-4.3		0.6	
274	Canopy Cover	5	3	G14-09-CL5-L3	-14.3		48.7		-3.6		0.6	
274	Canopy Cover	5	5	G14-09-CL5-L5	-14.3		48.2		-3.1		0.6	
274	Canopy Cover	6	1	G14-09-CL6-L1	-14.8		47.4		-2.5		1.4	
274	Canopy Cover	6	3	G14-09-CL6-L3	-14.6	0.1	47.9	0.0	-2.0		1.4	
274	Canopy Cover	6	5	G14-09-CL6-L5	-14.4		48.0		-1.5		1.6	
307	Canopy Cover	4	1	G14-10-CL4-L1	-14.0		-		-0.5		1.0	0.0
307	Canopy Cover	4	3	G14-10-CL4-L3	-13.8		-		-0.3		0.8	
307	Canopy Cover	4	5	G14-10-CL4-L5	-13.9		-		-0.4	0.0	0.8	

Day on Julian Calendar[1]	Site	Plant Number[2]	Leaf Number[3]	Sample ID	δ^{13}C (‰, VPDV)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
					Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
307	Canopy Cover	4	6	G14-10-CL4-L6	-14.0		46.8		0.0		0.8	
307	Canopy Cover	5	1	G14-10-CL5-L1	-14.2		-		-2.2		0.6	
307	Canopy Cover	5	3	G14-10-CL5-L3	-14.1		-		-1.3		0.8	
307	Canopy Cover	5	7	G14-10-CL5-L7	-13.9		-		-0.7		0.8	
307	Canopy Cover	6	1	G14-10-CL6-L1	-14.4		-		-1.8		0.8	
307	Canopy Cover	6	3	G14-10-CL6-L3	-14.0		-		-0.9		0.9	
307	Canopy Cover	6	5	G14-10-CL6-L5	-14.0	0.1	-		-1.0		0.7	

[1] Day on Julian Calendar : date that plants were collected, e.g., 122 represents plants collected on May 2nd, 2014 for April, 154 represents plants collected on June 3rd, 2014 for May, 184 represents plants collected on July 3rd, 2014 for June, 213 represents plants collected on August 1st, 2014 for July, 248 represents plants collected on September 5th, 2014 for August, 274 represents plants collected on October 1st, 2014 for September, 307 represents plants collected on November 3rd, 2014 for October

[2] Plant No.: plant from which the leaf was collected

3 Leaf No: assigned leaf number with the oldest leaf labelled as leaf 1

Appendix D: Data for all samples of *A. breviligulata* and *C. longifolia* plants collected annually from 2006 to 2014 at Pinery Provincial Park.

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
A. breviligulata - Dune											
2006	1	1	G06-AB1-L1	-25.6		46.6		-2.1		0.6	
2006	1	3	G06-AB1-L3	-27.2		46.9		-1.7		0.5	
2006	1	5	G06-AB1-L5	-25.6	0.0	48.3	0.4	-1.2		0.5	
2006	1	7	G06-AB1-L7	-25.1		48.0		-1.1		0.5	
2006	2	1	G06-AB2-L1	-26.1		47.6		-2.1		0.5	
2006	2	3	G06-AB2-L3	-25.5		48.4		-2.2		0.4	
2006	2	5	G06-AB2-L5	-25.3		47.9		-2.4		0.4	
2006	2	7	G06-AB2-L7	-25.7		46.4		-5.7		0.4	
2006	3	1	G06-AB3-L1	-25.0		46.7		-2.3		0.5	
2006	3	3	G06-AB3-L3	-26.2		46.6		-2.1		0.6	
2006	3	5	G06-AB3-L5	-26.6		47.8		-2.2		0.4	
2006	3	7	G06-AB3-L7	-25.4		48.6		-1.6		0.4	
2006	3	9	G06-AB3-L9	-25.5		47.3		-1.8		0.3	
2007	1	2	G07-AB1-L2	-26.4		45.6		-0.9		0.3	
2007	1	3	G07-AB1-L3	-26.2		45.9		1.1		0.3	
2007	1	4	G07-AB1-L4	-25.8				0.2		0.4	
2007	2	1	G07-AB2-L1	-26.9		45.6		0.2		0.4	
2007	2	3	G07-AB2-L3	-26.4		47.0		1.2	0.0	0.3	0.2
2007	2	5	G07-AB2-L5	-26.1		45.4		1.5		0.3	
2007	3	1	G07-AB3-L1	-27.5		43.7		-0.8		0.3	
2007	3	3	G07-AB3-L3	-26.5		43.2		-0.3		0.5	
2007	3	5	G07-AB3-L5	-25.7		44.7		0.3		0.4	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2008	1	1	G08-AB1-L1	-27.1		45.3		-2.0		0.3	
2008	1	3	G08-AB1-L3	-27.1		45.3		-1.4		0.4	
2008	1	4	G08-AB1-L4	-27.4		46.1		-1.8		0.4	
2008	2	3	G08-AB2-L3	-28.2		44.8		-0.5		0.5	
2008	2	4	G08-AB2-L4	-27.3		46.5		0.4		0.4	
2008	2	5	G08-AB2-L5	-25.6		46.9		0.2		0.4	
2008	3	1	G08-AB3-L1	-26.9		45.6		-2.9		0.4	
2008	3	2	G08-AB3-L2	-26.1		45.8		-2.3		0.3	
2008	3	3	G08-AB3-L3	-25.8		45.6		-2.9		0.5	
2009	1	4	G14-09-AB1-L4	-26.7		47.2		1.5	0.0	0.4	0.2
2009	1	8	G14-09-AB1-L8	-26.5		48.2		0.4		0.5	
2009	1	9	G14-09-AB1-L9	-25.6	0.0	47.4	1.1	1.7		0.4	
2009	2	1	G09-AB2-L1	-25.0		46.0		-1.8		0.4	
2009	2	3	G09-AB2-L3	-25.9		46.8		1.5		0.4	
2009	2	5	G09-AB2-L5	-26.4		47.5		1.3		0.6	
2009	2	7	G09-AB2-L7	-25.7		47.7		1.8		0.9	
2009	3	1	G09-AB3-L1	-26.9		47.1		-2.0		0.3	
2009	3	9	G09-AB3-L7	-26.1		45.9		0.2		0.3	
2009	3	9	G09-AB3-L9	-25.7		47.5		0.4		0.3	
2009	3	11	G09-AB3-L11	-25.8		47.8		0.3		0.3	
2010	1	1	G10-AB1-L1	-26.4		46.3		-0.7		0.3	
2010	1	3	G10-AB1-L3	-25.8		46.7		-0.9		0.3	
2010	1	4	G10-AB1-L4	-25.8		46.2		-0.8	0.0	0.4	0.2
2010	1	5	G10-AB1-L5	-26.9		45.3		-1.3	0.0	0.6	0.3
2010	2	1	G10-AB2-L1	-26.9		47.5		0.2		0.3	
2010	2	3	G10-AB2-L3	-26.1		47.6		0.3		0.3	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2010	2	4	G10-AB2-L4	-26.3		48.2		0.0		0.3	
2010	3	1	G10-AB3-L1	-25.5				0.5	0.0	0.3	0.1
2010	3	2	G10-AB3-L2	-25.5		47.0		0.4	0.1	0.3	0.1
2010	3	3	G10-AB3-L3	-25.7		46.9		0.3		0.3	
2010	3	4	G10-AB3-L4	-26.3		46.3		0.2		0.4	
2013	1	1	G13-AB1-L1	-27.2		45.2		0.3		0.3	
2013	1	2	G13-AB1-L2	-26.7		45.2		0.4		0.3	
2013	1	3	G13-AB1-L3	-26.3		44.9		-0.4		0.4	
2013	2	1	G13-AB2-L1	-27.0		45.8		-0.8		0.4	
2013	2	2	G13-AB2-L2	-27.1	0.0	46.5	0.1				
2013	2	3	G13-AB2-L3	-26.8		45.5		-2.4		0.5	
2013	3	1	G13-AB3-L1	-26.6		45.3		0.4		0.4	
2013	3	2	G13-AB3-L2	-26.2		43.6		-0.1		0.6	
2014	1	1	G14-10-AB1-L1	-26.5				0.1		0.3	
2014	1	3	G14-10-AB1-L3	-25.9				0.2		0.3	
2014	1	4	G14-10-AB1-L4	-25.7				0.0		0.4	
2014	2	1	G14-10-AB2-L1	-26.6				0.0		0.3	
2014	2	3	G14-10-AB2-L3	-25.9				0.1		0.3	
2014	2	5	G14-10-AB2-L5	-25.6				-0.7		0.4	
2014	3	1	G14-10-AB3-L1	-26.9				0.2		0.3	
2014	3	3	G14-10-AB3-L3	-26.2	0.0		0.2	0.5		0.8	
2014	3	5	G14-10-AB3-L5	-25.1				-0.5		0.5	
C. longifolia- Dunes											
2006	1	3	G06-CL1-L3	-13.8		47.7		-5.2		0.6	
2006	1	5	G06-CL1-L5	-14.2		48.7		-4.5		0.7	
2006	1	7	G06-CL1-L7	-14.1		46.0		-4.3		0.8	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2006	1	9	G06-CL1-L9	-14.1				-4.2		1.0	
2006	2	1	G06-CL2-L1	-13.2		44.0		-4.7		0.6	
2006	2	3	G06-CL2-L3	-14.1		45.4		-3.9		0.5	
2006	2	5	G06-CL2-L5	-13.8		48.2		-3.3		0.6	
2006	2	7	G06-CL2-L7	-14.2		47.5		-3.3		0.7	
2006	3	1	G06-CL3-L1	-13.4	0.1	47.5	0.1	-5.2		0.5	
2006	3	4	G06-CL3-L4	-13.9		47.1		-5.0		0.5	
2006	3	5	G06-CL3-L5	-14.2		47.9		-4.8	0.0	0.6	0.3
2006	3	7	G06-CL3-L7	-14.0		46.5		-4.9		0.5	
2006	3	9	G06-CL3-L9	-13.9		48.0		-4.9		0.5	
2007	1	1	G07-CL1-L1	-14.4	0.1	47.0	0.3	-6.2		0.5	
2007	1	3	G07-CL1-L3	-14.8		46.8		-5.2		0.5	
2007	1	5	G07-CL1-L5	-15.0		46.6		-4.9		0.6	
2007	1	7	G07-CL1-L7	-14.5		45.5		-5.2		0.6	
2007	1	9	G07-CL1-L9	-14.2		45.3		-4.6		0.5	
2007	2	1	G07-CL2-L1	-13.6		46.1		-6.0		0.5	
2007	2	3	G07-CL2-L3	-14.6		47.1		-5.8	0.1	0.5	0.3
2007	2	5	G07-CL2-L5	-14.8		46.3		-5.8		0.8	
2007	2	7	G07-CL2-L7	-14.8	0.1	46.6	0.8	-5.7		0.5	
2007	2	9	G07-CL2-L9	-14.4		46.8		-5.6	0.1	0.5	0.3
2007	2	11	G07-CL2-L11	-14.1	0.1	46.6		-5.0		0.6	
2007	3	1	G07-CL3-L1	-13.1		46.8		-4.8		0.9	
2007	3	3	G07-CL3-L3	-14.2		48.0		-4.2		1.0	
2007	3	5	G07-CL3-L5	-14.7		46.8		-4.5	0.0	0.7	0.3
2007	3	7	G07-CL3-L7	-14.9		47.4		-5.8		1.1	
2007	3	9	G07-CL3-L9	-14.7		48.7		-4.9		1.0	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2007	3	11	G07-CL3-L11	-14.4		47.0		-6.0		0.9	
2007	3	13	G07-CL3-L13	-14.0		47.7					
2008	1	1	G08-CL1-L1	-14.2		46.5		-6.9	0.1	0.6	0.3
2008	1	3	G08-CL1-L3	-14.2		46.5		-5.3		0.7	
2008	1	5	G08-CL1-L5	-13.9		46.2		-5.5		0.8	
2008	1	7	G08-CL1-L7	-13.8	0.1	45.9	0.2	-5.7		0.8	
2008	1	9	G08-CL1-L9	-13.6		45.2		-5.1		0.9	
2008	2	1	G08-CL2-L1	-13.5		46.7		-6.9		0.8	
2008	2	3	G08-CL2-L3	-13.8		45.8		-5.8		0.6	
2008	2	5	G08-CL2-L5	-14.0		47.1		-5.9		0.7	
2008	2	7	G08-CL2-L7	-13.9		46.6		-5.5		0.7	
2008	2	9	G08-CL2-L9	-13.8		46.5		-5.3		0.7	
2008	3	2	G08-CL3-L2	-14.2		46.4		-5.1		0.6	
2008	3	3	G08-CL3-L3	-13.8		45.4		-5.3		0.7	
2008	3	4	G08-CL3-L4	-13.7		46.4		-5.3		0.7	
2008	3	5	G08-CL3-L5	-13.6		45.8		-4.9		0.7	
2008	3	6	G08-CL3-L6	-13.7		45.7		-4.7		0.8	
2008	3	9	G08-CL3-L9	-14.0		48.4					
2010	1	1	G10-CL1-L1	-14.8		44.2		-4.5		0.7	
2010	1	3	G10-CL1-L3	-14.6		45.3		-4.4		0.8	
2010	1	5	G10-CL1-L5	-14.3		45.1		-4.1		0.5	
2010	1	7	G10-CL1-L7	-14.3		45.4		-3.8		0.6	
2010	2	1	G10-CL2-L1	-13.5		47.0		-3.6		0.4	
2010	2	3	G10-CL2-L3	-13.8		46.4		-5.1	0.1	0.5	0.2
2010	2	5	G10-CL2-L5	-13.8		48.7		-4.2		0.7	
2010	2	7	G10-CL2-L7	-13.6		45.6		-2.6		0.5	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2010	3	1	G10-CL3-L1	-14.1		46.7		-4.1		0.9	
2010	3	3	G10-CL3-L3	-13.8	0.0	47.3	0.5	-4.3		0.5	
2010	3	5	G10-CL3-L5	-13.6		47.6		-4.5	0.0	0.7	0.4
2010	3	7	G10-CL3-L7	-13.8		45.6		-3.2		0.9	
2011	1	1	G11-CL1-L1	-13.2		48.3		-6.1		0.9	
2011	1	3	G11-CL1-L3	-13.5		45.6		-6.4		0.7	
2011	1	5	G11-CL1-L5	-14.1	0.1	48.2	0.0	-4.6		0.7	
2011	1	7	G11-CL1-L7	-13.9		47.9		-4.6	0.1	1.3	0.1
2011	1	9	G11-CL1-L9	-13.8		47.8		-4.6		0.5	
2011	1	11	G11-CL1-L11	-13.6		45.9		-4.6		0.3	
2011	2	1	G11-CL2-L1	-14.0		47.4		-4.4		0.7	
2011	2	3	G11-CL2-L3	-13.7		47.2		-4.5		0.7	
2011	2	5	G11-CL2-L5	-13.8		45.8		-4.2		0.8	
2011	2	7	G11-CL2-L7	-13.6	0.0	47.1	0.3	-4.3		0.6	
2011	2	9	G11-CL2-L9	-13.6		46.8		-2.6		1.2	
2011	3	1	G11-CL3-L1	-14.0		47.6		-4.9		0.7	
2011	3	3	G11-CL3-L3	-13.9		48.5		-4.5		0.7	
2011	3	7	G11-CL3-L7	-13.7		46.9		-4.8		0.5	
2012	1	1	G12-CL1-L1	-14.5	0.0	47.1	0.3	-4.3		0.6	
2012	1	3	G12-CL1-L3	-14.4		46.9		-3.4		0.6	
2012	1	5	G12-CL1-L5	-14.2		46.6		-3.0		0.7	
2012	1	7	G12-CL1-L7	-14.2		46.1		-2.4		0.8	
2012	1	9	G12-CL1-L9	-13.9		46.4		-1.9	0.2	1.3	0.7
2012	2	1	G12-CL2-L1	-14.4		46.7		-3.8		0.6	
2012	2	3	G12-CL2-L3	-14.0		46.9		-3.4		0.5	
2012	2	5	G12-CL2-L5	-14.1		43.9		-3.1		0.5	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2012	2	7	G12-CL2-L7	-14.0		46.1		-3.5		0.5	
2012	2	8	G12-CL2-L8	-14.1		45.2		-2.9		0.5	
2012	3	1	G12-CL3-L1	-14.6		47.2		-4.0		0.6	
2012	3	3	G12-CL3-L3	-14.6		47.6		-3.7		0.7	
2012	3	5	G12-CL3-L5	-14.2		47.5		-3.2		0.8	
2012	3	6	G12-CL3-L6	-14.5				-3.1		0.6	
2012	3	8	G12-CL3-L8	-14.2		46.1		-3.3		0.6	
2013	1	1	G13-CL1-L1	-14.0		45.4		-5.4		0.6	
2013	1	3	G13-CL1-L3	-14.2	0.0	45.3	0.6	-5.3		0.6	
2013	1	5	G13-CL1-L5	-14.0		45.0		-5.2		0.5	
2013	1	6	G13-CL1-L6	-14.0		44.6		-5.0		0.5	
2013	1	7	G13-CL1-L7	-14.0		46.0					
2013	2	1	G13-CL2-L1	-13.8		46.3		-7.1		0.6	
2013	2	3	G13-CL2-L3	-13.8		45.3		-7.1	0.0	0.7	0.4
2013	2	5	G13-CL2-L5	-13.7		45.2		-6.6	0.1	0.6	0.3
2013	2	7	G13-CL2-L7	-13.5		44.7		-6.0		0.5	
2013	3	1	G13-CL3-L1	-14.4		45.5		-4.9	0.1	0.9	0.5
2013	3	3	G13-CL3-L3	-14.2		45.6		-3.6	0.1	0.7	0.4
2013	3	5	G13-CL3-L5	-14.0		45.2		-4.7		0.7	
2013	3	7	G13-CL3-L7	-13.9		43.9		-4.8	0.0	0.6	0.3
2014	1	1	G14-10-CL1-L1	-14.1		47.3		-3.9		0.6	
2014	1	3	G14-10-CL1-L3	-13.7		46.2		-3.8		0.6	
2014	1	5	G14-10-CL1-L5	-13.7	0.1	46.2	1.1	-3.8		0.6	
2014	1	7	G14-10-CL1-L7	-13.7				-3.8		0.8	
2014	2	1	G14-10-CL2-L1	-14.1		47.9		-5.4		0.5	
2014	2	3	G14-10-CL2-L3	-13.9		47.5		-5.5		0.5	

Year	Plant No.[1]	Leaf Number[2]	Sample ID	δ^{13}C (‰, VPDB)		C (wt. %)		δ^{15}N (‰, AIR)		N (wt. %)	
				Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev
2014	2	5	G14-10-CL2-L5	-13.7		47.9		-5.5		0.6	
2014	2	7	G14-10-CL2-L7	-13.5		47.1		-4.9		0.5	
2014	3	1	G14-10-CL3-L1	-13.9		48.1		-3.8		0.6	
2014	3	3	G14-10-CL3-L3	-13.8		48.5		-3.9		0.5	
2014	3	5	G14-10-CL3-L5	-13.6		48.1		-3.2		0.5	
2014	3	7	G14-10-CL3-L7	-13.7		47.5		-2.8		0.6	

[2] Plant No.: plant from which the leaf was collected
[3] Leaf No: assigned leaf number with the oldest leaf labelled as leaf 1

Appendix E: Monthly April to September weather data for Thedford, Ontario from 2006 to 2014. Data was obtained from http://www.theweathernetwork.com.

Year	Month	Total Precipitation (mm)	Mean Daily Temperature (°C)	Mean Daily Maximum Temperature (°C)	Mean Daily Minimum Temperature (°C)
2006	April	77	8.8	15.0	2.5
	May	68	14.5	20.0	8.9
	June	55	18.0	23.7	12.4
	July	82	22.5	27.9	17.1
	August	84	20.3	25.7	14.8
	September	85	15.5	19.9	10.9
2007	April	44	6.8	11.7	1.9
	May	72	14.6	21.1	8.1
	June	25	19.9	26.9	13.0
	July	31	20.4	26.5	14.3
	August	71	20.9	26.5	15.3
	September	44	18.8	25.1	12.4
2008	April	28	9.9	16.2	3.6
	May	75	11.7	17.3	6.1
	June	126	20.1	25.4	14.8
	July	120	21.5	27.1	15.8
	August	107	19.5	25.0	13.9
	September	168	17.3	22.9	11.6
2009	April	122	8.1	13.7	2.5
	May	57	13.4	19.4	7.4
	June	106	16.8	22.1	11.5
	July	47	19.0	24.1	13.8
	August	69	20.4	25.5	15.1
	September	59	16.4	22.5	10.3
2010	April	63	10.7	16.7	4.7
	May	106	15.2	20.7	9.6
	June	60	19.3	24.7	13.9
	July	107	22.9	28.5	17.2
	August	10	22.9	29.0	16.8
	September	92	16.9	22.2	11.6
2011	April	89	6.6	11.6	1.5
	May	87	14.0	19.0	8.9
	June	69	18.3	23.4	13.1
	July	65	23.2	28.9	17.4
	August	131	20.9	26.1	15.7
	September	131	16.8	21.6	12.0
2012	April	42	6.9	12.1	1.7

	May	53	16.0	22.1	9.8
	June	75	20.3	26.4	14.1
	July	61	23.3	29.9	16.6
	August	49	21.1	27.3	14.9
	September	78	16.5	22.4	10.6
2013	April	171	6.2	11.3	1.1
	May	74	15.3	21.5	9.0
	June	99	18.7	23.7	13.7
	July	143	21.5	26.6	16.3
	August	39	19.7	25.3	14.1
	September	87	16.2	21.4	11.0
2014	April	62	6.4	12.4	0.5
	May	74	13.1	19.0	7.2
	June	30	18.7	30.3	12.8
	July	78	19.5	24.7	14.2
	August	77	19.6	24.5	14.7
	September	132	16.0	21.8	10.2

Appendix F: Daily weather parameters from April 1, 2014 to November 3, 2014 for Thedford Ontario used to calculate average monthly parameters for 2014. Data obtained from http://www.theweathernetwork.com.

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
April	1	0	8.9	16.5	1.4
April	2	0	1.9	5.6	-1.9
April	3	0	1.4	4.9	-2.1
April	4	5.1	6.0	10.4	1.7
April	5	0.2	1.8	6.8	-3.2
April	6	0	4.0	12.1	-4.2
April	7	6.4	2.2	6.5	-2.1
April	8	N/A	N/A	N/A	0.7
April	9	0	6.8	12.8	0.7
April	10	0	12.1	20	4.1
April	11	0	8.8	16.7	1.0
April	12	2.7	10.5	19.4	1.6
April	13	10.2	14.4	22.7	6.2
April	14	1.8	9.3	19.6	-1.0
April	15	1.0	-2.8	-0.8	-4.7
April	16	0	-0.8	5.5	-7.1
April	17	0	7.0	15.0	-1.0
April	18	1.5	4.1	7.2	1.0
April	19	0	3.5	7.0	0
April	20	0	10.1	21.3	-1.1
April	21	0.2	15.1	25.3	4.9
April	22	8.3	8.3	13.9	2.6
April	23	0.2	1.8	5.8	-2.3
April	24	0	3.5	10.9	-3.8
April	25	3.7	6.8	11.6	1.9
April	26	0	4.2	8.7	-0.3
April	27	0	3.8	7.6	0
April	28	0	9.3	14.8	3.8
April	29	20.9	13.1	17.5	8.6
April	30	3.4	11.2	13.2	9.2
May	1	0	10.6	13.6	7.6
May	2	3.0	10.2	13.6	6.7
May	3	6.1	9.8	13.7	6.0
May	4	1.0	6.3	10.9	1.6
May	5	0	5.2	10.2	0.1
May	6	0	6.1	10.1	2.1
May	7	0.2	9.3	16.2	2.5

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
May	8	0.2	17.8	26.6	8.9
May	9	3.2	20.5	26.0	15.0
May	10	0.2	14.7	21.7	7.7
May	11	0	14.4	24.2	4.4
May	12	10.4	18.4	22.9	14.0
May	13	10.4	22.6	28.7	16.5
May	14	7.6	11.3	16.6	6.1
May	15	16.3	12.2	18.0	6.4
May	16	0	5.5	8.5	2.5
May	17	0	8.8	13.0	4.6
May	18	0.2	11.4	18.8	4.1
May	19	0	15.6	21.9	9.2
May	20	2.4	8.8	17.9	4.6
May	21	7.6	16.5	22.9	10.1
May	22	0	11.7	17.0	6.4
May	23	0.2	9.4	14.2	4.7
May	24	0	11.3	16.3	6.4
May	25	0	17.5	26.9	8.1
May	26	0	21.6	29.2	13.9
May	27	5.1	19.9	28.1	11.7
May	28	0	N/A	N/A	N/A
May	29	0	12.3	16.7	7.8
May	30	0	15.3	23.0	7.6
May	31	0	16.6	23.6	9.5
June	1	0	19.9	28.5	11.2
June	2	2.7	23.3	29.3	17.2
June	3	0	20.1	27.3	13.0
June	4	0	15.1	192	10.9
June	5	0	12.7	18.3	7.1
June	6	0	15.6	25.1	6.1
June	7	0	17	25.5	8.4
June	8	0	14.1	17.4	10.7
June	9	0	14.6	18.7	10.4
June	10	0	14.3	18.3	10.4
June	11	2.3	20.1	26.5	13.8
June	12	0	22.8	27.3	18.3
June	13	0	15.8	21.0	10.7
June	14	0	11.9	15.6	8.3
June	15	0	16.3	26.1	6.4
June	16	0	24.1	30.8	17.4
June	17	0	24.3	32.7	15.8

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
June	18	12.6	19.4	24.9	14.0
June	19	0.4	15.2	19.1	11.3
June	20	0	16.4	23.1	9.6
June	21	0	18.3	23.3	13.3
June	22	0	19.4	26.7	12.0
June	23	0	20.4	28.0	12.9
June	24	10.7	20.9	25.4	16.4
June	25	0	16.3	18.6	13.9
June	26	0	18.3	22.0	14.5
June	27	0	19.9	26.2	13.7
June	28	0	22.9	31.6	14.3
June	29	1.6	25.8	30.4	21.2
June	30	0	25.9	30.7	21.2
July	1	9.7	24.9	29.1	20.8
July	2	0	21.4	26.9	16
July	3	0.8	15.2	17.6	12.7
July	4	0	16.2	20.9	11.5
July	5	0	18.3	26.7	9.9
July	6	0	21.4	28.1	14.8
July	7	26.6	24.1	29.2	18.9
July	8	11.5	21.1	24.9	17.2
July	9	0	17.3	20.7	13.9
July	10	0	17.4	21.8	13.0
July	11	0	18.7	25.7	11.7
July	12	4.9	22.1	29.1	15.0
July	13	0.6	23.0	28.8	17.2
July	14	2.0	20.9	25.8	16.1
July	15	1.1	18.8	22.5	15.1
July	16	0	14.6	17.4	11.8
July	17	0	17.9	23.3	12.5
July	18	0	18.0	24.8	11.2
July	19	0.5	17.9	21.1	14.6
July	20	0	19.3	23.8	14.8
July	21	0	21.1	29.0	13.2
July	22	0	23.6	31.0	16.2
July	23	0	21.4	26.6	16.3
July	24	0	16.4	21.4	11.5
July	25	0	17.4	25.4	9.5
July	26	0	22.0	27.9	16.1
July	27	7.3	23.1	29.1	17.1
July	28	7.0	15.8	17.8	13.9

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
July	29	0.4	16.9	21.7	12.0
July	30	0	17.4	21.8	12.9
July	31	5.4	18.9	25.7	12.0
August	1	9.9	20.9	27.7	14.0
August	2	0.3	19.9	24.5	15.2
August	3	0	19.5	25.7	13.3
August	4	3.7	22.9	27.9	17.9
August	5	12.3	19.1	22.7	15.5
August	6	0	18.1	22.5	13.6
August	7	0	17.5	22.3	12.7
August	8	0	17.1	22.5	11.6
August	9	0	17.5	23.8	11.2
August	10	0	19.6	26	13.1
August	11	29.4	21.1	24.9	17.2
August	12	2.8	20.4	24.9	16.0
August	13	0	17.4	22.6	12.3
August	14	0	15.3	19.7	10.8
August	15	0	15.9	21.8	10.1
August	16	1.1	17.4	23.8	11.0
August	17	0	17.6	21.9	13.3
August	18	0	17.4	22.0	12.9
August	19	8.4	21.3	27.0	15.6
August	20	5.8	22.9	27.2	18.5
August	21	0	21.6	25.7	17.4
August	22	0	19.9	21.6	18.2
August	23	1.6	20.3	23.3	17.2
August	24	0	20.4	24.2	16.5
August	25	0	22.6	28.0	17.2
August	26	0	25.0	31.0	19.0
August	27	0	17.7	21.3	14.1
August	28	N/A	N/A	N/A	N/A
August	29	0	17.9	24.4	11.5
August	30	3.7	24.3	29.5	19.0
August	31	0	23.7	27.9	19.5
September	1	8	23.9	27.9	20.0
September	2	1.0	20.6	26.0	15.2
September	3	0	19.9	26.8	13
September	4	0	22.7	28.8	16.6
September	5	43.3	25.3	32.0	18.5
September	6	5.9	16.4	20.0	12.7
September	7	0	16.4	21.9	10.8

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
September	8	0	16.6	24.4	8.9
September	9	0	21.1	26.1	16.1
September	10	28.2	20.2	23.9	16.5
September	11	0	17.3	23.3	11.2
September	12	2.4	12.6	15.2	9.9
September	13	10.3	9.9	13.7	6.2
September	14	0	9.8	16.0	3.7
September	15	1.9	11.7	16.5	6.9
September	16	0	12.9	17.9	8.0
September	17	0	12.7	19.2	6.2
September	18	0	10.9	16.1	5.7
September	19	0	11.6	19.6	3.6
September	20	8.1	17.6	24.9	10.4
September	21	15.9	15.4	20.6	10.3
September	22	0	11.4	15.1	7.8
September	23	0	13.9	21.4	6.4
September	24	0	15.8	23.7	7.9
September	25	0	16.4	23.4	9.3
September	26	0	15.3	23.1	7.4
September	27	0	15.3	22.1	8.6
September	28	0	15.3	21.4	9.1
September	29	1.6	16.8	25.0	8.5
September	30	5.1	14.0	16.5	11.5
October	1	0	15.4	19.6	11.3
October	2	0.5	17.9	22.4	13.4
October	3	9.7	16.1	21.7	10.6
October	4	3.5	8.2	10.8	5.5
October	5	0	8.8	11.6	5.9
October	6	0.8	13.1	17.5	8.7
October	7	0.2	11.3	15.9	6.7
October	8	0	11.1	14.8	7.3
October	9	0	8.4	14.9	1.9
October	10	0	7.8	12.5	3.1
October	11	0	6.5	12.2	0.7
October	12	0	8.0	15.7	0.3
October	13	0.8	15.3	20.3	10.3
October	14	7.1	18.1	22.2	14.0
October	15	1.1	15.2	19.3	11.1
October	16	1.2	13.2	16.7	9.6
October	17	0.6	12.5	16.3	8.7
October	18	2.1	7.3	8.7	5.9

Month	Day	Daily Precipitation (mm)	Daily Mean Temperature (°C)	Daily Maximum Temperature (°C)	Daily Minimum Temperature (°C)
October	19	0	6.8	10.5	3.2
October	20	1.8	10.2	12.9	7.4
October	21	1.6	9.1	10.3	7.9
October	22	0	8.8	11.6	6.1
October	23	0	6.5	12.0	1.1
October	24	0	8.6	16.4	0.7
October	25	0	13.3	18.7	7.8
October	26	0	8.3	14.6	2.0
October	27	0	9.8	18.9	0.7
October	28	0	8.3	19.3	2.0
October	29	0	8.6	11.1	6.0
October	30	0	7.1	10.2	4.0
October	31	18.0	5.4	7.3	3.5
November	1	1.0	3.2	4.9	1.5
November	2	0.2	4.3	7.9	0.7
November	3	0	8.6	13.6	3.6

Appendix G: $\delta^{13}C$ of Atmospheric CO_2 collected on from the dunes and under canopy cover in Pinery Provincial Park.

	Dunes			Under Canopy Cover		
Sampling Date	**$\delta^{13}C$**	**Mean $\delta^{13}C$**	**Std. Dev.**	**$\delta^{13}C$**	**Mean $\delta^{13}C$**	**Std. Dev.**
June 3 2014	-8.51	-8.46	0.26	-8.94	-9.00	0.14
June 3 2014	-8.33			-8.87		
June 3 2014	-8.11			-9.23		
June 3 2014	-8.80			-8.94		
June 3 2014	-8.55			-9.00		
July 3 2014	-7.54	-7.77	0.13	-8.57	-8.08	0.28
July 3 2014	-7.83			-8.01		
July 3 2014	-7.79			-8.03		
July 3 2014	-7.87			-7.89		
July 3 2014	-7.82			-7.90		
Aug 1 2014	-7.03	-7.13	0.14	-7.27	-7.46	0.26
Aug 1 2014	-7.23			-7.78		
Aug 1 2014	-7.05			-7.68		
Aug 1 2014	-7.01			-7.36		
Aug 1 2014	-7.33			-7.18		
Sept 5 2014	-9.47	-9.34	0.23	-9.83	-9.07	0.57
Sept 5 2014	-9.39			-8.82		
Sept 5 2014	-8.94			-9.23		
Sept 5 2014	-9.37			-8.28		
Sept 5 2014	-9.51			-9.17		
Oct 1 2014	-8.38	-8.50	0.08	-9.22	-8.81	0.30
Oct 1 2014	-8.55			-8.99		
Oct 1 2014	-8.58			-8.48		
Oct 1 2014	-8.54			-8.77		
Oct 1 2014	-8.47			-8.57		
Nov 4 2014	-8.42	-8.40	0.09	-8.42	-8.34	0.08
Nov 4 2014	-8.41			-8.40		
Nov 4 2014	-8.38			-8.22		
Nov 4 2014	-8.52			-8.34		
Nov 4 2014	-8.27			-8.31		
Dec 1 2014	-9.08	-9.04	0.11	-8.70	-8.89	0.26
Dec 1 2014	-9.00			-8.74		
Dec 1 2014	-9.17			-8.81		
Dec 1 2014	-8.91			-9.34		
Dec 1 2014				-8.87		

Curriculum Vitae

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