Stable Isotopic Compositions of Silica Phytoliths and Plant Water in Grasses: Implications for the Study of Paleoclimate

by

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Abstract

Information regarding climatic conditions during plant growth is preserved by the oxygen-isotope composition of biogenic silica (phytoliths) deposited in grasses. The O-isotope composition of phytoliths is dependent on soil-water $\delta^{18}O$ values, relative humidity, evapotranspiration and temperature during plant growth. The compositions of plant water from several grass species at Pinery Provincial Park were examined to determine the variability in $\delta^{18}O$ and δD values from which biogenic silica may precipitate. Here, stem water was unfractionated from soil-water $\delta^{18}O$ values. Hence, the $\delta^{18}O$ values of stem silica can provide a proxy of the soil water available for root uptake during the growing season. Phytolith and plant-water $\delta^{18}O$ values for C3 (*Ammophila breviligulata*) and C4 (*Calamovilfa longifolia*) grasses from southwestern Ontario were also used to compare the isotopic fractionation between biogenic silica and water in various plant tissues. Temperatures calculated from $\delta^{18}O_{silica}$ and $\delta^{18}O_{plant\ water}$ values in non-transpiring tissues matched measured growing temperatures. The isotopic heterogeneity of water within individual leaves and the diurnal variations in $\delta^{18}O_{leaf\ water}$ values that result from transpiration processes complicate interpretations. Water within the sheath, and lower and upper leaf tissues experiences continual evaporation, hence becoming progressively enriched in ^{18}O and D as it moves towards the tip of the leaf. However, the water from which leaf silica precipitates has not acquired the extreme ^{18}O-enrichment predicted using steady-state models, or measured for mid-day leaf water. There may be a secluded water fraction within the leaf, which experiences smaller diurnal variations in isotopic composition than leaf water at sites of evaporation.

C. longifolia was collected across North America to investigate the effects of climate and soil-water $\delta^{18}O$ values on the oxygen-isotope composition of phytoliths. Phytoliths at all sites have a similar pattern of $\delta^{18}O$ values within an individual plant, but the isotopic separation between leaf and stem silica increases as relative humidity decreases. Once the phytoliths are transferred to the soil, the temperature and soil-water

$\delta^{18}O$ signals carried by the phytoliths from stems, sheaths and rhizomes may be masked by the $\delta^{18}O$ values of phytoliths from leaves and inflorescence, which are variably further enriched, depending on relative humidity. The difference between growing temperature and that calculated using measured $\delta^{18}O$ values for stem silica and local meteoric water became larger as relative humidity decreased, likely because of evaporative ^{18}O-enrichment of soil water.

Key words: stable isotopes, oxygen, hydrogen, silica, phytoliths, plant water, soil water, meteoric water, grasses, prairie, climate, evapotranspiration, Pinery Provincial Park, North America

Co-authorship

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Chapter 1. Introduction

1.1. OBJECTIVES

To understand the impact of human activity on the current trends in global climate change it is necessary to determine the amplitude of natural climatic fluctuations by studying past climate changes. For example, the historical climate record of the northern Great Plains appears to coincide with uncommonly favourable conditions. However, over the last 2000 years the natural fluctuation in temperature and amount of precipitation in this area has been greater than that experienced since human cultivation of these lands. In general, the climate was cooler and more humid but commonly experienced longer and more intense periods of drought than over the past century (Laird et al., 1996; Lemmen et al., 1997).

The development of proxy climate records is the only way to extend the relatively short historical climate record. Proxies are indirect measures of past climate based on paleoenvironmental reconstruction. Urey (1947) first demonstrated the thermodynamic properties of isotopic substances and the temperature dependence of isotopic fractionation between marine carbonates and the waters in which they formed. He showed that the isotopic composition of ancient materials could preserve information regarding the temperature of formation. Similar methods of paleoclimate reconstruction in the terrestrial environment are complicated by the wide range in isotopic composition of meteoric water, which is correlated with continental climatic variables such as temperature and amount of precipitation (Dansgaard, 1964; Rozanski et al., 1993). Successful reconstruction of terrestrial paleoclimate has been achieved through the isotopic analysis of materials such as fossil cellulose (Yakir et al., 1994; Feng and Epstein, 1995), inland saline and fresh water diatoms (e.g. Laird et al., 1996), land snail carbonates (Yapp, 1979; Goodfriend, 1991), sagittal fish otoliths (Patterson, 1998), speleothems (Schwarcz,

1986), phosphates from mammalian bone and enamel (Luz et al., 1990; Fricke et al., 1998) and soil carbonates (Cerling and Quade, 1993).

The purpose of this study is to determine if the oxygen-isotope composition of biogenic silica phytoliths deposited in terrestrial plants can provide a useful record of the climatic conditions under which the plants grew. In an equilibrium system the oxygen-isotope composition of plant silica should be dependent on the cumulative effects of temperature and plant-water $\delta^{18}O$ values over the growing season. If plant silica is formed in equilibrium with plant water, the size of the equilibrium oxygen isotopic fractionation between silica and water at any particular location in the plant will be controlled by temperature. In addition, the oxygen isotopic composition of phytoliths will be influenced by factors that determine the $\delta^{18}O$ values of plant water, such as relative humidity, transpiration rate, and the isotopic composition of the soil water that is supplied to the plant. The degree to which soil water can become enriched in ^{18}O with respect to precipitation through surface evaporation is dependent on relative humidity (Allison et al., 1984; Walker and Brunel, 1990). Relative humidity can also affect the degree to which water in the leaves and other transpiring tissues becomes enriched in ^{18}O and D by equilibrium and kinetic fractionation associated with transpiration (Flanagan and Ehleringer, 1991; Farquhar and Lloyd, 1993).

An understanding of the variations in biogenic silica $\delta^{18}O$ values that occur under known, natural climatic conditions is a prerequisite to interpretation of such data for ancient soils and sediments. The purpose of this thesis is to evaluate the systematics and suitability of phytoliths as a paleoclimatic indicator by examining the effects of temperature, relative humidity and $\delta^{18}O_{soil\ water}$ values on the oxygen isotope composition of biogenic silica phytoliths in living grasses of the North American prairies. This study also examines the variability in $\delta^{18}O$ and δD values of the plant water from which biogenic silica precipitates, as this is a major control on the range of phytolith isotopic compositions within a single grass plant.

1.2. OUTLINE

The oxygen-isotope composition of silica phytoliths is acquired in equilibrium with plant water (Shahack-Gross et al., 1996). Accordingly, the $\delta^{18}O$ values of plant silica will depend on the cumulative effects of diurnal and seasonal changes in temperature and plant water $\delta^{18}O$ values over the growing season. In addition, phytolith compositions will be influenced by factors that determine the $\delta^{18}O$ values of plant water, such as relative humidity, transpiration rate, and the oxygen isotopic composition of the soil water that is supplied to the plant. Accordingly, phytoliths and plant waters were extracted from various tissues of two grass species in order to understand the relative effects of these variables on the $\delta^{18}O$ values of silica deposited throughout a plant. In particular, Chapter 2 compares the oxygen isotopic compositions of silica phytoliths from *Ammophila breviligulata* and *Calamovilfa longifolia* grown under field conditions at Pinery Provincial Park, and plant waters extracted from greenhouse grown samples of the same species.

Further research was then undertaken to investigate the relative influences of temperature, relative humidity and the $\delta^{18}O$ values of soil water on the oxygen-isotope composition of phytoliths over a wider range of climatic conditions. These results are presented in Chapter 3, which examines the variability in $\delta^{18}O$ values of silica phytoliths deposited in one species, *C. longifolia*, across the east-west and north-south span of the North American Great Plains.

Finally, to better understand the range in oxygen isotopic composition of phytoliths from a single grass plant, it was necessary to determine in more detail the variability in $\delta^{18}O$ and δD values of the water from which biogenic silica precipitates. These results are provided in Chapter 4, which describes the oxygen and hydrogen isotopic variability of water in the tissues of several grass species grown in Pinery Provincial Park and discusses the implications for phytolith $\delta^{18}O$ values.

1.3. BACKGROUND

The following section provides background information regarding the physiology of the grass species examined in this study, as well as a general discussion of water movement and silica deposition in plants. For readers that do not possess a biological background a glossary of biological terms used in this chapter is provided in Appendix A.

1.3.1. Native prairie grasses

Grasslands are the dominant vegetation type of the North American continent. They cover an area ~1000 kilometers wide between western Indiana and the foothills of the Rocky Mountains, and ~3800 kilometer long from Alberta, Saskatchewan and Manitoba to southern Texas (Barbour et al., 1980). The vegetation of the prairies has been greatly altered by man over the past century. However, for thousands of years prior to human cultivation of these lands, the dominant vegetation was composed of grasses, forbs and woody shrubs, with a notable absence of trees (Weaver, 1968). Prairie vegetation has evolved to withstand grazing, frequent fires and climatic conditions associated with long and short term droughts (Joern and Keeler, 1995). Herbaceous perennials, typical of prairie vegetation, have adapted to these stresses by developing underground storage/perennating structures and growing points slightly below ground level where they are protected from fire and grazing. Prairie grasses have also developed extensive deep rooting systems that are able to tap deeper supplies of soil moisture during periods of drought.

The variation in grassland vegetation throughout the prairies is sensitive to changes in climate. There is a significant east-west moisture gradient across the North American prairies. Precipitation ranges between 100 to 200 millimeters on the western margin of the prairies and 600 to1000 millimeters on the eastern boundary (Bragg, 1995; Joern and Keeler, 1995). Two-thirds of the annual precipitation occurs during the active growing season for grasses (April to September). The dry conditions, which occur in the

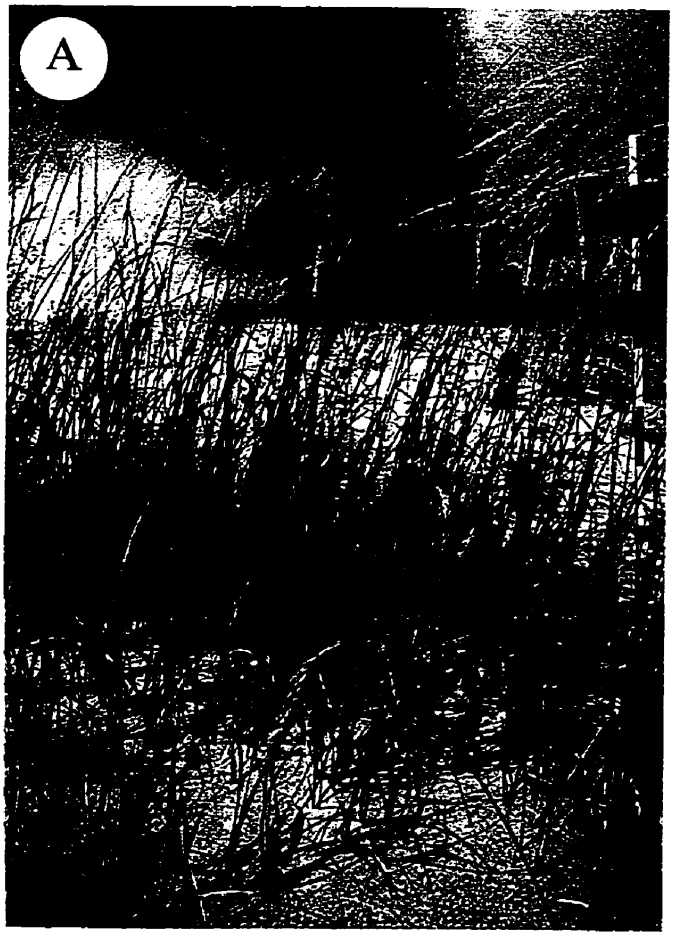
rain shadow created by the Western Cordillera on the western boundary of the Great Plains, support short-grass and desert grassland vegetation. An increase in available moisture on the eastern boundary of the Great Plains supports a greater production of biomass, and the vegetation here is predominated by tall-grass prairie. The central portion of the North American prairies lies between these two extremes, experiencing intermediate amounts of precipitation and supporting mixed or mid-grass prairie vegetation. Latitudinal gradients in the proportion of cool- versus warm-season grasses are driven by differences in temperature, season length, and maximum day length (Joern and Keeler, 1995). The dominant temperature gradient across the North America prairies increases in average July temperatures from northwest (20°C) to southeast (30° C; Bragg, 1995).

Two species, *Calamovilfa longifolia*, a C4 grass, and *Ammophila breviligulata*, a C3 grass, were selected for detailed study of the variations in the stable isotopic compositions of phytoliths and plant water. The following species descriptions are compiled from Hitchcock (1950), Phillips (1963), Weaver (1968) and Maun (1985).

C. longifolia, commonly known as sandreed grass, is a perennial, warm-season, tall grass with solitary culms (50 to 180 cm tall; Fig. 1-1a). Growth begins in early spring, and seed stalks appear from July to September. Leaf blades are firm, elongate and flat. They are 4 to 8 mm wide near the base tapering to a long fine point. The sheaths of *C. longifolia* are pressed closely to the stem and are covered with fine hairs. Panicles are 15 to 35 cm long, rather narrow but sometimes slightly spreading. This species produces strong, sharply pointed, scaly, creeping rhizomes. The roots of *C. longifolia* form a dense mat of wiry roots in the top meter of the soil. The roots extend laterally up to 1.5 meters and may reach a depth of 3 meters. *C. longifolia* can be found in sand hills, sandy prairies or open woods from Michigan to Alberta, south to Indiana, Colorado and Idaho.

A. breviligulata is commonly known as American beach grass or marram grass (Fig. 1-1b). The culms form in tufts, 70 to 100 cm tall, which are covered in broad

Figure 1-1. Photographs of A) *C. longifolia* growing on an unstabilized dune at Sprucewoods Provincial Park, Manitoba; and B) *A. breviligulata* growing on a stabilized slope on the southern shore of Lake Michigan. The measuring stick is marked in units of 20 cm.

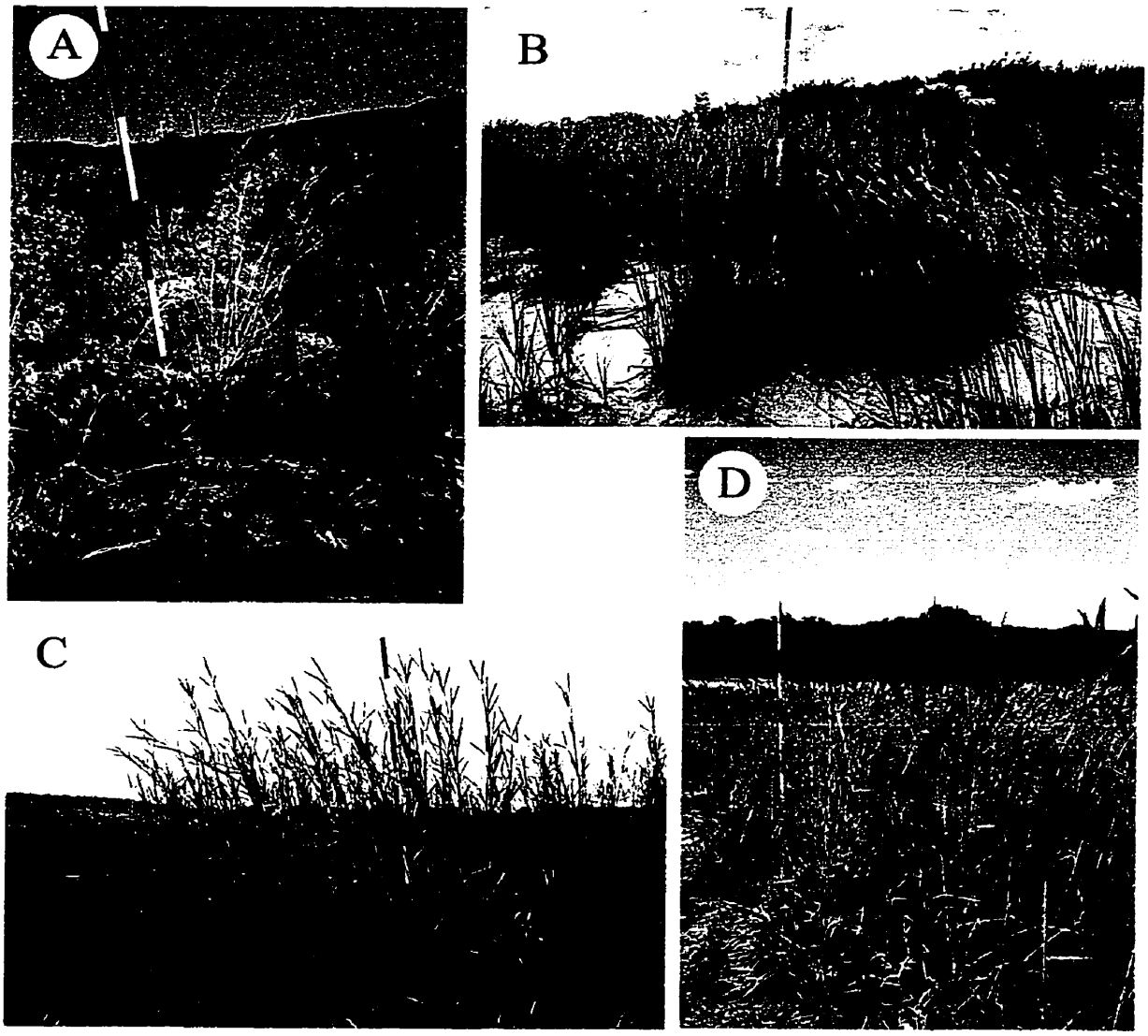

A


B

overlapping sheaths. Leaf blades are elongate, and may be flat or involute. *A. breviligulata* also has strong, deep, and laterally extensive creeping rhizomes, making it an important sand binding grass. The panicles are pale, dense, spike-like structures 15 to 30 centimeters long. *A. breviligulata* occurs on sand dunes along the Atlantic coast from Newfoundland to North Carolina and on the shores of the Great Lakes. Both *C. longifolia* and *A. breviligulata* grow vertically and increase in vigour in response to sand accretion and the associated increase in soil nutrient content (Maun, 1985).

Other grasses selected for isotopic analysis of plant water include the C3 grass, *Stipa spartea* and C4 grasses *Andropogon scoparius*, *Andropogon gerardi* and *Sorghastrum nutans*. The common name for *S. spartea* is porcupine grass. It is a native cool-season perennial grass (Fig. 1-2a). Growth begins in the late fall and plants may remain green all winter. Maximum growth occurs between late March and early June. Seed production occurs in late June and the grass remains dormant for the remaining summer months. This species grows approximately one meter tall. The leaves are 20 to 30 centimeters long and 3 to 5 millimeters wide. The panicles are narrow, nodding and may be up to 15 to 20 centimeters long. The root systems of *S. spartea* are finely branching and reach a depth of 1.2 meters. *A. scoparius*, also known as little bluestem, is a native, perennial warm-season grass of the mixed-prairie. The culms occur in tufted clumps with freely branched upper portions (Fig. 1-2b). Leaf blades are 3 to 6 millimeters wide and tend to fold. Plants of this species may be green, glaucous or purplish in colour and grow 50 to 150 centimeters tall. Flowers, 3 to 6 centimeters long, are covered with fine hairs or down and grow laterally from the axis of the inflorescence. The dense rooting system may reach 1.5 to 2.4 meters in depth and spread laterally 45 centimeters. *A. gerardi*, or big bluestem, is a common native, perennial, warm-season grass of the tall-grass prairie (Fig. 1-2c). Growth begins in early April. The culms are 1 to 2 meters tall and robust, often in large leafy clumps. The lower sheaths and blades may be covered in fine hairs. The leaf blades of *A. gerardi* are flat, elongate and 5 to 10

Figure 1-2. Photographs of A) *S. spartea* (yellow grass) growing in the Sandhills region of Nebraska; B) *A. scoparius* growing at Pinery Provincial Park, Ontario; C) *A. gerardi* growing at the USDA South Plains experimental Ranch, Fort Supply, Oklahoma; and D) *S. nutans*, growing on a seed farm in Fertile, Minnesota. The measuring stick is marked in units of 20 cm.


A
B
D
C

millimeters wide. The roots of *A. gerardi* saturate the top 60 centimeters of the soil, and may reach depths of up to 4 meters. *S. nutans* is a native, perennial warm season-grass of the tall-grass prairie, commonly referred to as Indian grass (Fig. 1-2d). Leaf blades are 10 to 30 centimeters long and 0.5 to 1 centimeters wide tapering to a narrow base. The dense, golden, plume-like inflorescence may grow 10 to 30 centimeters long. *S. nutans* has short scaly rhizomes. Culms of this species grow 1 to 2.5 meters tall, while rooting systems may reach a depth of 2.5 meters.

For the purpose of assessing the variation in isotopic composition of silica and water within different grass tissues, samples were divided into six parts (Fig. 1-3). The stem forms the central axis of the grass, bearing the leaves, buds and flowers and providing mechanical support to the plant. The inflorescence is a flowering structure, or panicle, that consists of more than one flower. Leaves are green, expanded organs generated from alternating nodes on either side of the stem. The leaf tissues are the sites where the majority of photosynthesis occurs. The base of the leaf blade that encloses the stem is known as the sheath. Sheaths surround the stem, serving to protect new growth and provide support to the thin stem. Underground biomass consists of both roots and rhizomes. The rhizomes, which usually persist from season to season, are horizontal, creeping, underground stems that bear roots and may produce new shoots. The plant is anchored by fibrous roots through which water and nutrients enter the plant.

1.3.2. Water movement in grasses

More than 90% of water entering a plant passes through and evaporates (Stern, 1985). Water travels into the roots along the path of least resistance along cell walls and between root cells to the endodermis. Here, water and dissolved materials are forced to move through the differentially permeable cell membrane of the endodermis. Resistance to water movement is much lower once the water enters the vascular cylinder and the xylem. From the vascular tissue, water travels in the liquid phase through the cell walls

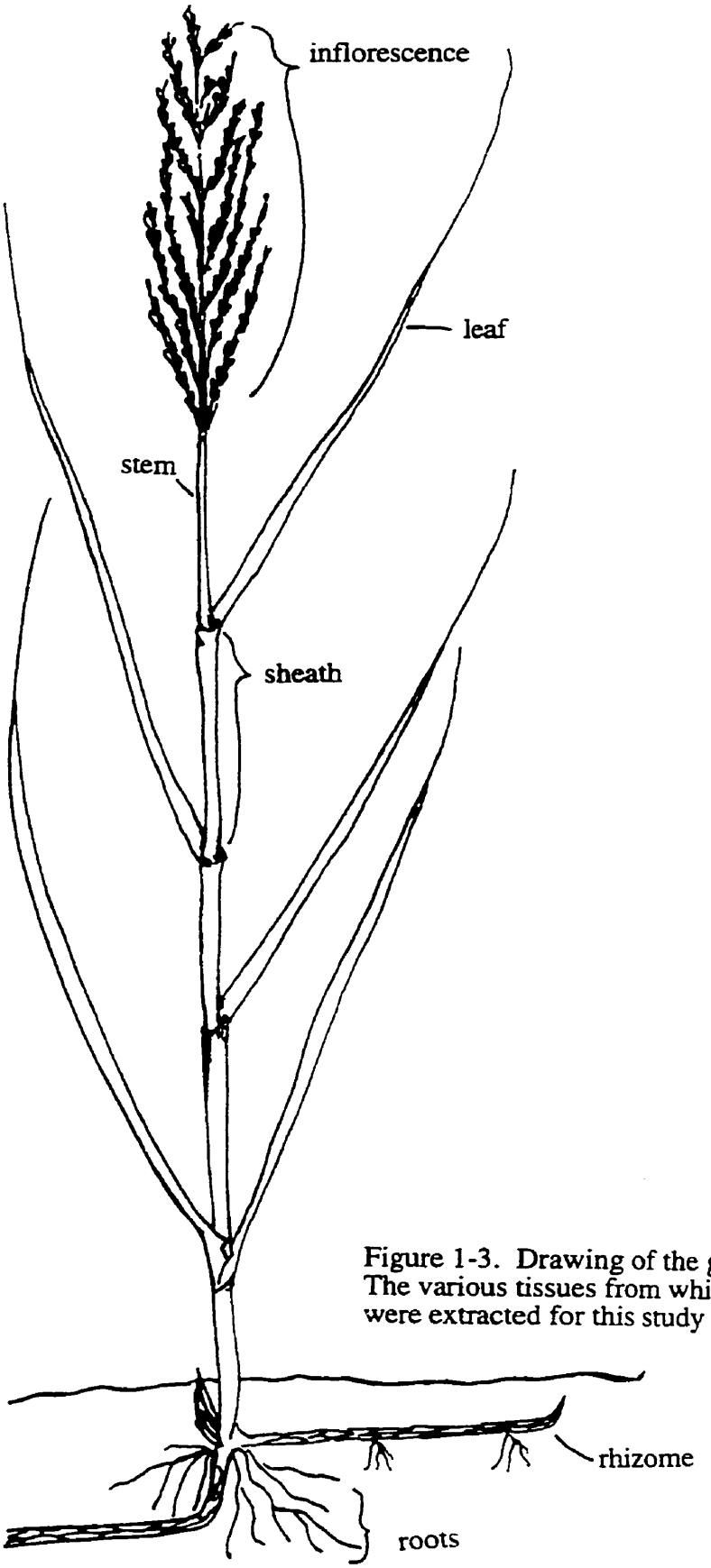


Figure 1-3. Drawing of the grass plant *C. longifolia*. The various tissues from which plant water and silica were extracted for this study are labeled.

and between mesophyll cells to the sub-stomatal cavities of the leaves where it evaporates and travels out into the atmosphere. The vascular system is divided into fine veins within the leaves, such that almost every cell is within one or two cells of vascular tissue, but no vascular tissue is exposed directly to the intercellular spaces adjacent to the stomatal pores (Barbour et al., 1980; Rayan and Matsuda, 1988). The process of water vapour loss from the interior of the leaf is called transpiration and is controlled by the opening and closing of the stomatal pores.

Water moves through the plant in response to water-potential gradients (caused mainly by transpiration) between the leaf's evaporating surface and the soil water. As water molecules are removed from the surface of the leaf, a pull or tension occurs from one molecule to the next, that draws water through the xylem cells. During periods of low transpiration solute accumulation in the xylem of the roots increases the osmotic potential between water in the soil and the root, and water will flow through the plant as a result of root pressure. Water is also involved in the translocation of food substances in solution through the phloem, often in a direction opposite to flow in the xylem (Sutcliffe, 1979). The hydrostatic pressure gradient between the source of nutrients in the leaves and the sinks in other plant tissues causes water and dissolved substances to flow along the phloem sieve tubes.

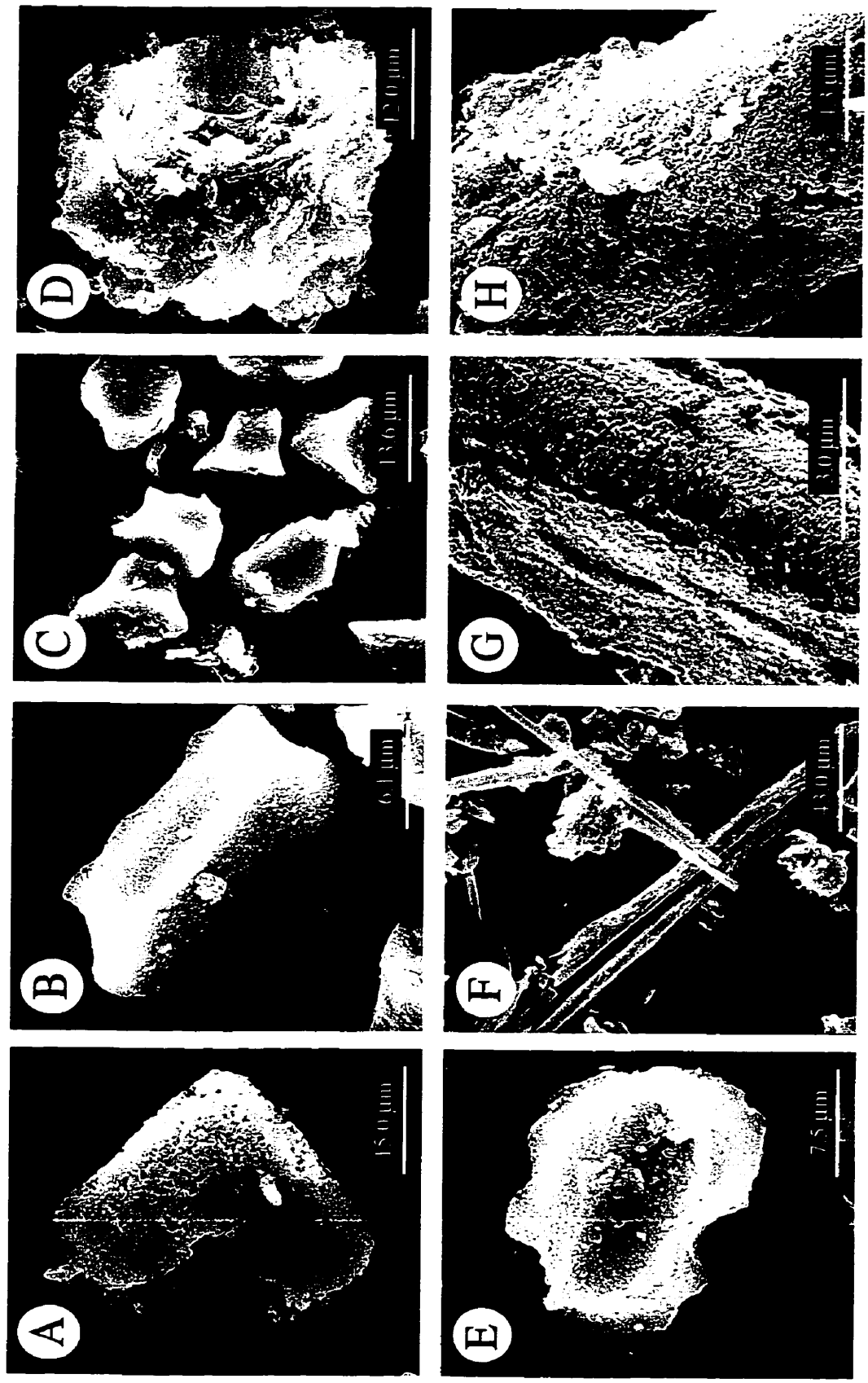
1.3.3. Silica phytoliths

Biogenic silica forms in plants as porous opal-A (SiO_2, commonly occurring as $Si(OSi\equiv)_n(OH)_{4-n}$ units; Mann et al., 1983), and contains approximately 1 % occluded organic matter (Jones and Beavers, 1963; Wilding, 1967; Kelly et al., 1991). In most plant species, particularly those belonging to the Gramineae family, silica is deposited in intercellular spaces, in cell walls and within cells as thin sheets, fragments or as discrete bodies known as phytoliths (Metcalfe, 1960; Sangster and Parry, 1981). Silica is deposited in all tissues of a grass plant (Brown, 1984). The majority of silica in roots

polymerizes within the endodermis. For example, silica is deposited in the darker layers of the endodermal cell walls, which are rich in phenols and suberin, and intracellular silica deposits occur on localized regions of the endodermal cell walls and project into the cell lumen. Silica deposits may also occur in the intercellular spaces of the large cortical cells immediately outside the endodermis of the root (Sangster and Parry, 1981; Sangster and Hodson, 1986). In the leaf, the majority of silica is deposited in the epidermal cells including silica cells (short cells), trichome (hair), bulliform, stomatal and long cells (Kaufman et al., 1981). The cell wall delineates the morphology of the intercellular silica deposits and as a result the silicified short cells have characteristic morphologies that can be distinguished to the species level. Grass phytoliths have been classified in three morphological groups (Twiss et al., 1969). The Panicoid class includes dumb-bell shaped phytoliths, which are generally produced in grasses belonging to the sub-family Panicoideae (Lanning and Eleuterus, 1989). Festucoid class phytoliths can be distinguished by circular, rectangular, elliptical, acicular, crescent, crenate and oblong shapes that are often formed in grasses of the sub-family Festuceae (Pearsal, 1989). Grasses of the sub-family Eragrostoideae (Chloridoideae) often produce phytoliths suites classified as Chloridoid, which can be recognized by the presence of saddle-shaped phytoliths and the absence of dumbbell or oval shaped phytoliths (Lanning and Eleuterus, 1989). In general C4 grasses deposit more silica than C3 grasses and have a greater number of dumb-bell shaped phytoliths and silicified bulliform cells and long cells (Kaufman et al., 1985).

SEM photographs of phytoliths extracted from *C. longifolia* and *A. breviligulata* are illustrated in Figure 1-4. Phytoliths from the stems of *A. breviligulata* have a variety of morphologies including cubic (Fig. 1-4a), oval (Fig. 1-4b), and trapezoidal (Fig. 1-4c). Many of the phytoliths extracted from the rhizome tissues of *A. breviligulata*, had a nondescript shape (Fig. 1-4d). Other rhizome phytoliths had very distinct shapes, such as the saddle-shaped phytolith shown in Figure 1-4e. Figure 1-4f shows an example of

Figure 1-4. SEM photomicrographs of silica phytoliths extracted from *A. breviligulata* and *C. longifolia* grown at Pinery Provincial Park, Ontario. A) Cubic stem phytolith from *A. breviligulata*, B) Oval-shaped stem phytolith from *A. breviligulata*, C) Trapezoid-shaped stem phytoliths from *A. breviligulata*, D) Non-distinct rhizome phytolith from *A. breviligulata*, E) Saddle-shaped phytolith extracted from the rhizomes of *A. breviligulata*, F) Silicified long cells from the leaves of *C. longifolia*, G) Plate-like and fibrillar silica structures seen on long-cell phytoliths extracted from the leaves of *C. longifolia*, H) Globular silica structures (arrow) in the silicified long cells of *A. breviligulata.*


A
150 µm
B
61 µm
C
136 µm
D
130 µm
E
75 µm
F
430 µm
G
30 µm
H
13 µm

silicified long cells extracted from the leaves of *C. longifolia*. In general, some phytolith shapes can be found in all plant tissues of an individual species except the roots (Brown, 1984). However, not all shapes relevant to a given species will be found in all plant parts. One plant species may be associated with a suite of phytolith morphologies, but several morphologies may be shared by other species.

Once the plant matter has decayed and the phytoliths are transferred to the underlying soil, the overall characteristic of a phytolith assemblage can be diagnostic of the former presence of trees, grasslands, paleolsols and agricultural practices. The occurrence of phytoliths is very widespread. Each square meter of grassland produces billions of phytoliths annually, which can be redistributed by grazing, bioturbation, fires and wind to provide a regionally homogenous soil-phytolith assemblage (Fredlund and Tieszen, 1997). Consequently, phytoliths are available where other microfossils may be absent or scarce. For example, traditional palynology is limited by the scarcity of locations in which pollen is preserved. This makes it difficult to use pollen to interpret regional changes in vegetation in response to climate change (Fredlund and Tieszen, 1994). By comparison, phytoliths that have resisted dissolution and retained their original shapes have been recovered from Quaternary and archaeological soils and sediments (Powers and Gilbertson, 1987; Mulholland, 1989; Fredlund and Tieszen, 1994; Rosen and Weiner, 1994). Recognizable phytoliths have even been discovered in Paleocene and Eocene sediments (Baker, 1960; Jones, 1964; Kodama et al., 1992). Experimental work has shown that biogenic silica can remain stable and resist dissolution for thousands of years (Wilding, 1967; Bartoli and Wilding, 1980; Drees et al., 1989). Even in equatorial rainforest soils where a large fraction of phytolith silica is rapidly recycled, Alexandre et al. (1997a) showed that sufficient material is preserved to provide a useable record. Morphological studies of soil-phytolith assemblages are widely used in archaeology to determine the vegetation, general climate and temperature at the time of soil formation

(Twiss et al., 1969; Rovner, 1971; Piperno, 1984; Mulholland, 1989; Powers et al., 1989; Fredlund and Tieszen, 1997; Alexandre et al., 1997b).

1.3.3.1. ***The formation of silica phytoliths***

When pH is less than 9, the predominant silicon species in solution is the small non-polar molecule silicic acid, $Si(OH)_4$ (Raven, 1983; Sjöberg, 1996). The concentration of silicic acid in natural waters ranges from 0.0001 mg/l in surface seawaters to 40 mg/l in soil water (Farmer, 1986). In plants, silicic acid can be present at concentrations of 5 to 1000 µmol/l (Birchall, 1995). Plants can be divided into three groups based on their habit of silica accumulation (Jones and Handreck, 1965). Some plants, commonly wetland grasses, actively take up silicic acid from the soil and these may contain up to 10% (dry weight) silica. Active uptake is evident when the concentration of silicic acid in the xylem is several times higher than in the soil water. Dicotyledons, by comparison, actively exclude silicic acid from their metabolism and generally contain less than 0.1% silica by dry weight. Plants that take up silicic acid passively generally have silica contents that are consistent with the concentration of silicic acid in the soil water and amount of water movement through the plant. Hence, silica content increases as water movement through the plant increases as the transpiration rate rises (Rosen and Weiner, 1994). Most dry-land grass species, such as those chosen for this study, take up silica passively and contain on average 1% silica (dry weight). However, some cereals have been shown to actively take up silicic acid from the soil water under greenhouse conditions when transpiration rates are low (Jarvis, 1987; Walker and Lance, 1991).

Aqueous silicic acid, $Si(OH)_4$, is transported through the plant along the transpiration stream (Raven, 1983). Polymerization is driven by local increases in the supersaturation of silica induced by the removal of water through transpiration, the degradation of Si complexes, a change in pH and/or reaction with ionized surfaces of certain organic compounds (Kaufman et al., 1981; Perry and Mann, 1989). Silica bodies in the epidermal cells of leaves are believed to polymerize as a result of increasing the

concentration of silicic acid through transpiration. Experiments have shown that preventing a plant from transpiring by coating the leaves with oil will prevent silica deposition in these tissues (Simkiss and Wilbur, 1989). Emmer wheat crops that have been irrigated produce a higher percentage of large cross-shaped phytoliths and multi-celled phytoliths, reflecting the greater water movement through the plant that results from higher rates of transpiration (Rosen and Weiner, 1994).

In tissues where water is not being removed by transpiration, silica deposition may be the result of metabolic processes that are sensitive to changes in pH. The concentration of silicic acid in the xylem can exceed saturation. However, silica precipitation does not occur because the silicic acid may (i) form complexes with organic compounds such as phenols or a tropolene derivative (Kaufman et al., 1981; Sangster and Hodson, 1986), (ii) form hydrogen bonds with O, NH_2 and COOH (Simkiss and Wilbur 1989) or (iii) form soluble Si chelates. These complexes remain stable until pH increases above 7 or enzymatic processes destroy the chelating agent. Once the complexes decompose, the silicic acid is liberated in a highly localized area initiating silica polymerization (Kaufman et al., 1981). Silica deposition is prevented at the nodes of the stem, the junction between the leaf blade and leaf sheath and in the pulvini (Kaufman et al., 1981). The intercalary meristem in the nodes of the stem produces GA3 gibberellic acid, which plays a role in promoting rapid growth and elongation in young cells. This creates a chemical environment with a pH of less than 5 that is not favourable for silica deposition. The pulvini shows strong acidification reactions to geotropism and the low pH environment discourages silica precipitation (Kaufman et al., 1981).

The chemical environment for silica deposition within a plant can be variable. Although no bonds are formed between silica and organic matter (Sangster and Parry, 1981), hydrogen bonds may form between the silicic acid and an organic matrix, such as glycols or cellulose (Hodson and Evans, 1995). As a result, polymerization is often associated with organic components of a cell. For example, cell wall deposits are

associated with cellulose and polysaccharide matrices, cuticular deposits are associated with fatty acids and lipids and intracellular deposits are associated with protein complexes (Sangster and Parry, 1981; Perry and Mann, 1989). The aggregation of silica onto these structures reflects the charges present at the surface of the organic matrix, which acts as a template for silica nucleation (Perry and Mann, 1989). Within the cell lumen of a grass macro-hair, initial silicification creates sheet-like deposits associated with cellulose and arabinoxylan (Perry and Mann, 1989). As these two organic components decline and the amounts of glucan and mannan increase, the silica is deposited as a globular structure. In the final stages of macro-hair silicification when polysaccharide deposition is completed, the silica forms as a fibrillar structure (Perry and Mann, 1989). These structures have also been observed in silicified long cells of *C. longifolia* and *A. breviligulata* (Figs. 1-4g and 1-4h).

Cells that are not normally silicified may become so as they age. The reasons are unknown. Younger cells may chemically inhibit the polymerization of SiO_2, or senescent cells may accumulate cellular debris providing more nucleation centers to induce silica precipitation (Simkiss and Wilbur, 1989). Once the silica is deposited it cannot be translocated to other cells or tissues within the plant (Raven, 1983). Hence, as silica accumulation continues the older portions of the plant become more silicified than the younger tissues, and the overall silica content of the grass increases as the plant matures (Johnston et al., 1967, Shahack-Gross et al., 1996).

1.3.3.2. *The role of plant silica*

Although silicon is not considered to be an essential nutrient for plants, it is present in macronutrient concentrations in tissues and plays a beneficial role in plant metabolism. The concentration of Si in soil solutions is in the same range as essential plant nutrients K^{+}, Ca^{+2} and SO_4^{-2}, and in great excess of P concentrations (Epstein, 1994). The presence of silicic acid enhances the uptake of ferric hydroxide in iron-deficient plants (Birchall, 1995). The occurrence of silicon in plants can reduce the toxic

effects of aluminum and manganese (Hodson and Sangster, 1993; Birchall, 1995; Hodson and Evans, 1995). In addition, manganese is believed to be the controlling factor of phosphorus availability in plants. Reduced manganese uptake in the presence of Si increases the nutrient efficiency of phosphorus, especially in plants growing on soils with low phosphorus concentrations (Ma and Takahashi, 1990). The uptake of silicic acid also decreases a plant's ability to take up Na^+, thereby increasing its resistance to salinity (Epstein, 1994). The presence of silica in plants also provides several mechanical advantages. Because silica is highly stable and resistant to decay, and because its formation is more energy efficient than the incorporation of carbohydrates into cell walls, silica is used by many plants for structural support (Raven, 1983). As a result of increased mechanical strength, plants with a high silica content resist drooping. Erect plant shoots intercept more light, which results in increased photosynthetic capacity, carbon assimilation and overall plant yield (Raven, 1983; Epstein, 1994). The presence of silica-impregnated cellulose in the walls of the leaf epidermis has also been demonstrated to reduce transpiration (Lanning and Eleuterus, 1981). Some plants may also have evolved silica bodies as a defense to the evolution of herbivores. Silica can cause abrasion of enamel on teeth and the ingestion of plant silica is involved in several pathological conditions in animals (McNaughton and Tarrants, 1983; Cid et al., 1989).

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Chapter 2. The oxygen isotopic compositions of silica phytoliths and plant water in grasses[1]

2.1. INTRODUCTION

We describe here the oxygen isotopic behaviour of biogenic silica deposited in living samples of *Calamovilfa longifolia* (Hook) Scribn., a native grass following the C4 photosynthetic pathway, which was collected in three locations across North America. Results are also reported for *Ammophila breviligulata* Fern., a C3 grass planted together with *C. longifolia* in sandy soils and dunes in southwestern Ontario (Fig. 2-1). Grasses, including *C. longifolia*, comprised much of the North American prairie vegetation for thousands of years, prior to human cultivation of these lands (Weaver, 1968). The wide climatic range of these grasses, combined with their substantial contribution of biogenic silica to the underlying soils, may provide an opportunity for continental paleoclimatic reconstruction through stable isotope analysis of the silica. However, an understanding of the variations in biogenic silica $\delta^{18}O$ values that occur under known, natural climatic conditions is a prerequisite to interpretation of such data for ancient soils and sediments.

In most plant species, particularly those belonging to the Gramineae family, silica is deposited in cells and intercellular spaces as discrete bodies known as phytoliths (Metcalfe, 1960). Aqueous silicic acid, $Si(OH)_4$, is transported through the plant along the transpiration stream (Raven, 1983), where polymerization occurs as a combined result of an increase in the concentration of silicic acid through transpiration, an increase in the ionic activity of Na^+ or K^+, a change in pH, and/or reaction with ionized surfaces of certain organic compounds (Kaufman et al., 1981; Perry and Mann, 1989). The silica forms as porous opal-A ($SiO_2 \cdot nH_2O$, commonly occurring as $Si(OSi \equiv)_n(OH)_{4-n}$ units; Mann et al., 1983), and contains approximately 1% occluded organic matter (Jones and Beavers, 1963; Wilding, 1967; Kelly et al., 1991). At the end of the growing season, the organic plant

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matter decays but the silica phytoliths accumulate as a metastable component of the underlying soil (Fredlund and Tieszen, 1994). Many archaeologists catalogue soil-phytolith assemblages in terms of morphology, size and frequency to deduce the plant families and general climatic conditions at the time of soil formation (Twiss et al., 1969; Rovner, 1971; Piperno, 1984; Mulholland, 1989; Pearsall, 1989; Powers et al., 1989; Fredlund and Tieszen, 1994). In this paper, we explore whether additional information about climatic conditions during plant growth is preserved by the oxygen-isotope composition of these phytoliths, building on preliminary observations for *C. longifolia* and *A. breviligulata* first outlined in Webb and Longstaffe (1997).

The oxygen-isotope composition of phytoliths is dependent on soil-water $\delta^{18}O$ values, relative humidity/evapotranspiration, and temperature throughout the period of silica formation. The $\delta^{18}O$ value of the water that enters a plant through its root system is determined by the isotopic composition of soil water. Local meteoric water such as precipitation and shallow groundwater, which are the feed-stock for soil water, varies in oxygen- and hydrogen-isotope compositions in a systematic fashion described in general by the Meteoric Water Line (MWL; Craig, 1961); latitude, altitude and temperature play an important role in determining the distribution of local meteoric water δ-values along the MWL. However, soil water can deviate in composition from the MWL as a result of evaporation at or near the soil surface. This effect is most common in arid environments and pertinent in grasslands where the active rooting zone (*i.e.*, 60-100 cm for *C. longifolia*; Weaver 1968; Maun, 1985) can be situated close to the shallower regions most affected by ^{18}O-enrichment of soil water (Allison et al., 1984).

Once soil water has entered a plant through its roots, this water can be enriched in ^{18}O by transpiration in tissues such as the leaves and inflorescence. The extent of this change depends on plant physiology and relative humidity, with ^{18}O-enrichment increasing as relative humidity decreases (Leaney et al., 1985; Flanagan and Ehleringer, 1991; Walker and Lance, 1991; Farquhar and Lloyd, 1993). The size of the equilibrium oxygen isotopic

fractionation between a silica phytolith and plant water at any particular location in the vegetation is controlled by temperature.

There is a paucity of easily accessible paleoclimatic indicators preserved in continental environments; silica phytoliths may help to fill this gap. Phytoliths that have resisted dissolution and retained their original shapes have been recovered from Quaternary and archaeological soils and sediments (Powers and Gilbertson, 1987; Mulholland, 1989; Fredlund and Tieszen, 1994; Rosen and Weiner, 1994). Recognizable phytoliths have even been discovered in Paleocene and Eocene sediments (Baker, 1960; Jones, 1964; Kodama et al., 1992). Experimental work has shown that biogenic silica can remain stable and will resist dissolution under favourable burial conditions (pH less than 9) for thousands of years (Wilding, 1967; Bartoli and Wilding, 1980; Drees et al., 1989). Even in equatorial rainforest soils where a large fraction of phytolith silica is rapidly recycled, Alexandre et al. (1997) showed that sufficient material is preserved to provide a useable record. This behaviour and the widespread availability of phytoliths gives them the potential to join the short list of materials whose isotopic compositions may retain information about ancient terrestrial climatic conditions.

2.2. METHODS

2.2.1. Sample collection

In the fall of 1994, *A. breviligulata* was collected from the University of Western Ontario's experimental farm, 9 kilometers north of London, Ontario, Canada (site 1a). *C. longifolia* was obtained at the same time from Pinery Provincial Park, located on the eastern shore of Lake Huron about 50 km north of London, Ontario (site 1b) (Fig. 2-1). Preliminary results for these samples have been described in Webb and Longstaffe (1997). In October 1995, new samples of *A. breviligulata* and *C. longifolia* were taken from Pinery Provincial Park. Sites 1a and 1b share the same local climate. Additional samples of *C.*

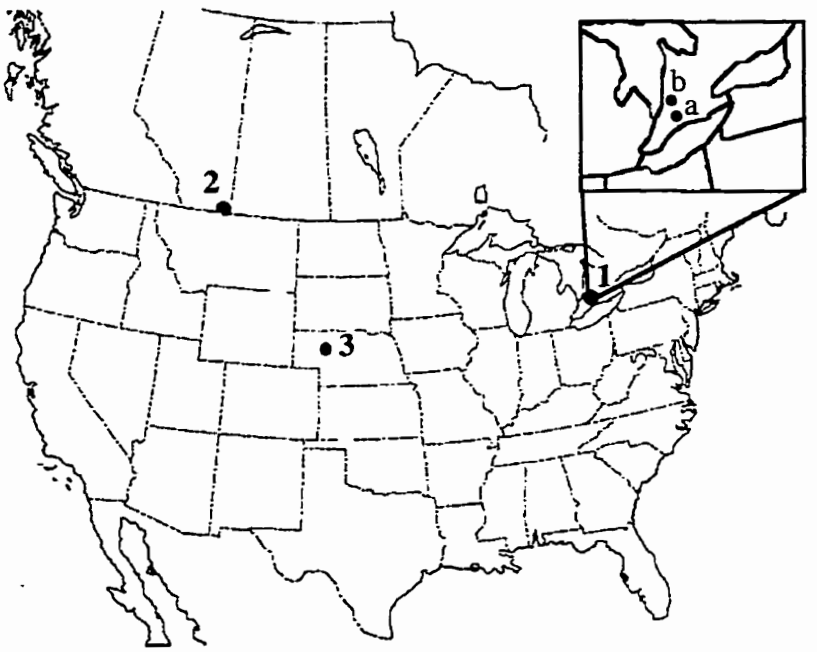

Figure 2-1. Sample locations in North America: 1a) London, Ontario, Canada; 1b) Pinery Provincial Park, Ontario, Canada; 2) Onefour, Alberta, Canada; 3) Thedford, Nebraska, U.S.A.

longifolia were collected during the fall of 1995 from the Agriculture Canada Station at Onefour, Alberta (site 2) and the Sandhills area west of Thedford, Nebraska (site 3). Each location represents a different ecological region: (1) humid temperate domain: warm summer-continental division (Pinery Provincial Park, Ontario); (2) dry domain: semi-arid steppe, Great Plains short grass (Onefour, Alberta), and (3) humid temperate domain: sub-humid, tall grass prairie (Thedford, Nebraska) (Bailey and Cushwa, 1981). Collection at a given site in a given year took place on a single day, and was timed such that the plant organic matter, and the biogenic minerals contained therein, represented natural growth for the entire season. Samples of local meteoric water were obtained from rivers, streams, lakes, rain and/or groundwater located in close proximity to each grass sample.

It is well known that plant-water stable isotope compositions vary substantially from pre-dawn to mid-day, and from day to day, in response to local conditions. To gain some appreciation for this variation in the southwestern Ontario samples, plant-water oxygen and hydrogen isotopic compositions were obtained for the periods of least (pre-dawn) and most intense (mid-day) transpiration, using greenhouse-grown *C. longifolia* and *A. breviligulata.* Several plants of each species were grown from seedlings for approximately 18 months in six-inch plastic pots filled with sand from Pinery Provincial Park. The plants were watered from the surface on a daily basis with tap water of a known isotopic composition. No steps were taken to prevent evaporation from the surface of the soil, but daily watering prevented excessive water stress on the plants. Tissues from the inflorescence, leaves, sheaths, stems, rhizomes and roots were then collected from two individual, adjacent, mature plants of each species during the same twenty-four hour period, one just before dawn and the other at mid-day. These tissues were sealed immediately in dry glass tubes.

2.2.2. Silica Extraction

Silica was extracted from dozens of adjacent individual plants in order to obtain sufficient quantities for analysis. This material represents an average for opal-A deposited in each grass species over its lifetime (one growing season to five years) at a given location. To determine how silica deposition, and the oxygen isotopic composition of the silica and plant water, varied within the grass, each species was divided into six parts: root, rhizome, stem, sheath, leaf and inflorescence. Samples were washed in distilled water to remove detrital minerals and dried at 65°C for a minimum of 36 hours. Dried samples of grass tissues (20 to 100 mg) were digested in an excess of 99% sulfuric acid for an average of two hours or until dissolved completely. The organic matter was then reacted with 30% hydrogen peroxide until only silica remained (Geis, 1973). Wet ashing techniques were considered preferable to dry ashing, which has been shown to partially recrystallize opal-A to cristobalite (Jones and Milne, 1963). The silica sample was then washed four to five times with distilled water, once with 1.0M HCl and again three times with distilled water using high-speed centrifugation. Once cleaned, the samples were freeze-dried.

Samples were examined by X-ray diffraction, and most showed no evidence of phases other than opal-A. Traces of quartz were present in some samples, especially those from roots in which phytolith concentrations were low and the potential for contamination from soil minerals was high. Gypsum and anhydrite were present in the leaf-silica samples from Pinery Provincial Park in southwestern Ontario. These phases are believed to be artifacts produced by reacting sulfuric acid with biogenic calcium oxalate, which can also be precipitated in leaves. Any oxalate present in the leaf tissue would have been destroyed during sample preparation. Sulphate minerals were removed by dissolution in HCl.

2.2.3. Isotopic Analysis

All stable isotope results are expressed in the standard δ-notation, relative to VSMOW for oxygen and hydrogen (Coplen, 1994) where

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000 \ (‰) \qquad \text{(Eqn. 2-1)}$$

and R represents D/H or $^{18}O/^{16}O$. Dual inlet, triple-collecting, gas-source, Optima or Prism II mass spectrometers were used for all measurements.

For oxygen-isotope analysis, two milliliters of each meteoric- and tap-water sample were equilibrated with CO_2 overnight at 25°C (Epstein and Mayeda, 1953). Hydrogen gas for isotopic analysis was produced by reducing (under vacuum) two microlitres of water with metallic zinc at 500°C for twenty minutes (Coleman et al., 1982). Reproducibility was better than ±0.1‰ for oxygen and ±1‰ for hydrogen. Water was extracted from grass samples under vacuum for forty-five minutes while being heated at 100°C. Three microlitres of the extracted water were equilibrated with a known amount of CO_2 for five days at 25°C (Epstein and Mayeda, 1953, as modified by Kishima and Sakai, 1980). Reproducibility of $\delta^{18}O_{plant\ water}$ values averaged ±0.3‰. The same three microlitres of water were reacted with metallic zinc to produce H_2 gas for hydrogen-isotope analysis, as described above. Reproducibility of $\delta D_{plant\ water}$ values averaged ±1‰.

Silica samples were heated under vacuum at 300°C for twelve hours prior to transfer, under a flow of dry nitrogen gas, to nickel reaction-vessels. The samples were then heated at 300°C under vacuum for a further two hours prior to the addition of bromine pentafluoride. Oxygen was liberated from silica by reaction with BrF_5 at 600°C (Clayton and Mayeda, 1963), and prepared for mass spectrometric analysis by conversion to CO_2 gas by reaction with incandescent graphite. During the course of these experiments, the $\delta^{18}O$ value of the laboratory's standard quartz was consistent with a value of +9.7±0.2‰ for standard silica sand (NBS-28).

Oxygen-isotope analysis of phytoliths presents some special problems. Post-formation oxygen isotopic exchange between amorphous silica and water has been discussed by many researchers (Mopper and Garlick, 1971; Kawabe, 1978; Mikkelsen et al., 1978; Labeyrie and Juillet, 1982; Wang and Yeh, 1985; Schmidt et al., 1997; Brandriss et al., 1998). Such exchange has the potential to occur in nature after the

phytoliths have accumulated in soil, and also in the laboratory as the samples are prepared for analysis. For our samples, the major concern was oxygen-isotope exchange between unstable Si-O bonds and hydroxyl groups in the opal-A during dehydration prior to oxygen-isotope analysis.

To evaluate the significance of this problem, the isotopic exchange procedure for opal-A described by Labeyrie and Juillet (1982) and Juillet-Leclerc and Labeyrie (1987) was applied to our samples. These results were then compared with those obtained for non-exchanged samples extracted from living grasses collected one year earlier (Webb and Longstaffe, 1997). In the exchange method, separate aliquots of the same silica sample, maintained at 200°C, were exchanged with the vapour produced at 0°C over two different, isotopically labeled waters ($\delta^{18}O$ = +41.6‰ and -0.9‰) in an evacuated environment. This process is intended to fix the oxygen isotopic composition of exchangeable oxygen in the opal-A at a known composition. After allowing exchange to proceed for six hours, the samples were sintered under vacuum at 1100°C for eighteen hours, to partially recrystallize the silica. FTIR spectrometry has shown that phytolith dehydration is completely reversible at ≤200°C, and that partial rehydration still occurs upon heating to 400°C (Alexandre, 1996). According to Unger (1994), the removal of hydroxyl groups creates strained siloxane groups, which can rehydroxylate with ease; these groups begin to convert to a stable configuration at ~500°C. However, our experiments suggest that a much higher temperature for sintering is needed to eliminate rehydration and the associated back-exchange of this opal-A.

Following Juillet-Leclerc and Labeyrie (1987), the oxygen-isotope composition of the non-exchangeable oxygen (δ_{silica}), and the percentage of exchangeable oxygen (X) in each phytolith sample, was calculated using the two results ($\delta_{measured\ 1}$, $\delta_{measured\ 2}$) obtained for the exchanged samples and the values determined for the exchanged oxygen ($\delta_{exchanged\ 1\ or\ 2}$):

$$\delta_{exchanged} = \delta_{H_2O\ liquid} + (\delta_{H_2O\ vapour} - \delta_{H_2O\ liquid}) + (\delta_{exchanged} - \delta_{H_2O\ vapour}), \quad \text{(Eqn. 2-2)}$$

where $\delta_{H_2O\ vapour} - \delta_{H_2O\ liquid}$ at 0°C equals -11.5‰ (Mopper and Garlick, 1971), and $\delta_{exchanged} - \delta_{H_2O\ vapour}$ at 200°C is 13.5‰ (Labeyrie and Juillet, 1982), and:

$$\delta_{silica} = [\delta_{measured\ 1} - X(\delta_{exchanged\ 1})] / (1-X), \qquad \text{(Eqn. 2-3)}$$

$$\delta_{silica} = [\delta_{measured\ 2} - X(\delta_{exchanged\ 2})] / (1-X). \qquad \text{(Eqn. 2-4)}$$

When the same exchanged silica sample was reanalyzed, the reproducibility of $\delta_{measured}$ averaged ±0.1‰ (fourteen pairs). When the entire exchange process was duplicated using separate aliquots of the same sample, the reproducibility of $\delta_{measured}$ averaged ±0.2‰ (eight pairs). Finally, for three samples in which the entire process was repeated at least twice using both the ^{18}O-rich and ^{18}O-poor labeled water, the average reproducibility of δ_{silica} was better than ±0.3‰. By comparison, reproducibility of $\delta^{18}O$ values for phytolith samples that were not subjected to the exchange procedure, including those first described by Webb and Longstaffe (1997), was substantially worse, averaging ±1.2‰.

2.3. RESULTS

Average growing season precipitation, temperature, and daily minimum and maximum relative humidity are listed in Table 2-1 for each location. Data for two different growing season intervals have been tabulated in Table 2-1, since *A. breviligulata* prefers cooler conditions for growth and begins to develop earlier in the spring than *C. longifolia* (Maun and Baye, 1989). It is fair to note, however, that the majority of silica in grasses is deposited in mature cells (Johnston et al., 1967; Perry and Mann, 1989). Hence, the potential exists for a substantial increase in silica formation later in the growing season, with attendant effects on the oxygen-isotope composition of the phytolith assemblage in the grass (e.g., Shahack-Gross et al., 1996). There is a large difference in the oxygen-isotope composition of meteoric water among these localities ($\delta^{18}O$ = -14.5 to -7.2‰), as recorded in the values obtained for surface and near-surface reservoirs at the end of the 1995 growing season.

Table 2-1. Sample locations and climatic information*.

	Pinery/London, Ontario	*Onefour, Alberta*	*Thedford, Nebraska*
Latitude	43° 15'N	49° 07'N	42° 02'N
Longitude	81° 51'W	110° 28'W	100° 49'W
†**Temperature** (°C)			
May-Aug.	20	16	20
July-Aug.	22	19	25
†**Minimum RH** (%)			
May-Aug.	54	35	41
July-Aug.	57	34	35
†**Maximum RH** (%)			
May-Aug.	92	79	89
July-Aug.	95	78	89
Precipitation (mm)			
Annual	919	384	694
May-Aug.	297	181	368
July-Aug.	171	70	112
§δ^{18}**O values of meteoric water** (‰, VSMOW)			
	-7.2	-14.5	-10.5

*Climatic information from Environment Canada, and the on-line Climate Visualization System, National Climatic Data Center. †Average daily values; RH = relative humidity. §Average values from local rivers, lakes, precipitation and shallow groundwater, extrapolated to the meteoric water line as needed.

The silica content of dry grass tissues from these sites varied from 0.1 to 6.7%, depending on locality and the part of the plant analyzed (Table 2-2). In general, the silica content was higher in tissues through which water transpires (sheath, leaves and inflorescence). *C. longifolia* and A. *breviligulata* each contained, on average, 1.1% silica over the total plant. Higher values reported for the 1994 samples from sites 1a and 1b by Webb and Longstaffe (1997) arose from methodological errors, and should be disregarded.

The $\delta^{18}O$ values of exchanged samples, and percentage of exchangeable oxygen, are summarized in Table 2-3 for silica phytoliths from various plant tissues of *C. longifolia* and *A. breviligulata*. On average, 5% of the phytolith oxygen in *C. longifolia* and A. *breviligulata* is exchangeable. We expect that this value will be typical of phytoliths from most grass species, and note that it is similar to that found for silica diatoms (Knauth and Epstein, 1982; Juillet-Leclerc and Labeyrie, 1987). The $\delta^{18}O$ values obtained for non-exchanged phytoliths collected in 1994 (Webb and Longstaffe, 1997) differ from exchanged samples collected in 1995 by –0.7 to +1.1‰ for *C. longifolia* and -3.7 to +4.7‰ for A. *breviligulata* (Tables 2-4a, 2-4b). Whether these differences are a signal of different growing conditions in 1994 versus 1995, a consequence of sampling at the London versus Pinery Provincial Park sites, or an artifact of a low number of samples, remains to be learned. However, these variations plus the substantially improved reproducibility of measured $\delta^{18}O$ values following exchange demonstrate that the effort to control the oxygen isotopic composition of exchangeable oxygen in grass phytoliths is warranted.

The isotopic results for plant water extracted from the greenhouse-grown grasses are summarized in Table 2-5. Average plant-water δ-values were calculated as unweighted averages of pre-dawn and mid-day δ–values. Bulk leaf-water δ-values were calculated assuming 95% lower leaf water and 5% upper leaf water, as estimated from the volume and shape of the leaves. During sampling of *C. longifolia* on July 27, 1996, temperature varied from 14 to 32°C, and relative humidity changed from 86 to 37% between pre-dawn and

Table 2-2. Silica content of *C. longifolia* and *A. breviligulata.*

Location	% Silica*					
	Inflore-scence	Leaves	Sheath	Stems	Rhizomes	Roots
C. longifolia						
Pinery, Ontario	1.9	3.3	2.4	0.3	0.5	0.1
Onefour, Alberta	2.8	6.7	4.4	0.4	0.4	0.1
Thedford, Nebraska	3.0	4.2	4.4	0.6	0.5	0.1
Average	2.6	4.7	3.7	0.4	0.5	0.1
†Relative contribution of total plant silica	5.0	41.2	32.5	3.2	15.1	3.0
A. breviligulata						
Pinery, Ontario	1.2	1.4	2.7	0.5	0.9	0.4
†Relative contribution of total plant silica	0.3	39.2	25.1	0.7	24.0	10.7

*Relative to dry weight grass. †Avg. silica content x avg. percentage of estimated total biomass based on personal observation and Maun (1985).

Table 2-3. O-isotope results (‰, VSMOW) and % exchangeable oxygen of silica phytoliths.

		Inflorescence		Leaf		Sheath		Stem		Rhizome	
Location	Species	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*
Pinery	*C. longifolia*	31.8	6.5	32.4	3.8	27.7	5.1	28.1	6.8	27.5	6.1
Pinery	*A. breviligulata*	28.3	4.1	32.0	3.8	29.2	2.5	28.6	3.2	28.4	2.5
Onefour	*C. longifolia*	32.5	7.6	32.7	4.4	24.5	5.1	24.2	7.0	24.8	6.9
Thedford	*C. longifolia*	32.5	3.7	33.3	9.5	26.8	4.4	26.1	5.6	26.6	5.7

*Average amount of oxygen affected by exchange.

Table 2-4a. O-isotope results (‰, VSMOW) for exchanged phytoliths*.

Plant Part	†$\delta^{18}O$ Exchanged Silica Phytoliths	§Avg. $\delta^{18}O$ Plant Water	$\Delta^{18}O_{SiO_2\text{-}H_2O}$	¥Isotopic Temperature
C. longifolia (Pinery)				
Inflorescence	31.8	**-0.6**	32.4	**27**
Upper leaf		12.4	*20.0*	*62*
Lower leaf		-2.4	*34.8*	*20*
Bulk leaf	32.4	-1.7	34.1	22
Sheath	27.6 ±0.1	-5.0	32.6	26
Stem	28.1	-5.9	34.0	23
Rhizome	27.5 ±0.2	**-6.4**	33.9	**23**
A. breviligulata (Pinery)				
Inflorescence	28.3			
Upper leaf		4.6	*27.4*	*41*
Lower leaf		-2.9	*34.9*	*20*
Bulk leaf	32.0	-2.5	34.5	21
Sheath	29.2 ±0.1	-4.5	33.7	23
Stem	28.6 ±0.6	-6.5	35.1	20
Rhizome	28.4	**-6.0**	34.4	**21**

*Phytoliths were extracted from several individuals, and represent silica precipitated over the 1995 growing season. Phytoliths in rhizome tissues may represent silica deposition over several years of growth. †Each value was calculated from data pairs obtained by exchanging separate aliquots of each sample with ^{18}O-rich and ^{18}O-poor water; errors indicate spread of calculated results when multiple analyses of at least one exchanged sample were performed. §From Table 2-5; **bold** values indicate mid-day sampling only. ¥Geothermometer of Shahack-Gross et al. (1996); values in *italics* were calculated assuming that bulk leaf silica $\delta^{18}O$ values are representative of both upper and lower leaf phytoliths; values in **bold** were calculated using mid-day water compositions.

Table 2-4b. O-isotope results (‰, VSMOW) for unexchanged phytoliths*.

Plant Part	†$\delta^{18}O$ Unexchanged Silica Phytoliths	§Avg. $\delta^{18}O$ Plant Water	$\Delta^{18}O_{SiO_2-H_2O}$	¥Isotopic Temperature
C. longifolia: (Pinery)				
Inflorescence	32.9 ±1.4	**-0.6**	33.5	**24**
Upper leaf		12.4	*19.3*	*64*
Lower leaf		-2.4	*34.1*	*22*
Bulk leaf	31.7 ±0.8	-1.7	33.4	24
Sheath	27.8 ±0.7	-5.0	32.8	26
Stem	28.2	-5.9	34.1	22
Rhizome	28.0 ±0.6	**-6.4**	34.4	**21**
A. breviligulata: (London)				
Inflorescence	33.0			
Upper leaf		4.6	*27.4*	*41*
Lower leaf		-2.9	*34.9*	*20*
Bulk leaf	32.0 ±0.3	-2.5	34.5	21
Sheath	29.6	-4.5	34.1	22
Stem	27.9 ±1.1	-6.5	34.4	21
Rhizome	24.7 ±1.7	**-6.0**	30.7	**32**

*Phytoliths were extracted from several individuals, and represent silica precipitated over the 1994 growing season (Webb and Longstaffe, 1997).
†Errors indicate spread of results for separate aliquots. §,¥As in Table 2-4a.

Table 2-5. O- and H-isotope results (‰, VSMOW) for plant water*.

	Mid-day		Pre-dawn		Average Daily	
Plant Part	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD
†*C. longifolia*						
Inflorescence	-0.6	-47				
Upper leaf	21.8 (1)	-6 (1)			12.4	-24
	17.6 (2)	-6 (2)	5.0 (3)	-42 (3)		
Lower leaf	1.0 (1)	-45 (1)	-5.7 (4)	-53 (4)	-2.4	-48
	0.1 (2)	-46 (2)	-5.2 (3)	-55 (3)		
Bulk leaf	2.0 (1)	-43 (1)			-1.7	-47
	1.0 (2)	-44 (2)	-4.7 (3)	-54 (3)		
Sheath	-4.3 (1)	-55 (1)	-5.6 (4)	-54 (4)	-5.0	-54
	-4.3 (2)					
§Stem	-5.9,-6.7	-52,-57	-5.4	-54	-5.9	-54
Rhizome	-6.4	-56				
§Root	-5.3	-50	-4.6,-4.9	-48,-54	-5.0	-51
¥*A. breviligulata*						
Upper leaf	17.4 (1)	-13 (1)	0.7 (3)	-42 (3)	4.6	-33
	-0.6 (2)	-36 (2)				
Lower leaf	-0.1 (1)		-4.1 (3)	-51 (3)	-2.9	-50
	-3.2 (2)	-48 (2)				
Bulk leaf	0.8 (1)		-3.9 (3)	-50 (3)	-2.5	-48
	-3.1 (2)	-47 (2)				
Sheath	-5.3 (1)	-52 (1)	-3.7 (3)	-50 (3)	-4.5	-52
		-56 (2)				
§Stem	-6.4,-6.0	-56,-55	-6.0,-7.6	-57,-55	-6.5	-56
Rhizome	-6.0	-54				
Root	-5.4	-52	-5.9	-50	-5.6	-51

*One-day cycle; grass grown in London greenhouse; tap water: $\delta^{18}O$ = -7.2‰, δD = -57‰. †*C. longifolia* - mid-day temperature & relative humidity = 32°C & 37%; pre-dawn = 14°C & 86% RH; leaf (1): 8th leaf from soil, height above soil = 35 cm, length = 55 cm; leaf (2): 4th leaf, 22 cm above soil, length = 68 cm; leaf (3): 5th leaf, 15 cm above soil, length = 62 cm; leaf (4): 3rd leaf, 12 cm above soil, length = 51 cm. §Duplicate results for separate extractions from similar parts of the same plant; e.g., another piece of stem. ¥A. breviligulata - mid-day = 25°C & 56% RH; pre-dawn = 14°C & 86% RH; leaf (1): 3rd leaf, 9 cm above soil, length = 32cm; leaf (2): 4th or 5th leaf, 15 cm above soil, length = 43cm; leaf (3): 2nd leaf, 11cm above soil, length = 25cm.

mid-day collection of water. The daily variation was less extreme during sampling of *A. breviligulata*, one week later (14-25°C, 86-56% relative humidity). Such conditions are typical of those encountered at the experimental farm and Pinery Provincial Park at this time of the year, although still larger variations may occur under entirely natural conditions. Tap water provided to the grasses in the greenhouse was obtained from Lake Huron, and had average $\delta^{18}O$ and δD values of -7.2±0.3‰ (n=110) and -57±3‰ (n=87), respectively, for weekly samplings from February 1994 to November 1996.

2.4. DISCUSSION

2.4.1. Distribution of Silica

The majority of the silica phytoliths in these grasses formed in the leaves and sheaths (Table 2-2). This distribution is indicative of passive transport of silicic acid via the transpiration stream, which is typical of the Gramineae family (Raven, 1983). Less than one third of the plant silica was deposited in roots and rhizomes, and this measurement may be inflated because of the potential for detrital quartz contamination in root samples. The absolute amount of silica precipitated in the rhizomes is higher, on average, than in the roots (0.5 versus 0.1% in *C. longifolia*, 0.9 versus 0.4% in *A. breviligulata*; Table 2-2). Nevertheless, roots and rhizomes still contribute only a modest fraction of the total silica contributed to the soil over several growing seasons. Underground tissues (roots and rhizomes) can survive over several growing seasons (up to five years at the depths sampled in this study), and their silica content represents phytolith production over that period of time (Maun, 1985; Maun and Baye, 1989). Most above-ground tissues, by comparison, are regenerated each year. This much higher turnover rate relative to roots and rhizomes leads to a substantially higher delivery of above-ground phytoliths to the soil assemblage, assuming that preservation is similar for all phytoliths from the same plant. As a result, the time-integrated contribution of root and rhizome silica ought to be substantially lower than

the range (18-35%) reported in Table 2-2. This may be an important consideration in the interpretation of phytoliths from soil profiles. For example, although the underground biomass constitutes up to 58% of total plant biomass for *A. breviligulata* and 69% for *C. longifolia* at the Pinery site (Maun, 1985), contamination of underlying, older phytolith assemblages by younger silica from roots and rhizomes that penetrate these soils will be relatively modest. We cannot rule out, however, that underground tissues produced by other species may contain a greater fraction of their total phytolith production (Geis, 1978; Mulholland, 1989).

2.4.2. Isotopic Behaviour of Plant Water

To understand the range in isotopic composition of phytoliths throughout a plant, it is first necessary to determine the variability in δ-values of the water from which biogenic silica may have precipitated. Water supplied to soil may be altered in isotopic composition prior to phytolith crystallization by evaporation from the soil surface and by transpiration through the plant's leaves. We observed little difference among pre-dawn or mid-day water δ-values for plant parts (roots, rhizomes, stems) in which transpiration was unimportant (Table 2-5). In fact, plant-water δ-values for the C4 grass (*C. longifolia*) remained relatively constant throughout the grass during the pre-dawn period when transpiration rates are low. Nevertheless, root, rhizome and stem waters are enriched in ^{18}O by 1 to 2 permill relative to the water provided to these soils ($\delta^{18}O = -7.2‰$). Since there is no known fractionation associated with uptake of soil water by roots, or its subsequent movement up the stem (Allison et al., 1984; White et al., 1985), the soil water must have become enriched in ^{18}O through evaporation at or near the soil surface. In the greenhouse, plants are potted individually and watered on a daily basis. Under such conditions of low water stress, soil water may have a long residence time, during which it is subject to slow evaporation. In addition, small pot size and poor aeration do not enable healthy root development. The root water sampled here is slightly enriched in ^{18}O relative

to stem (or rhizome) water. This behaviour likely indicates that these roots were not fully active in water uptake, and as a result, suffered evaporative water loss.

Because our plant-water sampling method consumes the grass, we used adjacent individuals to obtain results for pre-dawn and mid-day conditions. This approach assumes that each plant had a similar water source, transpiration rate and hence plant-water isotopic behaviour. For non-transpiring portions of the grasses, this assumption seems to be valid. The $\delta^{18}O$ and δD results for pre-dawn and mid-day stem waters vary very little for adjacent plants (-5.9±0.4 ‰ and -54±0‰ for *C. longifolia*; -6.5±0.3‰ and -56±0‰ for *A. breviligulata*; Table 2-5).

This type of validation is more difficult for transpiring plant tissues, especially leaves, but the similarity of plant-water results for several individuals suggests that our sampling approach is suitable (Webb and Longstaffe, unpublished data). Nevertheless, some plant-water isotopic behaviour is a consequence of the unique morphology of an individual plant. Walker and Lance (1991) showed that separate leaves of a barley plant have different isotopic compositions that are related to their position on the stem. At mid-day, the flag leaf is the most enriched relative to older leaves, which grow closer to the base of the plant. This difference may occur because of higher transpiration rates in younger leaves, which are exposed to more turbulent boundary layer conditions at the top of the canopy. Alternatively, it may reflect progressive enrichment of water in higher leaves, which are fed not only by stem water but also by water that was enriched in D and ^{18}O within leaves situated further down the stem. The two mid-day samplings of *C. longifolia* leaves exhibit this pattern. The leaf sampled closest to top of the plant is more enriched in ^{18}O than leaves sampled further down the plant (Table 2-5). The results for *A. breviligulata* are not so consistent. One mid-day sample of upper leaf water is slightly depleted of ^{18}O relative to leaves further down the same plant, and to a comparable pre-dawn sample from an adjacent individual (Table 2-5). We do not know why this occurs, except to note that the limited spatial segregation among stem, sheath and leaves in *A.*

breviligulata is very different from the clear separation in *C. longifolia* or barley plants in general.

These details notwithstanding, a much larger range in plant-water δ-values occurs in the sheaths, leaves and inflorescence of *C. longifolia* and *A. breviligulata* than in their non-transpiring tissues. This behaviour reflects the influence of relative humidity on evaporation, which is generally described by the Craig and Gordon (1965) model for a standing body of water. That relationship, with some modifications, has been used to describe the isotopic variations in plant water that occur during transpiration (Leaney et al., 1985; Yakir et al., 1989; Yakir et al., 1990; Flanagan and Ehleringer, 1991; Walker and Lance, 1991; Farquhar and Lloyd, 1993; Wang and Yakir, 1995). Water in the transpiring tissues is enriched in D and ^{18}O as a consequence of both equilibrium and kinetic isotope fractionation. Equilibrium fractionation is dependent on the temperature at which the liquid changes to vapour. The extent of kinetic isotope fractionation depends on the ratio of vapour pressures between the different isotopic species of water, and conditions at the boundary layer between the transpiring tissue and the atmosphere. Kinetic isotope fractionation is larger during periods when temperature is high, boundary layer conditions are most stagnant, and the ratio of vapour pressure in the atmosphere to that in the leaves is lowest. Consequently, maximum D- and ^{18}O-enrichment of the plant water occurs during maximum rates of transpiration, which generally occur at the lowest relative humidity (Walker and Lance, 1991). The enrichment in ^{18}O that occurs between upper leaf water and stem water can be enormous. In our samples, differences of up to 28‰ were measured for *C. longifolia* during a change in relative humidity of 49%, and 24‰ for *A. breviligulata* during a slightly less extreme daily variation in relative humidity and temperature.

It should be possible to predict these changes in the isotopic composition of leaf water. In theory at least, the flux of water through a leaf is high and the instantaneous volume of water is very small. Such conditions should allow leaf water to reach isotopic

steady state with stem water. The steady-state isotopic composition of the transpiring body of water in a leaf can be expressed by the equation:

$$\delta_l = \varepsilon^* + \varepsilon^k + \delta_s + h\,(\delta_a - \varepsilon^k - \delta_s) \qquad \text{(Eqn. 2-5)}$$

where $\varepsilon^* = (1-\alpha)1000$ is the equilibrium fractionation factor (Majoube, 1971), ε^k is the kinetic fractionation factor (Merlivat, 1978), δ_l, δ_s and δ_a are the $\delta^{18}O$ or δD values of the water in the leaf, stem and atmospheric vapour respectively, and h is relative humidity. However, the model described by Equation (2-5) commonly produces $\delta^{18}O$ and δD values that are higher than measured total leaf water values (Flanagan and Ehleringer, 1991; Walker and Lance, 1991; Bariac et al., 1994). This discrepancy may indicate that turnover rates for leaf water are slower than the daily change in ambient conditions for plants, which prevents attainment of steady-state conditions. Wang and Yakir (1995) have described plants that did not reach isotopic steady-state even when exposed to constant ambient conditions for more than three hours. It has also been suggested that leaf water is not well mixed. In one scenario, ^{18}O- and D-enriched water in isotopic steady-state is diluted by non-fractionated water from leaf veins or symplastic water in leaf cells, which may exchange with, but remains separate from transpiring water pools (Leaney et al., 1985; Yakir et al., 1989, 1990). In another scenario, Farquhar and Lloyd (1993) proposed a single leaf-water pool, in which enriched water vapour at the sites of evaporation back-diffused to mix with unfractionated water entering a leaf. In this model, physical parameters that describe the leaf and the path length of transpiring water are used to help account for the discrepancy between modeled and observed leaf-water δ-values. Buhay et al. (1996) suggested that variations in the kinetic isotope fractionation-factor (ε^k) arising from leaf size and morphology could also account for some of the differences between measured and modeled O- and H-isotope compositions, as could leaf temperature for some of the discrepancies in δD values.

Leaf-water δ-values measured for *C. longifolia* and *A. breviligulata* are compared in Figure 2-2 to those calculated for steady-state conditions from stem water δ-values.

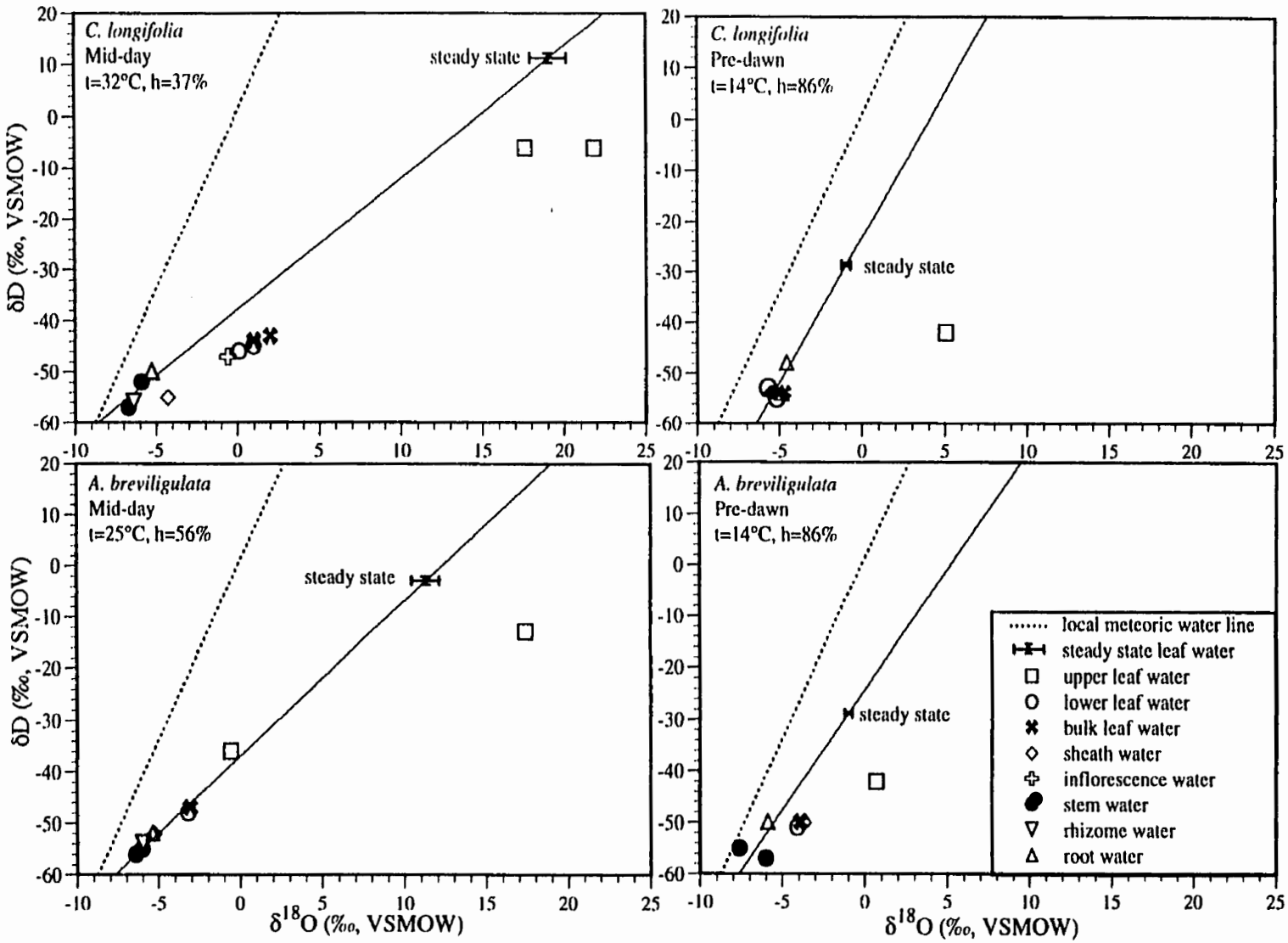

Figure 2-2. The δD versus $\delta^{18}O$ values of plant water for *C. longifolia* and *A. breviligulata* grown in a London, Ontario greenhouse. The solid line represents mixing between average stem-water compositions and steady-state values calculated for leaf water that evolved from stem water. The error bars on the steady-state values represent the variation produced when ε^k is adjusted to account for different wind speeds (20-60 cm/s) and leaf size.

Separate calculations using tap water δ-values produced similar results. The value of δ_a used in the calculation was obtained by assuming equilibrium with greenhouse tap water ($\delta^{18}O$ = -7.2‰, δD = -57‰) at the temperature of sample collection. This is a reasonable approximation, given that the plant canopy is sparse, and that this humid location is surrounded by two Great Lakes, which supplied the tap water (Lake Huron, $\delta^{18}O$ = -7.2‰, δD = -57‰; Lake Erie $\delta^{18}O$ = -7.1‰, δD= -52‰). The value of ε^k was also modified to account for leaf size and morphology (Buhay et al., 1996). However, wind-speed velocity over the leaf surface was estimated, not measured; the error bars on the steady-state values for leaf water reflect this uncertainty. In addition, possible corrections for underestimation of leaf temperature, which typically is higher than air temperature surrounding the plants, were not applied.

The root, rhizome, sheath and lower leaf waters plot on, or close to a mixing line between stem-water and steady-state, leaf-water δ-values for both pre-dawn and mid-day conditions (Fig. 2-2). The situation for leaf water is more complicated. The $\delta^{18}O$ and δD values obtained for the upper portions of individual leaves (~5% of bulk leaf water) are significantly higher than the lower portions (~95% of bulk leaf water) (Table 2-5), and generally do not fall on the mixing line. In many cases, the upper leaf water is more enriched in ^{18}O than predicted by Equation (2-5). Such behaviour can be attributed to the supply of progressively ^{18}O- and D-enriched water from the base to the tip of the leaf, via successive leaf-water pools (Wang and Yakir, 1995). At the end of a string of such pools, upper leaf water can become significantly more enriched in ^{18}O than predicted for steady-state conditions. The underestimation of leaf temperature, which has consequences for the vapour concentration-gradient in the leaf boundary-layers, may also have contributed to this discrepancy (Buhay et al., 1996).

By comparison, the δD values of upper leaf water are lower than predicted, despite the potential underestimation of leaf temperature. Buhay et al. (1996) described a further temperature-dependent effect that can cause leaf water to be depleted of D relative to that

predicted for evaporative enrichment. They suggest that hydrogen-isotope exchange with leaf tissue, dissolved constituents in leaf cell-fluids, and(or) the water reservoirs that feed the leaves may be responsible for low leaf-water δD values, with greater depletion occurring at lower temperatures.

The one analysis of plant water from the inflorescence of *C. longifolia* lies on the mixing line in Figure 2-2, and does not show the large enrichments in D and ^{18}O observed for upper leaf water. This flower had not yet emerged from the sheath, and hence was insulated from extensive transpiration. The δ-values of water from fully emergent flowers would be expected to show much larger transpiration-related effects.

It is reasonable to expect that the isotopic composition of plant water and materials derived from it, such as cellulose and phytoliths, would be affected by photosynthetic pathway (Leaney et al., 1985; Yakir et al., 1994). C4 plants are favoured in water-stressed climates because they can thrive with lower rates of transpiration. In principle, low stomatal conductance produces a more stagnant boundary layer within the stoma. This increases the residence times of water in leaves, thus making possible successive episodes of their enrichment in D and ^{18}O (Leaney et al., 1985; Cooper and DeNiro, 1989). However, Flanagan et al. (1991) found no difference in the isotopic pattern for leaf water from C3 versus C4 plants in their field studies. Direct comparison of the results for *C. longifolia* and *A. breviligulata* is not possible since these samples were not collected under identical conditions. But no obvious difference exists in the isotopic behaviour of leaf water in these two plants. Differences in the D- and ^{18}O-enrichment of leaf water between species likely depend more on path length of water movement through the leaf to the sites of evaporation than on photosynthetic pathway (Farquhar and Lloyd, 1993; Flanagan, 1993).

2.4.3. Oxygen Isotopic Behaviour of Phytolith Silica

The previous discussion described the wide range of plant-water δ-values possible in transpiring tissues of *C. longifolia* and *A. breviligulata*. The impact of that variability on the oxygen isotopic composition of silica phytoliths from these grasses is considered next. The control of relative humidity on the $\delta^{18}O$ values of silica phytoliths was first recognized by Bombin and Muehlenbachs (1980). They concluded that paleotemperature estimates using the oxygen-isotope compositions of ancient phytoliths would be impossible without independent knowledge of relative humidity. A major purpose of this discussion is to evaluate their conclusion.

The oxygen-isotope compositions of phytoliths and plant water from the corresponding portion of the grass are compared in Figure 2-3 for samples of *C. longifolia* and *A. breviligulata* from southwestern Ontario. Under these climatic conditions, where the minimum average daily relative humidity is fairly high (Table 2-1), only water from the leaves, and potentially the inflorescence, is subject to significant ^{18}O-enrichment during transpiration. These tissues also contain the phytoliths that have the highest $\delta^{18}O$ values (Table 2-3). However, the degree of ^{18}O-enrichment of phytoliths from transpiring versus non-transpiring tissues is much smaller than measured for plant water from comparable plant parts, particularly when mid-day δ-values are considered. This difference likely occurs because the oxygen isotopic composition of biogenic opal represents a cumulative weighted average for phytoliths formed in a particular portion of a plant over its lifetime. In such a system, daily fluctuations in plant-water $\delta^{18}O$ values are smoothed to average values over time. Our data also show that the diurnal variation in relative humidity is too rapid for most leaf water to attain the isotopic compositions predicted for steady-state conditions. Instead, the majority of leaf and inflorescence phytoliths crystallized from water that is buffered from the extremes of ^{18}O- enrichment during transpiration.

Silica phytoliths from the rhizomes, stems and sheaths of *C. longifolia* and *A. breviligulata* collected in 1995 are consistently enriched in ^{18}O relative to greenhouse plant-

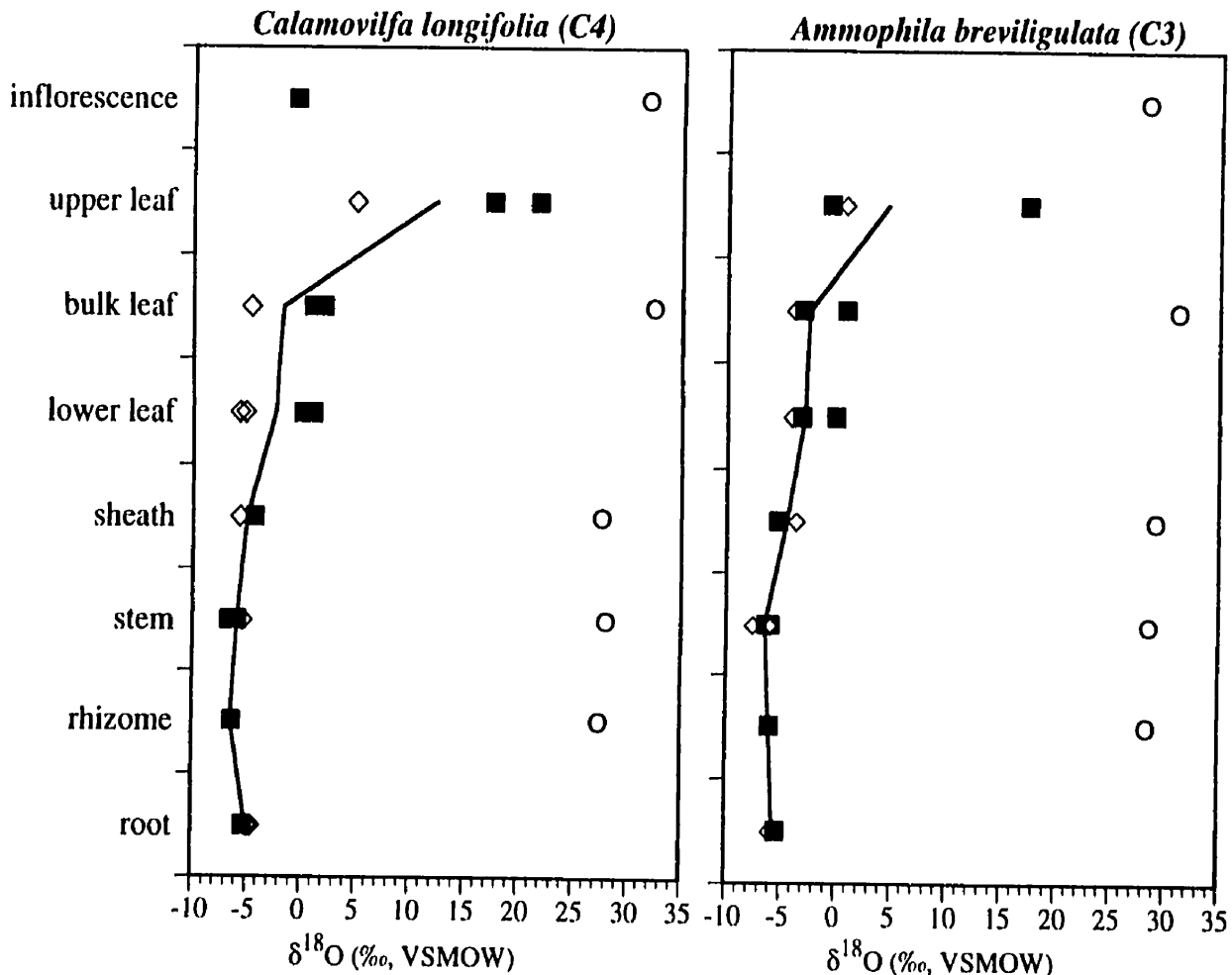

Figure 2-3. Comparison of $\delta^{18}O$ values for plant silica (O) and plant waters (◇ - pre-dawn; ■ - mid-day) for various parts of *C. longifolia* and *A. breviligulata* collected in southwestern Ontario (data from Tables 2-4a and 2-5). The solid line illustrates the average plant-water composition (*C. longifolia*, 14-32°C, 86-37% relative humidity; *A. breviligulata*, 14-25°C, 86-56% relative humidity).

water by ~34‰ under the growing conditions at Pinery Provincial Park and London (Table 2-4a). A virtually identical value was obtained for the 1994 samples (Webb and Longstaffe, 1997), despite the non-ideal analytical procedures used to obtain these isotopic results (Table 2-4b). The constancy of this value is notable, given that it describes two different grass species, grown during two different years at two different southwestern Ontario localities, and reflects the range of temperatures and non-transpired water compositions throughout the life of each plant. This result suggests both that the plant-water compositions within non- (or weakly) transpiring tissues showed little variability between 1994 and 1995, and that the silica crystallized at isotopic equilibrium with the plant water.

Leaf and inflorescence phytoliths, which presumably formed at similar temperatures, ought to be characterized by a similar spread of $\Delta^{18}O_{silica\text{-}water}$. If so, leaf and inflorescence phytoliths from *C. longifolia* and *A. breviligulata* in southwestern Ontario should have precipitated from leaf water with a $\delta^{18}O$ of about -2‰ (excepting one anomalously low inflorescence value for *A. breviligulata*) (Tables 2-4a, 2-4b). Such compositions are characteristic of the daily average bulk leaf or lower leaf water rather than upper leaf water (Table 2-5). It seems that most phytoliths formed in the lower leaf where the majority of tissue occurs, and transpiration-induced ^{18}O-enrichment is much smaller (Webb and Longstaffe, 1997). Such behaviour, coupled with the failure of leaf water to reach steady-state conditions in these grasses, serves to dampen considerably the ^{18}O-enrichment of silica phytoliths that otherwise might be expected in transpiring tissues.

Phytoliths from *C. longifolia* and *A. breviligulata* in southwestern Ontario have very similar $\delta^{18}O$ values (Tables 2-4a, 2-4b; Fig. 2-3), despite the variation in morphotypes and density of phytolith assemblages between C3 and C4 plants (Kaufman et al., 1985; Lanning and Eleuterius, 1989). In general, C4 plants have greater photosynthetic capacity, and increased nitrogen- and water-use efficiency in high light, high temperature and low humidity environments, which gives them a competitive edge over C3 plants in typical

grassland climates (Teeri and Stowe, 1976; Berry and Downton, 1982). However, in the temperate environment of Pinery Provincial Park, the domains of these two species overlap on the open sand dunes (Elfman et al., 1985), and this is reflected in the similarity of phytolith and plant-water isotopic behaviour. The relatively small differences in phytolith $\delta^{18}O$ values that are observed between the two species likely arise from microclimatic variations, in which water stress is not an important issue. More detailed study is required to reveal the extent of phytolith isotopic variability at an individual locality.

While the ^{18}O-enrichment of phytoliths from transpiring tissues is much smaller than might have been predicted directly from plant-water δ-values, variations arising from differences in relative humidity can still be discerned clearly. In southwestern Ontario, where the average minimum and maximum daily relative humidities are highest among the sites studied (54-95%, Table 2-1), the difference in oxygen isotopic composition between phytoliths from transpiring versus non- (or weakly) transpiring tissues of *C. longifolia* is about ~4‰ (Fig. 2-4, Table 2-3). At Onefour, Alberta, which has a much lower range of average minimum and maximum relative humidities (34-79%, Table 2-1), the difference in $\delta^{18}O$ values is ~8‰. At Thedford, Nebraska, the average relative humidity lies between these extremes, and the difference in $\delta^{18}O$ values is ~6‰. Phytoliths from strongly transpiring tissues at all three localities have similar $\delta^{18}O$ values. However, results for other sites suggest that this outcome is fortuitous (Webb and Longstaffe, unpublished data).

Figure 2-4 and Table 2-3 also illustrate that the silica $\delta^{18}O$ values for non- (or weakly) transpiring tissues (rhizome, stem, sheath) are relatively constant at the Alberta and Nebraska localities, as well as in southwestern Ontario. Such behaviour is predictable, since transpiration does not play a direct role in determining the oxygen-isotope composition of phytoliths from these plant parts. However, each location has a unique $\delta^{18}O$ value for rhizome, stem and sheath phytoliths from *C. longifolia*, which is a function

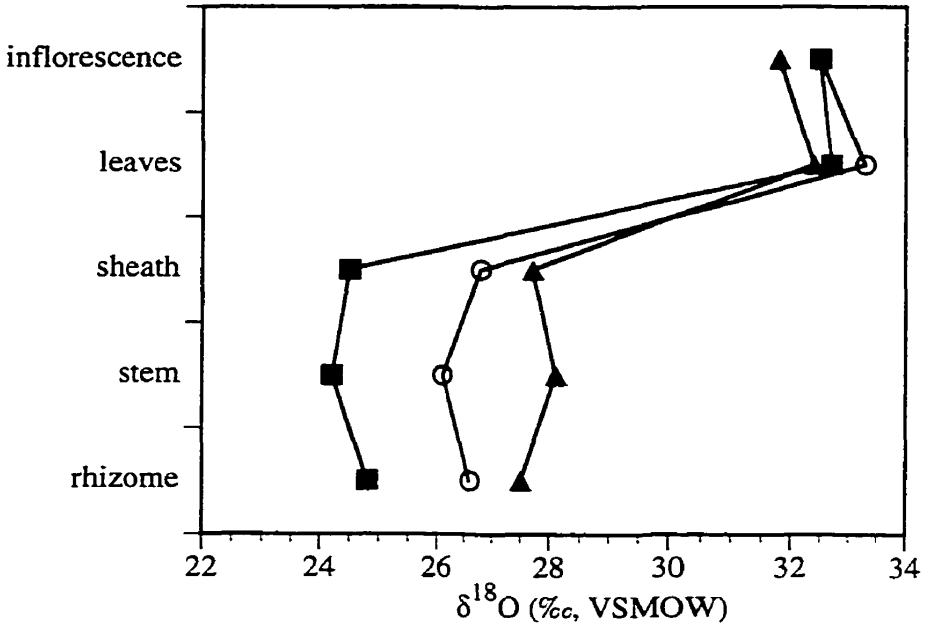

Figure 2-4. Phytolith $\delta^{18}O$ values for plant parts of *C. longifolia* collected at Onefour, Alberta (■), Thedford, Nebraska (O), and Pinery Provincial Park, Ontario (▲) (data from Table 2-3). The difference in $\delta^{18}O$ values between transpiring and non-transpiring tissues increases with decreasing average relative humidity (see Table 2-1 for climatic information).

of both the temperature of phytolith crystallization and the isotopic composition of soil water.

2.4.4. Oxygen-Isotope Geothermometry Using Phytolith Silica

The equilibrium oxygen-isotope fractionation between amorphous silica and water has been described by many equations (Labeyrie, 1974; Knauth and Epstein, 1976; Kawabe, 1978; Kita et al, 1985; Juillet-Leclerc and Labeyrie, 1987; Shemesh et al., 1992; Shahack-Gross et al., 1996; Brandriss et al., 1998). We have selected the equation of Shahack-Gross et al. (1996) to interpret the results of our study:

$$t\ (°C) = 5.8 - 2.8\ (\delta SiO_2 - \delta H_2O - 40). \qquad \text{(Eqn. 2-6)}$$

This relationship was derived for phytolith silica from wheat stems grown under monitored temperatures, and was chosen because the materials and methods used were most similar to our own. A preliminary attempt to interpret the data for the 1994 sampling of *C. longifolia* by applying the equation of Labeyrie (1974), which was developed using unexchanged silica diatoms, yielded significantly higher temperatures (Webb and Longstaffe, 1997).

At the Pinery Provincial Park site, temperatures of 23°, 23° and 26°C were calculated for the formation of rhizome, stem and sheath silica from *C. longifolia* collected in 1995, using the average daily plant-water compositions for the corresponding tissue (Table 2-4a). Very similar results were obtained for silica collected from this grass at the same locality in the previous year (21°, 22°, 26°C; Table 2-4b), although the latter data carry much larger errors. Slightly lower temperatures were obtained for *A. breviligulata* collected from the same site in 1995 (rhizome, 21°C; stem, 20°C; sheath, 23°C; Table 2-4a), and from London, Ontario in 1994 (stem, 21°C; sheath; 22°C; Table 2-4b), the latter results again being subject to much larger errors. These values match the late season growing temperature in this area very closely (22°C, Table 2-1), during which much of the silica is expected to precipitate (Shahack-Gross et al., 1996). The slight difference in temperature between the two species deduced from isotopic data cannot be deemed significant.

Nevertheless, it is intriguing to note that *A. breviligulata* has a longer and cooler average growing season, with photosynthetic activity peaking from April to early July (Maun and Baye, 1989), and that *C. longifolia* generally grows higher on the dunes, in areas of least shade. However, detection and confirmation of such microclimatic variations require a study more detailed than ours.

Similar determinations for leaf silica are complicated by the extreme variation in leaf-water $\delta^{18}O$ values described earlier. Unreasonable 'temperatures' (>40°C) result if the average upper leaf-water compositions are used in the calculation (Tables 2-4a, 2-4b). However, most leaf silica did not form in isotopic equilibrium with the very ^{18}O-enriched waters typical of the upper leaf. Lower leaf- or bulk leaf-water $\delta^{18}O$ values most closely match the water compositions required for silica formation in this tissue. Using these parameters, temperatures of 20-22°C and 20-21°C (Table 2-4a) can be calculated for *C. longifolia* and *A. breviligulata* collected from Pinery Provincial Park in 1995; similar results can be obtained using the less precise 1994 data set (22-24°C, 20-21°C; Table 2-4b). If we reverse this approach, and use the measured temperature and bulk leaf $\delta^{18}O_{silica}$ values to calculate the oxygen isotopic composition of leaf water involved in silica precipitation, we obtained the same value (~-2‰) deduced earlier using the value of $\Delta^{18}O_{silica\text{-}water}$ determined empirically at Pinery Provincial Park. Under natural ambient conditions, most leaf water from which the silica precipitates has $\delta^{18}O$ values far below the $\delta^{18}O$ values calculated for mid-day, steady-state conditions.

The results for the Pinery Provincial Park grasses show that the silica precipitated in equilibrium with plant water, and that the $\delta^{18}O$ values of phytoliths provide an accurate record of growing temperatures. However, before this method can be applied in studies of paleoclimate, either as a direct measure of temperature or an indirect indication of the isotopic composition of ancient meteoric water, several difficulties must be resolved. The first of these problems is the potential for variation between the isotopic composition of local meteoric water and the soil-water reservoir utilized by grasses.

Growing 'temperatures' calculated using stem-silica $\delta^{18}O$ values and local meteoric water $\delta^{18}O$ values are compared in Table 2-6 with actual temperatures for the southwestern Ontario, Nebraska and Alberta localities. For the Pinery Provincial Park samples, the calculated temperature for *C. longifolia* is very similar to the May-August average temperature, but somewhat lower than the temperature that most closely reflects the major period of silica crystallization in this grass. For *A. breviligulata*, there is a reasonably close match with the May-August average temperature; the slightly lower calculated temperature may in fact better represent the preponderance of biomass development in this species. This relatively good agreement between calculated and measured temperatures is likely to be characteristic of localities where mid-day relative humidity is high and rainfall is abundant. In such areas, soil moisture is least likely to be significantly enriched in ^{18}O relative to local meteoric water.

The deviation between calculated and measured temperatures is much larger for *C. longifolia* stem phytoliths from the Nebraska and Alberta sites (Table 2-6). We suggest that the major reason for this difference is that soil water utilized by these grasses is more enriched in ^{18}O relative to local meteoric water. As the daily average relative humidity and amount of precipitation decrease, the opportunity for evaporation from unsaturated soils increases, leading to a greater spread between soil water and meteoric water $\delta^{18}O$ values, particularly at the rooting depths of these grasses (Allison et al., 1984; Bariac et al., 1994). Such effects are amplified in coarse sandy soils with low water-holding capacity, which are typical of dune and many grassland environments that we have examined.

The soil-water $\delta^{18}O$ values needed to produce the measured late season growing temperatures at Thedford, Nebraska and Onefour, Alberta are about 3‰ higher than local meteoric water (Table 2-6). Such values are well within the range of soil-water ^{18}O-enrichment reported for such environments (e.g., Hsieh et al., 1998). Indeed, the relatively modest enrichment deduced here for these relatively arid environments may reflect in part the ability of these grasses to store water in their rhizomes, as well as the

Table 2-6. Comparison of calculated and measured temperatures for stem phytoliths.

Location	Species	*$\Delta^{18}O_{SiO2\text{-}H2O}$ for stems	*Calculated T (°C)	*May-Aug. T (°C)	*July-Aug. T (°C)	*Meteoric Water $\delta^{18}O$ (‰, VSMOW)	§Estimated Soil Water $\delta^{18}O$ (‰, VSMOW)
Pinery	*C. longifolia*	35.3	19	20	22	-7.2	-6.8 to -6.1
Pinery	*A. breviligulata*	35.8	18	20	22	-7.2	-6.3 to -5.6
Onefour	*C. longifolia*	38.7	9	16	19	-14.5	-12.2 to -11.1
Thedford	*C. longifolia*	36.6	15	20	25	-10.5	-8.8 to -7.0

*δ-values from Tables 2-1 and 2-3. §Geothermometer of Shahack-Gross et al. (1996); see text for discussion

depth to which portions of their living root-rhizome systems can extend. It may also be, in part, a consequence of successive evaporation-condensation cycles. A combination of low vegetative cover, progressive warming by the sun and poor thermal conductivity in sandy soils results in high surface temperatures, which drop rapidly both above and below the sand surface (Baldwin and Maun, 1983). On sunny days we have measured temperature gradients of up to 20°C, both two meters above and below the soil surface at Pinery Provincial Park. Lower ambient temperatures in the evening rapidly cool the soil surface, reversing the sub-surface temperature gradient. As a result, water vapour derived from deeper in the soil can be condensed in the shallower subsurface (Ranwell, 1972; Baldwin and Maun, 1983; Walker and Brunel, 1990). This process would contribute water that was depleted of D and ^{18}O relative to soil moisture present at sites of evaporation (Walker and Brunel, 1990). In addition, atmospheric moisture can condense on the soil surface and in soil pore-spaces if surface temperatures fall below the dew point (Ranwell, 1972). Such behaviour can cause soil water to be enriched in, or depleted of, ^{18}O and D, depending on local conditions.

Other factors also may have contributed to the low calculated growth temperatures that resulted from the pairing of stem-silica and meteoric water $\delta^{18}O$ values. First, the meteoric water samples from which we obtained our data were collected from shallow groundwater, rivers and lakes. These bodies are much less sensitive than soil water to input of late summer precipitation, which tends to be enriched in ^{18}O. Second, the weather stations from which we obtained the data in Table 2-1 were not located at the sites of grass collection; they ranged in distance from 10 km for Pinery Provincial Park to 100 km for Onefour, Alberta. However, it is unlikely that either factor significantly reduces the need to account for soil-water ^{18}O-enrichment in more arid regions. We have not completed a similar regional comparison for C3 plants but expect that the soil-water effect will be smaller, as C4 plants tend to grow in higher proportions in areas with high levels of sunlight and drier soils than C3 plants (Teeri and Stowe, 1976).

2.4.5. Implications for Soil-Phytolith Assemblages

The preceding discussions illustrate the magnitude of phytolith ^{18}O-enrichment that can be attributed to evaporative enrichment of soil water, and for leaf and inflorescence tissues, transpiration within the plant. Both processes are intimately related to aridity during phytolith formation. A means to assess the total enrichment arising from evapotranspiration will be needed before it is possible to interpret fully the $\delta^{18}O$ values of soil-phytolith assemblages, particularly those that retain a substantial fraction of biogenic silica formed in transpiring tissues. In the simplest case of a soil containing only grass phytoliths formed locally under identical climatic conditions, the oxygen-isotope composition of the assemblage will be a weighted average of phytoliths produced in transpiring versus non- (or weakly) transpiring tissues. For the grasses analyzed in this study, up to 40-45% of the phytoliths formed in strongly transpiring tissues (leaves and inflorescence; Table 2-2). Depending on relative humidity, the $\delta^{18}O$ value of the bulk phytolith assemblage from these samples would therefore be 2 to 4 ‰ higher than obtained using silica derived only from non- (or weakly) transpiring tissues (sheaths, stems and rhizomes).

Future efforts should be focused on effective methods to estimate with reasonable accuracy, the size and origin of ^{18}O-enrichment of soil-phytolith assemblages. In some cases, it may be possible to separate phytoliths produced in non-transpiring versus transpiring tissues based on physical shape. It may also be that the proportion of phytoliths originating from transpiring tissues decreases in soil assemblages with time. While no specific phytolith morphology is uniquely characteristic of a particular plant part, leaf-phytolith assemblages from the grasses investigated here tend to contain a higher percentage of rod-shaped "long-cell" silica bodies, which have a higher surface area than most stem or rhizome phytoliths. Such differences could lead to comminution and preferential dissolution of grass-leaf phytoliths in the ancient soil record. It may also be possible to infer relative humidity as well as temperature by a combined interpretation of δD

and $\delta^{18}O$ values of phytoliths, provided that some primary hydrogen is retained in the silica. Pairing of phytolith isotopic data with other proxies in soils whose isotopic composition is more directly related to soil-water composition, such as pedogenic minerals, should also provide significant insight into the temperature and relative humidity of ancient continental surface environments.

2.5. CONCLUSIONS

The oxygen-isotope compositions of silica phytoliths from modern samples of the grass species *C. longifolia* and *A. breviligulata* preserve valuable information about climatic conditions during plant growth. The $\delta^{18}O$ values of phytoliths deposited in non-transpiring tissues (rhizome, stem, sheath) are determined directly by the temperature of silica crystallization and the corresponding plant-water composition, and accurately record measured growing temperatures when the isotopic geothermometer of Shahack-Gross et al. (1996) is applied. The magnitude of any difference between the average $\delta^{18}O$ value of plant water in non-transpiring tissues and local meteoric water reflects the extent of evaporation experienced by the soil-moisture reservoir that feeds the grass, which increases with increasing aridity of the environment. Average relative humidity has an additional role in determining the oxygen isotopic compositions of phytoliths formed in leaves and other transpiring tissues. However, the transpiration-related ^{18}O-enrichment of leaf and inflorescence phytoliths (4 to 8‰ in the samples described here, increasing with decreasing relative humidity) is smaller than anticipated. Leaf silica is precipitated from plant water that has not achieved the extreme ^{18}O-enrichment predicted using steady-state models. With increased understanding of the limits on phytolith ^{18}O-enrichment likely to occur over a range of relative humidities, it may yet prove possible to use the $\delta^{18}O$ values of soil-phytolith assemblages from ancient grassland environments for paleoclimatic reconstruction.

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Chapter 3. Climatic influences on the oxygen isotopic composition of biogenic silica deposited in prairie grass

3.1. INTRODUCTION

Aqueous silicic acid ($Si(OH)_4$) enters plants through their root systems and is polymerized as amorphous opal-A in cells and intercellular spaces along the transpiration stream (Raven, 1983). These discrete silica bodies are known as phytoliths. Previous studies have demonstrated that the oxygen-isotope composition of phytoliths develops in isotopic equilibrium with the plant waters from which they precipitate (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000). Because the fractionation between plant silica and plant water varies as a function of temperature, the isotopic composition of phytoliths can be a good proxy for this variable. However, plant-water $\delta^{18}O$ values are dependent on soil-water $\delta^{18}O$ values and the susceptibility of different plant tissues to be affected by climatic variables, including relative humidity. Hence, the $\delta^{18}O$ values of phytoliths will vary within individual plants as well as between plants grown under differing climatic conditions in response to these parameters in addition to temperature dependent fractionation.

In the terrestrial environment, the $\delta^{18}O$ and δD values of the water from which silica is precipitated are dependent on climate and, as a result, can be quite variable. Soil water entering the roots of the plant determines the original isotopic composition of the plant water from which the silica is precipitated. Soil water can become enriched in ^{18}O with respect to precipitation through surface evaporation associated with low relative humidity (Allison et al., 1984). Relative humidity also affects the degree to which water in the leaves and other transpiring tissues becomes enriched in ^{18}O and D by kinetic and equilibrium fractionation associated with transpiration (Flanagan and Ehleringer, 1991; Farquhar and Lloyd, 1993).

Previously, the isotopic composition of phytoliths has been examined under controlled or closely monitored conditions for living grasses (Bombin and Muehlenbachs,

1980; Shahack-Gross et al., 1996) and in fossil soil-phytolith assemblages (Fredlund, 1993). In this study we examine the natural variation in $\delta^{18}O$ values of silica phytoliths within one grass species over a range of natural climatic regimes. If the integrated effects of temperature and relative humidity on the $\delta^{18}O$ values of plant silica can be shown to be predictable for living grasses, there is a great potential for the use of phytoliths as a paleoclimatic indicator.

Phytoliths are produced in all plant species but are particularly abundant in grasses (Metcalfe, 1960). The production of phytoliths over wide geographic and climatic gradients, plus their persistence in soil profiles, makes them available for paleoclimate studies in environments where other microfossils may be absent or scarce. Already, morphological studies of soil-phytolith assemblages are widely used in archaeology to determine the vegetation, general climate and temperature at the time of soil formation (Twiss et al., 1969; Rovner, 1971; Piperno, 1984; Mulholland, 1989; Powers et al., 1989; Alexandre et al., 1997b; Fredlund and Tieszen, 1997). Stable isotope investigations have the potential to provide more detailed climatic information, given a clear understanding of the relationships between the $\delta^{18}O$ values of phytoliths on one hand, and temperature, soil-water $\delta^{18}O$ values and relative humidity, on the other hand.

3.2. METHODS

3.2.1. Sample collection

In this study we investigate the oxygen-isotope behaviour of biogenic silica deposited in living samples of *Calamovilfa longifolia*, commonly known as prairie sandreed grass, from sites throughout the mid-continent of North America. Naturally grown samples of *C. longifolia* were collected over a two-week period at the end of each growing season (late August) in 1995 and 1996, across a transect that represents a wide range in climatic parameters (Fig. 3-1, Table 3-1). *C. longifolia*, a C4 plant, is present

across the mixed- and tall-grass prairie regions of the mid- and northern Great Plains in both Canada and the United States. This species was selected because it produces abundant phytoliths, thrives throughout a variety of climatic regimes and is generally found growing in sandy soils. The high infiltration rate of rain into the deeper layers of sandy soils reduces the evaporative loss of rainwater from the surface (Guiru et al., 1992). *C. longifolia* can produce roots to depths of three meters in order to use this deeper supply of soil water efficiently (Barnes and Harrison, 1982).

Samples were collected from 1) Pinery Provincial Park, Ontario, Canada; 2) Colorado Springs, Colorado, USA; 3) Long Branch Nature Preserve, Havanna, Illinois, USA; 4) Kellogg, Minnesota, USA; 5) Aroya, Colorado, USA; 6) Quinter, Kansas, USA; 7) Thedford, Nebraska, USA; 8) Bonner, Nebraska, USA; 9) Fertile, Minnesota, USA; 10) Cheyenne, Wyoming, USA; 11) the Agriculture Canada Station at Onefour, Alberta, Canada; 12) Dundurn, Saskatchewan, Canada; 13) Sprucewoods Provincial Park, Manitoba, Canada; 14) the Seminoe Dam, Rawlins, Wyoming, USA; 15) Broadus, Montana, USA; 16) the University of Alberta's research station at Kinsella Ranch, Kinsella, Alberta, Canada; and 17) Kortes Dam, Wyoming, USA (Fig. 3-1).

We expect that the oxygen-isotope composition of silica deposited in most other grass species will behave in a similar manner. Comparable relationships have been observed in C3 grasses *Triticum aestivum* (Shahack-Gross et al., 1996) and *Ammophila breviligulata* (Webb and Longstaffe, 2000). We predict that *C. longifolia* will be representative of the behaviour of the majority of silica deposited from grasses in the soils of the North American Great Plains over the last 10,000 years.

3.2.2. Silica extraction

Silica was extracted from dozens of individual plants that comprise each sample in order to obtain sufficient quantities for analysis. This material represents an average for opal-A deposited in each grass species over its lifetime (one to five growing seasons,

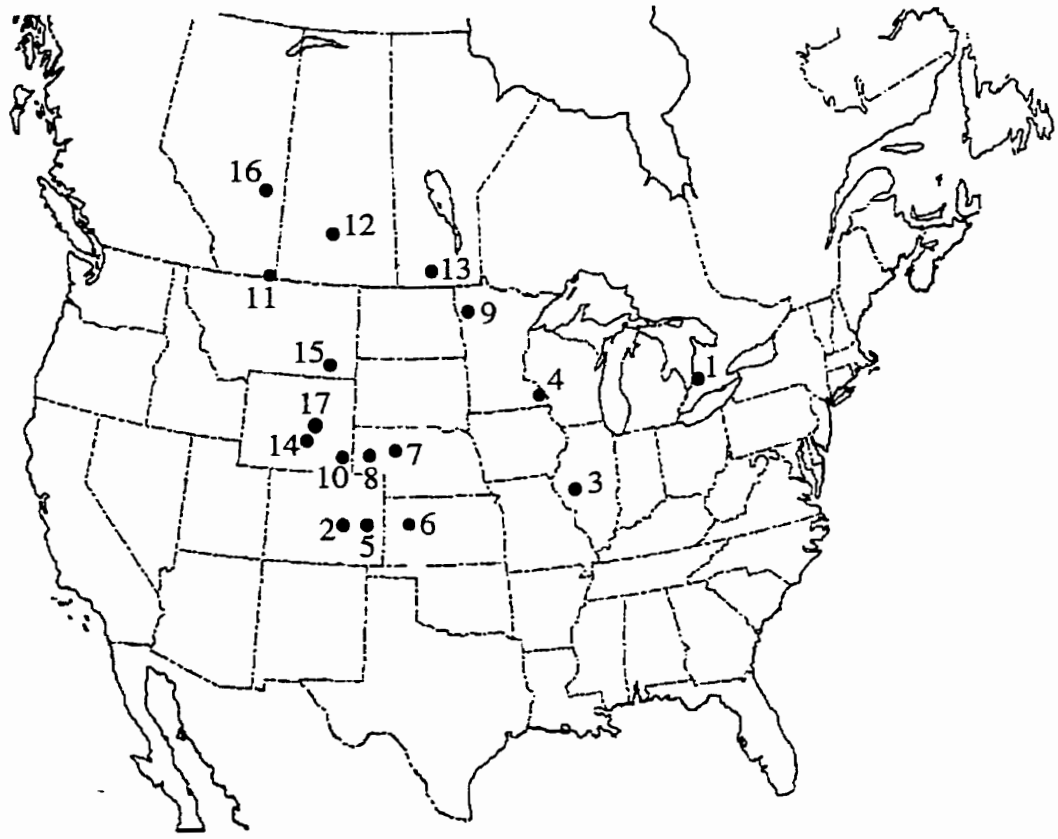

Figure 3-1. Sample locations across North America. 1) Pinery Provincial Park, ON; 2) Colorado Springs, CO; 3) Havanna, IL; 4) Kellogg, MN; 5) Aroya, CO; 6) Quinter, KS; 7) Thedford, NE; 8) Bonner, NE; 9) Fertile, MN; 10) Cheyenne, WY; 11) Onefour, AB; 12) Dundurn, SA; 13) Sprucewoods, MA; 14) Rawlins, WY; 15) Broadus, MT; 16) Kinsella, AB; 17) Kortes Dam, WY.

Table 3-1. Sample locations and climatic information *

Location	Latitude	Longitude	Year	†Temperature (°C)		†Max/min RH (%)		Precipitation (mm)			§δ"O values of meteoric water (‰, VSMOW)			¥calculated soil-
City, Province (State), Country				May-Aug.	July-Aug.	May-Aug.	July-Aug.	Annual	May-Aug.	July-Aug	surface water	ground water	summer precip.	water δ"O values
1. Pinery, ON, CAN	43°19'N	81°45'W	1995	20	22	92/54	95/57	919	297	171	-7.2	-10 (1)	-6.0	-6.9
early summer (May-June)			1996	15		90/53		1229	422		-7.2	-10 (1)	-5.8	-9.3
late summer (May -August)			1996	18	20	90/53	90/52	1229	422	138	-7.2	-10 (1)	-6.8	-9.2
			1997	17	19	97/59	97/61	900	410	211	-7.2	-10 (1)	-6.1	-8.5
2. Colorado Springs, CO, USA	38°51'N	104°45'W	1996	19	20	85/32	87/36	408	205	154	-5.8	-13.5		-7.8
3. Havanna, IL, USA	40°18'N	90°03'W	1996	21	23	94/54	94/52	797	411	153	-6.2	-6.4	-4.1 (2)	-7.3
4. Kellogg, MN, USA	44°18'N	91°59'W	1995	20	22	89/47	93/54	715	350	38	-8 (3)	-7 (4)	-6 (5)	-7.7
5. Aroya, CO, USA	38°51'N	103°07'W	1996	21	22	89/39	91/41	408	205	154	-7.2	-10.0		-7.6
6. Quinter, KS, USA	39°04'N	100°13'W	1996	22	24	94/49	94/48	635	441	240	-5.9	-9.7 (6)		-7.9
7. Thedford, NE, USA	42°02'N	100°49'W	1995	20	25	89/41	89/35	694	368	112	-10.5	-12.1	-5.8 (7)	-8.8
8. Bonner, NE, USA	41°56'N	103°01'W	1995	19	24	92/39	90/29	512	316	75		-12.1	-9.6 (16)	
9. Fertile, MN, USA	47°32'N	96°16'W	1995	18	20	90/46	92/50	620	345	231	-9.0	-12.7	-8 (8)	-10.0
10. Cheyenne, WY, USA	41°20'N	104°34'W	1995	16	21	84/38	75/24	478	290	36	-14.3	-17 (9)		-11.5
11. Onefour, AB, CAN	49°07'N	110°28'W	1995	16	19	79/35	78/34	384	181	70	-14.5 (10)	-18 (1&11)	-14.2 (16)	-12.2
12. Dundurn, SA, CAN	51°49N	106°30'W	1995	15	16	91/44	99/53	370	227	170	-11.5	-19 (1)	-13.6 (2)	-12.8
13. Sprucewoods, MA, CAN	49°52'N	99°22'W	1995	17	19	91/45	95/46	545	217	75	-11.0	-14.5		-12.3
14. Rawlins, WY, USA	42°03'N	106°56'W	1995	15	19	83/30	75/18	418	227	38	-15.6		-14.9 (16)	-13.2
15. Broadus, MT, USA	46°24'N	106°16'W	1995	20	24	84/36	79/30	375	218	76	-14.4	-19 (12)	-12.2 (16)	-11.7
16. Kinsella, AB, CAN	53°00'N	111°32'W	1995	14	14	91/47	95/54	389	222	143	-18.5 (13)	-18.9 (14)	-15.6 (15)	-14.0
17. Kortes Dam, WY, USA	42°03'N	106°56'W	1995	15	19	83/31	75/19	418	227	38	-16.4			-14.3

*Climatic information from Environment Canada, and the on-line Climate Visualization System, National Climatic Data Center. †Average of daily values; RH = relative humidity. §Average values from local rivers, lakes, precipitation and shallow groundwater measured in this study and (1) Fritz et al. (1987); (2) IAEA (1992); (3) Yapp, (1979); (4) median value from Siegel (1989); (5) Hunt et al. (1997); (6) this study and Clarke et al. (1998); (7) Fricke et al. (1998); (8) median value from LaBaugh et al. (1997); (9) Back et al. (1983); (10) this study and Hannagan et al. (1991); (11) Hendry and Schwartz (1988); (12) median value from USGS (1984); (13) this study and Maulé et al. (1994); (14) this study and Fortin et al. (1991); (15) Maulé et al. (1994); (16) Luz et al. (1990). A detailed description of water-sampling locations and water types is provided in Appendix B. ¥Soil-water values are calculated using the May to August temperatures and Equation (3-8) for temperature-dependent fractionation of phytolith silica (see text).

depending on the plant part) at a given location. To determine how silica deposition and the oxygen isotopic composition of the silica, varied within the grass, each sample was divided into six parts: roots, rhizomes, stems, sheaths, leaves and inflorescence. Samples were washed in distilled water to remove detrital minerals and dried at 65°C for a minimum of 36 hours. Dried grass samples were digested in 99% sulfuric acid for an average of two hours. The organic matter was then reacted with 30% hydrogen peroxide until only silica remained (Geis, 1973). Wet-ashing techniques were considered preferable to dry ashing, which has been shown to partially recrystallize opal-A or reduce the size of the phytoliths (Jones and Milne, 1963; Runge, unpublished manuscript). The silica sample was then washed four to five times with distilled water, once with 1.0M HCl and again three times with distilled water using high-speed centrifugation. Once cleaned, the samples were freeze-dried.

Samples were examined by X-ray diffraction, and most showed no evidence of phases other than opal-A. Traces of quartz were present in some samples, especially those from roots in which phytolith concentrations were low and the potential for contamination from soil minerals was high. Gypsum and anhydrite were present in the leaf-silica samples from Pinery Provincial Park in southwestern Ontario, Dundurn, Saskatchewan and the Seminoe Dam location near Rawlins, Wyoming. These phases are believed to be artifacts produced by reacting sulfuric acid with biogenic calcium oxalate, which can also be precipitated in the leaves of grasses. Any oxalate present in the leaf tissue would have been destroyed during sample preparation. Sulphate minerals were removed by dissolution in HCl.

3.2.3. Isotopic analysis

All stable isotope results are expressed in the standard δ-notation, relative to VSMOW for oxygen and hydrogen (Coplen, 1994) where

$$\delta = [(R_{sample} / R_{standard}) - 1] \times 1000 \ (‰) \qquad \text{(Eqn. 3-1)}$$

and R represents D/H or $^{18}O/^{16}O$. Dual inlet, triple collecting, gas-source Optima or Prism II mass spectrometers were used for all measurements. The difference in δ-values between two phases (a and b) is expressed as:

$$\Delta^{18}O_{a-b} = \delta^{18}O_a - \delta^{18}O_b. \qquad \text{(Eqn. 3-2)}$$

Meteoric water was collected from rivers, streams, lakes, rain and/or ground water in close proximity to grass-sample locations. Two milliliters of each sample was equilibrated with CO_2 overnight (Epstein and Mayeda, 1953) for oxygen-isotope analysis. Hydrogen gas for isotopic analysis was produced by reducing (under vacuum) two microlitres of water with metallic zinc at 500°C for twenty minutes (Coleman et al., 1982). Reproducibility was better than ±0.1‰ for oxygen and ±1‰ for hydrogen.

Silica samples were heated under vacuum at 300°C for 12 hours prior to transfer, under a flow of dry nitrogen gas, to nickel reaction-vessels. The samples were then heated at 300°C under vacuum for a further two hours prior to the addition of bromine pentafluoride. Oxygen was liberated from silica by reaction with BrF_5 at 600°C (Clayton and Mayeda, 1963), and prepared for mass spectrometric analysis by conversion to CO_2 gas by reaction with incandescent graphite. During the course of these experiments, the $\delta^{18}O$ value of the laboratory's standard quartz was consistent with a value of +9.7±0.2‰ for standard silica sand (NBS-28).

The isotopic exchange procedure for opal-A described by Labeyrie and Juillet (1982) and Juillet-Leclerc and Labeyrie (1987) was employed in order to account for oxygen-isotope exchange between unstable Si-O bonds and hydroxyl groups in the opal-A during dehydration prior to oxygen-isotope analysis. Separate aliquots of the same silica sample, maintained at 200°C, were exchanged with the vapour produced at 0°C over two different isotopically labelled waters ($\delta^{18}O_{H_2O}$ = +41.6‰ and –0.9‰) in an evacuated environment. This process is intended to fix the oxygen isotopic composition of exchangeable oxygen in the opal-A at a known composition. After allowing exchange to

proceed for six hours, the samples were sintered under vacuum at 1100°C for eighteen hours, to partially recrystallize the silica (Webb and Longstaffe, 2000).

The oxygen-isotope composition of the non-exchangeable oxygen (δ_{silica}) and the percentage of exchangeable oxygen (X) in each phytolith sample, was calculated using the two results ($\delta_{measured\ 1}$, $\delta_{measured\ 2}$) obtained for the exchanged samples and the values determined for the exchanged oxygen ($\delta_{exchanged\ 1\ or\ 2}$):

$$\delta_{exchanged} = \delta_{H_2O\ liquid} + (\Delta_{H_2O\ vapour\text{-}H_2O\ liquid}) + (\Delta_{exchanged\text{-}H_2O\ vapour}), \qquad \text{(Eqn. 3-3)}$$

where $\Delta_{H_2O\ vapour\text{-}H_2O\ liquid}$ at 0°C equals −11.5‰ (Mopper and Garlick, 1971), $\Delta_{exchanged\text{-}H_2O\ vapour}$ at 200°C is 13.5‰ (Labeyrie and Juillet, 1982) and:

$$\delta_{silica} = [\delta_{measured\ 1} - X\ (\delta_{exchanged\ 1})] / (1 - X), \qquad \text{(Eqn. 3-4)}$$

$$\delta_{silica} = [\delta_{measured\ 2} - X\ (\delta_{exchanged\ 2})] / (1 - X). \qquad \text{(Eqn. 3-5)}$$

When the same exchanged silica sample was reanalyzed, the standard deviation of $\delta_{measured}$ averaged ±0.2‰ (twenty-six pairs). When the entire exchange process was duplicated using separate aliquots of the same sample, the standard deviation of $\delta_{measured}$ averaged ±0.2‰ (eighteen pairs). For samples in which one of these steps was repeated, the measure of variance of the calculated δ_{silica} values was ±0.1‰. By comparison, reproducibility of oxygen-isotope values for phytolith samples that were not subjected to the exchange procedure was substantially worse, averaging ±1.2‰ (Webb and Longstaffe, 2000).

3.2.4 Statistical analysis

Comparisons between data sets were performed using single factor ANOVA, student t-tests and paired t-tests with a two-tailed probability (Dytham, 1999). Where $P>0.05$, the variation of the data within a set is comparable to the variation between sets. In this case, the groups of data have equal means and are considered to be derived from the same set. Where $P<0.05$, the data sets in comparison are distinct. All curves were fitted using standard regression.

3.3. RESULTS

Growing-season average daily temperatures and minimum and maximum relative humidities are listed in Table 3-1 for each location. Climatic data for the late growing-season interval (July to August) have also been included in Table 3-1, since the majority of silica phytoliths in most grass species are deposited late in the growing season after the plant cells have matured and begun to senesce (Johnston et al., 1967; Simkiss and Wilbur, 1989; Shahack-Gross et al., 1996). It is uncertain whether the climate signals recorded in the oxygen-isotope compositions of phytoliths collected from mature plants will reflect the climatic influences present over the entire growing season. Other studies have shown that the late growing-season climatic conditions have a dominant influence on $\delta^{18}O$ values of phytoliths as the majority of silica is formed during this period (Shahack-Gross et al., 1996). It has also been demonstrated that July mean daily temperatures can be correlated with diagnostic phytolith-assemblage morphologies. Fredlund and Tieszen (1997) developed a multiple linear regression model that links temperature with the frequency of unique phytolith morphologies that are associated with warm-climate C4 grasses and cool-season C3 grasses. Under the assumption that grassland vegetation in North America is growing in equilibrium with the local climate, the Fredlund and Tieszen (1997) study carries the implication that the formation and distribution of phytoliths is constrained to temperature ranges under which each grass species flourishes.

Our best estimations for the oxygen isotopic composition of meteoric water at each locality are also listed in Table 3-1; a complete summary of the $\delta^{18}O$ values of meteoric water collected at or near the sites at the time of grass sampling is provided in Appendix B. The variation in meteoric water $\delta^{18}O$ values reflects the diverse climatic conditions that exist among the sampling sites. Meteoric waters that are more enriched in ^{18}O generally occur in warmer sites and at lower latitudes as a result of fractionation processes associated with Rayleigh distillation within a moving air mass (Dansgaard, 1964).

The amount of silica contained in dry grass tissues from this species varied from 0 to 8.4%, depending on locality and the part of the plant analysed (Table 3-2). The average silica content was up to 3% higher in tissues through which water more actively transpires (sheaths, leaves and inflorescence). On average, *C. longifolia* contains 0.9% silica by weight (n=20). The average silica content was noticeably higher in arid than relatively humid locations (1.2%, n=9 versus 0.6%, n=11).

The $\delta^{18}O$ values of exchanged samples and the percentage of exchangeable oxygen are summarized in Table 3-3 for silica phytoliths from various plant tissues of *C. longifolia*. For all samples analysed, an average of 4.6±1.7% of phytolith oxygen was susceptible to exchange (Table 3-3). We expect that this value will be typical of most grass species and note that it is similar to that found for silica diatoms (Knauth and Epstein, 1982; Juillet-Leclerc and Labeyrie, 1987).

Differences in morphology and surface area of phytoliths formed in different tissues of the same plant have the potential to affect the amount of exchangeable oxygen present in phytoliths from a specific plant part. Paired t-tests performed using the data from different plant parts reveal that the percentage of exchangeable phytolith oxygen in the sheath is significantly lower than in the inflorescence or rhizome (P=0.005 and P=0.0017 respectively). However, there is no significant difference in the amount of exchangeable oxygen among the other silica fractions (P>0.05). No consistent pattern of oxygen-isotope exchangeability associated with grass phytoliths from different locations is obvious from the data. Nevertheless, there exists a statistical possibility that exchangeability does vary with location (single factor ANOVA, P=0.03).

3.4. Discussion

The oxygen-isotope composition of phytoliths is dependent on soil-water $\delta^{18}O$ values, relative humidity/evapotranspiration and temperature throughout the period of silica formation. Once soil water has entered a plant through its roots, this water can be

Table 3-2. Silica content of *C. longifolia.* A. Humid sites (max/min relative humidity >90/50). B. Arid sites (max/min relative humidity <90/50). C. Average values for all sample locations.

Location	Year	% weight silica *					
City, Province (State), Country		Inflorescence	Leaves	Sheaths	Stems	Rhizomes	Roots
A. Humid locations							
1. Pinery, ON, CAN	1995	1.9	3.3	2.4	0.3	0.5	0.1
early	1996	0.1	1.1	1.4	0.6	0.2	-
late	1996	0.3	1.2	0.6	0.2	0.0	-
	1997	-	2.5	2.2	0.6	0.1	-
3. Havanna, IL, USA	1996	0.4	1.2	1.5	0.2	0.2	0.1
4. Kellogg, MN, USA	1995	0.1	1.0	1.6	0.2	-	-
6. Quinter, KS, USA	1996	-	7.0	5.6	1.5	-	0.2
9. Fertile, MN, USA	1995	0.1	2.6	1.3	0.7	0.2	-
12. Dundurn, SA, CAN	1995	0.8	3.5	-	1.0	0.3	0.3
13. Sprucewoods, MA, CAN	1995	2.0	-	3.0	-	-	-
16. Kinsella, AB, CAN	1995	-	1.9	2.3	0.4	0.0	0.0
Average		0.7	2.5	2.2	0.6	0.2	0.1
†Relative contribution of total plant silica		2.4	38.9	33.6	7.9	9.9	7.3
B. Arid locations							
2. Colorado Springs, CO, USA	1996	1.7	5.9	3.1	0.5	0.4	0.1
5. Aroya, CO, USA	1996	1.3	2.6	4.0	0.7	0.4	-
7. Thedford, NE, USA	1995	3.0	4.2	4.4	0.6	0.5	0.1
8. Bonner, NE, USA	1995	1.9	2.8	3.7	-	0.3	0.0
10. Cheyenne, WY, USA	1995	3.7	-	8.4	1.4	0.6	0.3
11. Onefour, AB, CAN	1995	2.8	6.7	4.4	0.4	0.4	0.1
14. Rawlins, WY, USA	1995	2.7	2.3	-	0.4	0.4	0.1
15. Broadus, MT, USA	1995	2.1	5.6	6.1	3.2	0.8	0.0
17. Kortes Dam, WY, USA	1995	5.0	5.6	4.1	0.4	0.1	-
Average		2.7	4.5	4.8	1.0	0.4	0.1
†Relative contribution of total plant silica		4.7	35.6	38.1	6.9	11.8	2.7
C. All locations							
Average		1.8	3.4	3.3	0.7	0.3	0.1
†Relative contribution of total plant silica		4.2	36.5	36.0	7.3	11.7	4.3

*Relative to dry weight of grass. †Avg. silica content x avg. percentage of total biomass (inflorescence = 0.022, leaves = 0.10, sheaths = 0.10, stems = 0.091, rhizomes = 0.344 and roots = 0.344), as estimated from personal observation and Maun (1985).

Table 3-3. O-isotope results (‰, VSMOW) and % exchangeable oxygen of silica phytoliths from *C. longifolia*.

Location	Year	Inflorescence		Leaf		Sheath		Stem		Rhizome		Root		§Soil-phytolith
City, Province (State), Country		$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$	% ex.*	$\delta^{18}O$
1. Pinery, ON, CAN	1995	31.8	6.5	32.4	3.8	27.7	5.1	28.1	6.8	27.5	6.1			28.9
early summer	1996			28.8	4.4	27.5	2.6	27.4	5.3	28.5	5.5			27.5
late summer	1996	29.8	7.0	28.7	4.7	26.7	5.0	26.4	4.4	29.1	5.3			27.3
	1997	32.8	2.4	31.7	5.7	27.2	3.7	27.5	3.2	28.1	4.8			28.6
2. Colorado Springs, CO, USA	1996	30.6	3.9	33.2	2.4	26.8	3.9	27.5	4.9	24.2	5.7	22.8	2.1	28.3
3. Havanna, IL, USA	1996	30.0	8.6	32.3	2.8	29.0	3.1	27.3	2.3					29.6
4. Kellogg, MN, USA	1995			29.7	2.2	26.6	1.0	27.2	4.6					27.4
5. Aroya, CO, USA	1996	35.3	4.7	35.1	4.0	27.4	3.5	27.0	5.6	25.5	5.0			29.5
6. Quinter, KS, USA	1996			29.3	3.7	27.5	5.5	26.3	4.9					27.4
7. Thedford, NE, USA	1995	32.5	3.7	33.3	9.5	26.8	4.4	26.1	5.6	26.6	5.7			28.7
8. Bonner, NE, USA	1995	36.0	6.2	33.4	2.6	25.3	3.2			27.4	5.2			28.3
9. Fertile, MN, USA	1995			28.6	1.9	25.4	3.6	25.7	1.6					26.1
10. Cheyenne, WY, USA	1995	37.2	4.9	36.3	5.9	24.8	5.4	24.8	3.2	25.3	4.6	23.4	6.3	28.8
11. Onefour, AB, CAN	1995	32.5	7.6	32.7	4.4	24.5	5.1	24.2	7.0	24.8	6.9			27.2
12. Dundurn, SA, CAN	1995	29.0	8.7	29.3	3.4	25.1	4.9	23.9	4.9	23.9	4.1	22.9	3.7	25.9
13. Sprucewoods, MA, CAN	1995	30.6	6.9			25.6	4.2	23.7	5.2					26.6
14. Rawlins, WY, USA	1995	37.1	2.6	36.9	4.8			23.6	2.3	24.7	1.5			28.9
15. Broadus, MT, USA	1995	34.7	5.9	33.1	5.9	22.6	4.0	23.3	3.0	22.8	4.3			26.3
16. Kinsella, AB, CAN	1995			28.2	4.1	24.3	1.6	23.0	5.5					25.0
17. Kortes Dam, WY, USA	1995	38.1	7.1	38.9	4.8	26.6	5.1	22.5	3.5	25.0	7.1			30.2

*Average amount of oxygen affected by exchange. § Calculated from Equation (3-10); see text.

enriched in ^{18}O by transpiration in tissues such as the leaves and inflorescence. The extent of this change depends on plant physiology and relative humidity, with ^{18}O-enrichment increasing as relative humidity decreases. The size of equilibrium oxygen-isotope fractionation between phytoliths and plant water at any particular location in the vegetation is controlled by temperature. The degree to which these factors influence the $\delta^{18}O$ values of phytoliths within different tissues and between grasses grown under different climatic conditions is discussed next.

3.4.1. Plant physiology

At all sample locations, the highest concentrations of phytoliths within the grass are found in the leaves and sheaths (Table 3-2, Fig. 3-2). The silica concentration-gradient within an individual sample of *C. longifolia* is dependent on the degree to which the different tissues participate in transpiration. As water is removed from the surface of the leaf during the transpiration process, water remaining in the leaf becomes enriched in solutes, including silicic acid. Once the concentration of silicic acid exceeds supersaturation, or pH conditions become favourable, silica is precipitated in the tissues where the majority of water is being removed (Simkiss and Wilbur, 1989).

Transpiration rates increase under conditions of low relative humidity, resulting in elevated rates of silica precipitation within plants at arid sites. In a previous study, Johnston et al. (1967) found no correlation between the abundance of silica and climatic parameters. In this study, however, the average silica content of transpiring tissues (leaf, sheath and inflorescence) is much higher at sites that are relatively arid than in localities with a higher relative humidity (wt. % silica = 2.7 to 4.8 % versus 0.7 to 2.5%; Table 3-2, Fig. 3-2). Much lower average silica abundances occur in non-transpiring tissues (stem, rhizome), but again these parts of the grass are much richer in silica in arid regions (0.4 to 1.0 %) than in more humid areas (0.2 to 0.6%) (Table 3-2, Fig. 3-2). In these tissues, transpiration does not have a direct effect on the degree of silica precipitation. However,

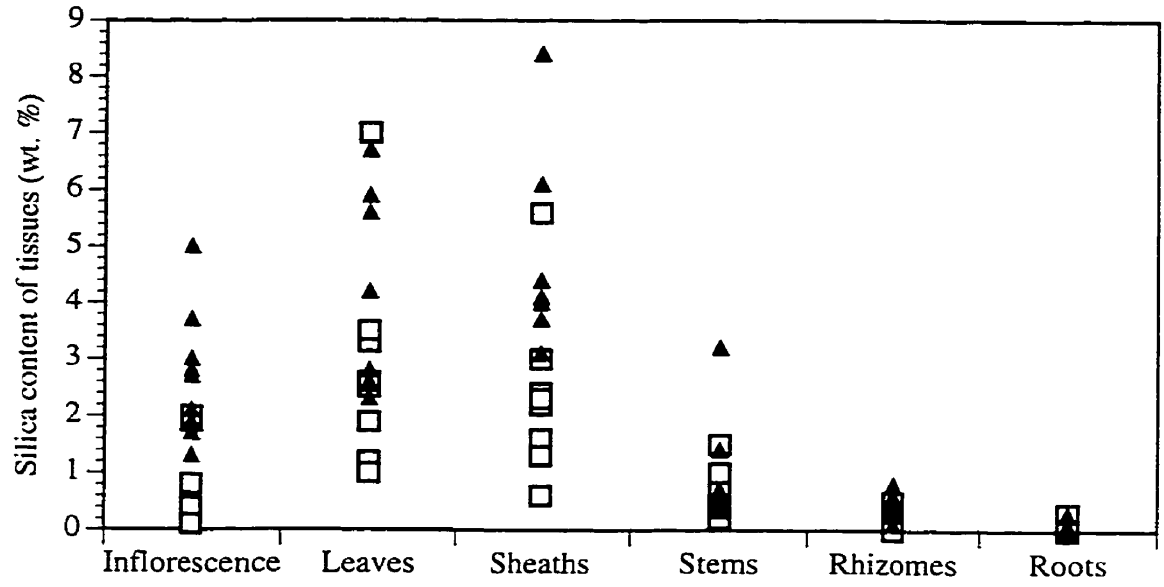

Figure 3-2. Silica content (weight %) of tissues of *C. longifolia* collected from (□) humid and (▲) arid locations across North America. Data from Table 3-2.

it is likely that, in arid regions, silica content is noticeably higher in the rhizomes, stems and sheaths (i.e. Broadus, Montana) because of increased movement of silicic acid-rich root water through the plant, which is required to replenish water lost during transpiration (Rosen and Weiner, 1994). The overall average silica content of *C. longifolia* is twice as high in arid regions (1.2%) than humid regions (0.6%). The relative contribution of silica from different plant parts to the overall phytolith assemblage, however, is similar, regardless of growing conditions (Table 3-2).

In light of these general observations, the negative correlations obtained between silica concentration and amount of precipitation or relative humidity are as expected, with silica concentrations increasing with aridity (Fig. 3-3). However, the imperfect correlations may suggest that the availability of silicic acid in the soil also affects the rate of phytolith production within a plant. The silicic acid concentration in soil water is a function of the availability and solubility of minerals, including amorphous silica, in a soil, as well as the residence time and pH of soil water (Raven, 1983; Söjberg, 1996; Alexandre et al., 1997a). Summer precipitation, which feeds the roots of *C. longifolia*, is expected to have a limited residence time in the unsaturated zone of the soil at each of our sites. Soil water present in the active rooting zone is quickly taken up by root systems, evaporated from the soil surface or rapidly drained to the ground-water reservoir, given the large pore spaces and high infiltration rates characteristic of sandy soils (Maulé et al., 1994; Berndtsson et al., 1996).

The sandy soils on which these samples were grown were analysed by X-ray diffraction and found to have the same basic mineralogy (in order of abundance: quartz, plagioclase and potassium feldspar, with traces of calcite). However, the presence of amorphous silica in the soil is not detectable by X-ray diffraction and consequently the soil-phytolith concentrations are unknown. Previous studies of the dissolution of biogenic opal in temperate climates (Bartoli and Wilding, 1980) revealed that the dissolution rate of silica phytoliths (0.5-3.0 mg Si/L) was not sufficient to explain

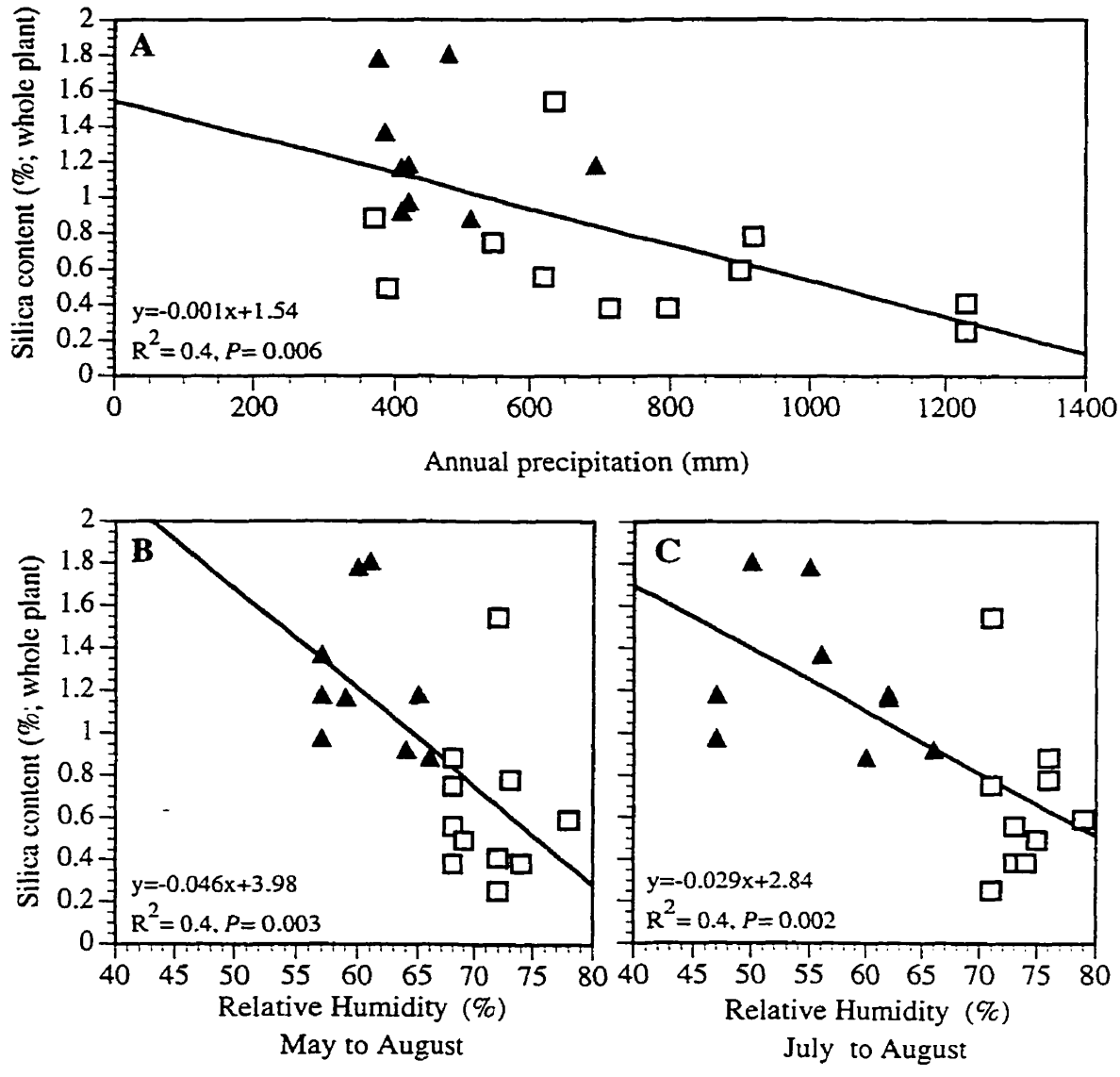

Figure 3-3. (A) Silica content of whole grass samples (weight %) versus annual precipitation (mm). (B & C) Silica content of whole grass samples (weight %) versus average daily relative humidity (%). Symbols represent grasses from relatively humid (□) and arid (▲) locations across North America. The silica content of whole grass samples from each location was determined by multiplying the % silica content of each tissue by the percent contribution of that tissue to the total biomass (Table 3-2), and nomalizing to 100%. In the cases where silica content was unavailable for specific tissues, the average silica concentration for that tissue from humid or arid locations accordingly, were substitituted for that tissue.

completely, the $Si(OH)_4$ concentrations commonly found in soil water (1-40 mg Si/L; Farmer, 1986). By comparison, the dissolution of amorphous silica in tropical soils provides the majority of silicic acid present in soils (Alexandre et al., 1997a). Under these conditions, the presence of phytoliths in a soil can increase the amount of silicic acid available for plant up-take and phytolith precipitation regardless of relative humidity conditions. The production of silicic acid in soil water from the dissolution of silica phytoliths is an important consideration for future research regarding the silica content in plants as well as the stability of fossil-soil phytolith assemblages.

To summarize, it appears that the concentration of silica within *C. longifolia* is dependent on the concentration of $Si(OH)_4$ in the soil water and relative humidity, and that the distribution of silica within the plant is related to transpiration processes. This pattern of silica deposition is indicative of passive uptake and transport of silicic acid along the transpiration stream (Jones and Handreck, 1965). There seems to be no active biological control on its distribution (Epstein, 1994). Although a passive mechanism of phytolith precipitation suggests that there is no metabolic oxygen-isotope fractionation associated with the formation of phytoliths, systematic variations in $\delta^{18}O$ values do exist for silica formed in different tissues of the same plant. This variation is dependent on the degree to which the plant tissues are affected by climatic variables such as relative humidity and temperature, and is discussed next.

3.4.2. Relative humidity

Tissues affected by transpiration (i.e. leaves, inflorescence) contain partially evaporated waters that are enriched in ^{18}O and D relative to soil waters. The degree of this enrichment increases with the rate of transpiration and aridity, fluctuating on a daily and seasonal basis. Leaf-water ^{18}O enrichment can be calculated from steady-state models based on relative humidity, temperature, equilibrium and kinetic fractionation and the size and morphology of the leaf (Leaney et al., 1985; Flanagan and Ehleringer, 1991; Farquhar

and Lloyd, 1993; Buhay et al., 1996). Previously, we have demonstrated that silica in the leaves of *C. longifolia* is not precipitated from the highly ^{18}O-enriched leaf-water predicted from steady-state models for mid-day conditions (Webb and Longstaffe, 2000). Instead, leaf silica appears to form from water with an isotopic composition similar to average daily lower-leaf water (measured) or average daily bulk-leaf water (calculated) (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000). In contrast, the water that resides in non-transpiring tissues (stems and rhizomes) is not involved in transpiration and therefore not influenced directly by relative humidity. Since there is no significant fractionation of soil-water $\delta^{18}O$ values during uptake through plant roots, these plant parts contain water with an isotopic value similar to that of the soil water, regardless of daily or seasonal changes in relative humidity (White et al., 1985; Flanagan and Ehleringer, 1991).

We make the assumption that all above-ground plant tissues (inflorescence, leaf, sheath and stem) at the same location grow at the same temperature, given that other studies in which the difference between leaf and stem temperature was measured found the difference to be negligible (Shahack-Gross et al., 1996). It follows then, that the variations in the $\delta^{18}O$ values of silica from above-ground plant tissues are a function of the water from which the silica precipitated.

There is no significant difference between the $\delta^{18}O$ values of the inflorescence and leaf silica (paired t-test, $P= 1.0$); it appears that the water in both of these tissues experiences similar amounts of ^{18}O-enrichment as a result of transpiration. There is also no significant difference in $\delta^{18}O$ values between phytoliths formed in sheaths, stems and rhizomes (Table 3-3; paired t-tests for sheath-stem, $P= 0.06$; stem-rhizome, $P= 0.4$; sheath-rhizome, $P= 1.0$).

Sheath tissues have stomata and contain high concentrations of silica, suggesting that the sheaths are active in transpiration. Hence, sheath waters ought to experience at least some ^{18}O enrichment as a result of transpiration. However, such enrichment does

not seem to manifest itself in the oxygen isotopic composition of the sheath phytoliths, which have $\delta^{18}O$ values very similar to those formed in the entirely non-transpiring tissues. The sheaths have fewer stomatal pores than the leaves, reducing the number of sites where water loss and associated ^{18}O-enrichment of the residual water can occur. In addition, the observed increase in the $\delta^{18}O$ values of leaf water from the base to the tip of the leaf has been attributed to a supply of progressively ^{18}O-enriched water via successive leaf-water pools (Wang and Yakir, 1995). Sheath water represents the beginning of this string of pools and therefore sheaths contain water with $\delta^{18}O$ values most similar to stem water. The potential for ^{18}O-enrichment of plant water increases when transpiration rates are high. Under these circumstances, the rate of water movement from the stem through the sheath increases in order to supply sufficient water to the leaf. The increased influx of non-fractionated stem water into the sheath may counteract any back diffusion of ^{18}O- and D-enriched molecules from sites of evaporative enrichment, minimizing the enrichment of water in sheath tissues during transpiration (Farquhar and Lloyd, 1993). Hence, we regard the sheath tissues as weakly transpiring and recognize their affinity, in terms of their $\delta^{18}O_{silica}$ behaviour, with the strictly non-transpiring rhizomes and stems.

Based on these observations, we conclude that leaf silica is deposited in equilibrium with mildly ^{18}O-enriched leaf water, with the amount of enrichment between leaf silica and stem silica being equal to the average enrichment between average-daily bulk leaf water and soil water:

$$\delta_{stem\ silica} - \delta_{soil\ water} = \delta_{leaf\ silica} - \delta_{leaf\ water}. \qquad \text{(Eqn. 3-6)}$$

The average degree of ^{18}O-enrichment in leaf water and consequently leaf silica is dependent on the transpiration rate and relative humidity. As relative humidity decreases, the rate of plant transpiration increases and leaf water becomes increasingly enriched in ^{18}O. The ^{18}O-enrichment of leaf silica relative to stem silica for the samples analysed in this study has been plotted against daily average relative humidity in Figure

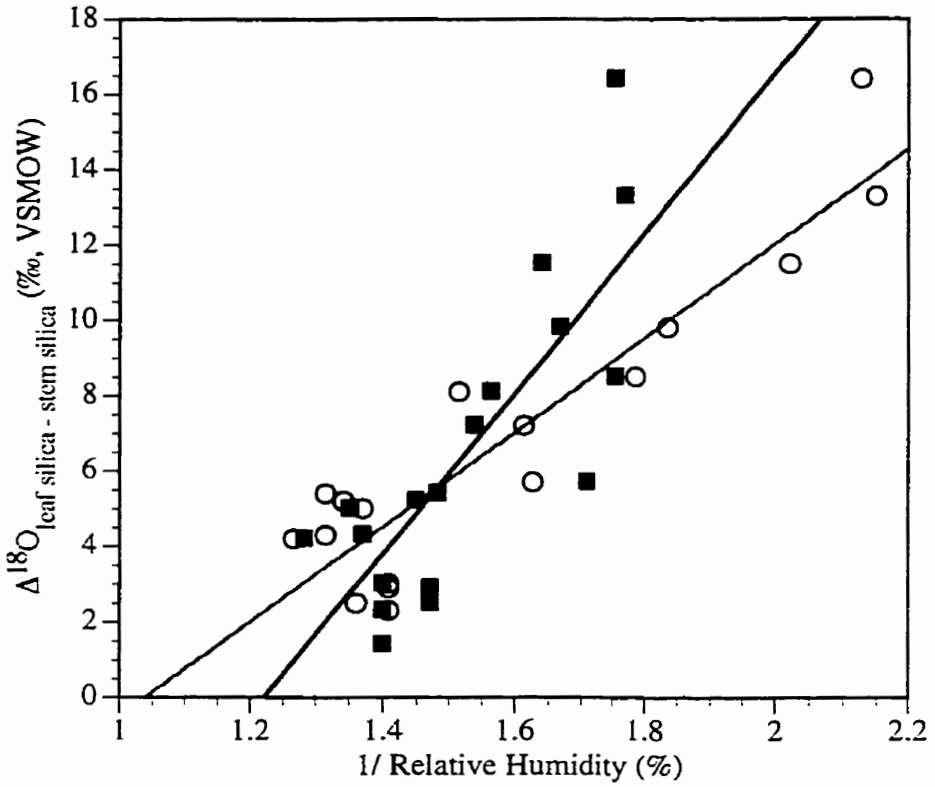

Figure 3-4. Relationship between values of $\Delta^{18}O_{leaf\ silica\ -\ stem\ silica}$ and average daily relative humidity for the May to August (■; $\Delta^{18}O_{leaf\ silica\ -\ stem\ silica} = 21.3/h - 26.0$, $R^2 = 0.6$, $P = 6.4 \times 10^{-5}$) and July to August (O; $\Delta^{18}O_{leaf\ silica\ -\ stem\ silica} = (12.5/h) - 13.0$, $R^2 = 0.9$, $P = 1.4 \times 10^{-7}$) growing periods.

3-4. The best correlation is observed with late-season (July-August), daily average relative humidity (h) (R^2=0.9, P=3.7x10^{-7}):

$$\Delta^{18}O_{\text{leaf water-soil water}} = \Delta^{18}O_{\text{leaf silica-stem silica}} = (12.5/h) - 13; \qquad \text{(Eqn. 3-7)}$$

This expression is very similar to that reported by Yapp (1979) for the evaporation of body fluids in terrestrial snails, whose carbonate shells were used in paleoclimate studies. In previous phytolith studies, the evaporation relationship developed by Yapp (1979) was used successfully to calculate the $\delta^{18}O$ values of leaf water involved in leaf-silica formation (Bombin and Muehlenbachs, 1980). The correlation observed in Figure 3-4 is better using the July to August data because the spread of relative humidity values during the late growing season better represents the differences in water stress at the individual sites (Fig. 3-4).

3.4.3. Temperature

It has been demonstrated previously that plant silica is formed in oxygen isotopic equilibrium with plant water (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000). It follows that the fractionation between plant silica and plant water ($\Delta^{18}O_{\text{silica-plant water}}$) in individual plant parts should arise solely from temperature effects. In non-transpiring plant tissues (stems, rhizomes, roots) where plant water is not kinetically enriched during transpiration, $\delta^{18}O_{\text{plant water}}$ values are equal to $\delta^{18}O_{\text{soil water}}$ values. Hence, any differences in $\Delta^{18}O_{\text{silica-soil water}}$ values observed for these tissues ought to be the result of a change in temperature. Such variations are apparent between values of $\delta^{18}O_{\text{silica}}$ among stem, rhizome and root tissues within a single plant (Table 3-3).

Although rhizome and stem phytoliths likely precipitate from soil water of very similar oxygen isotopic compositions, their $\delta^{18}O_{\text{silica}}$ values are not the same for the majority of samples of *C. longifolia* analyzed (Fig. 3-5a). This difference likely reflects a slight variation in the temperature of silica precipitation in the stems versus the rhizomes. The underground tissues are buffered from the extreme variations in temperature

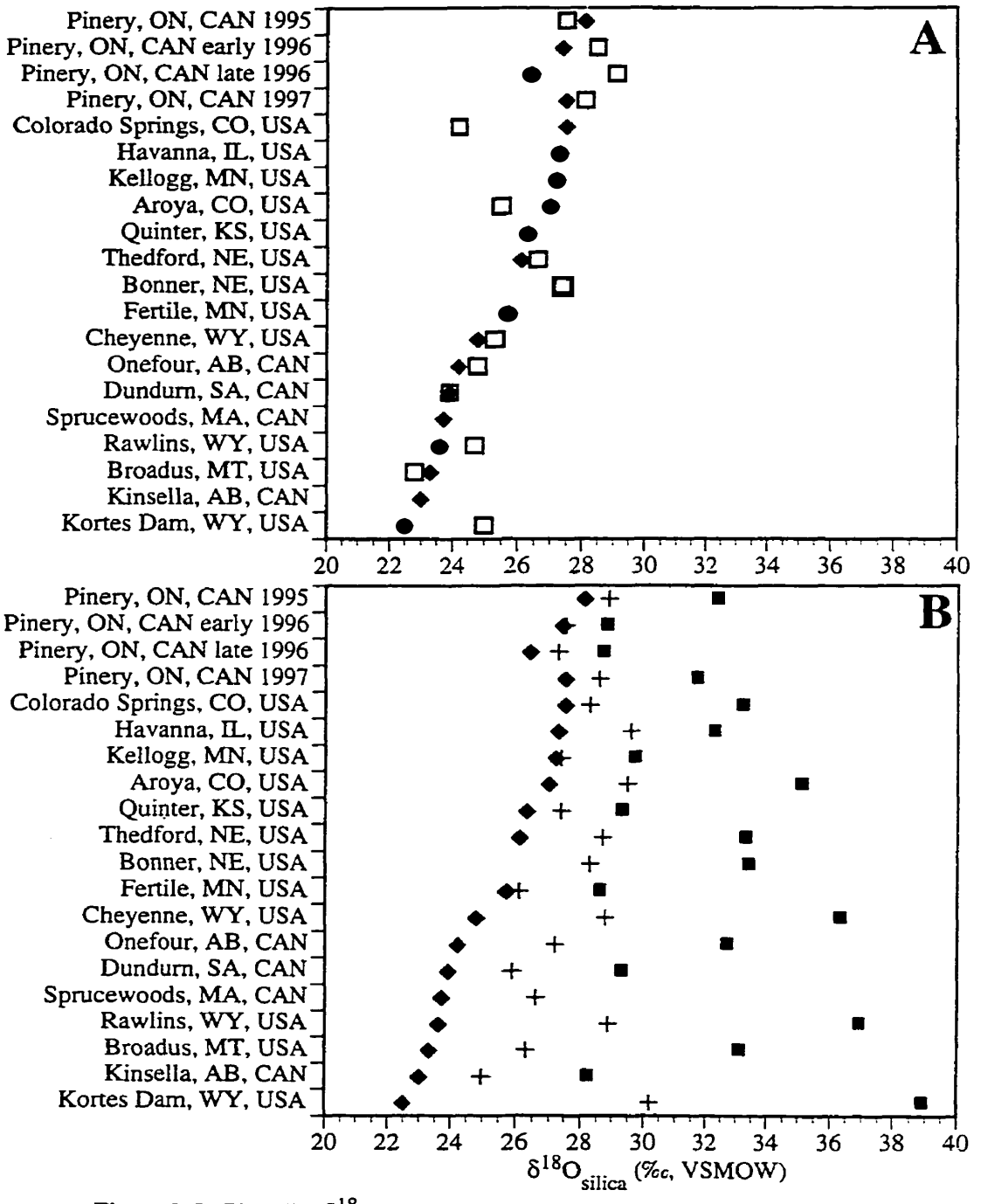

Figure 3-5. Phytolith $\delta^{18}O$ values for *C. longifolia* collected at different sites. A) Symbols represent phytoliths extracted from (◆) stem and (□) rhizome tissues. B) Symbols represent phytoliths extracted from (■) leaves, (◆) stems and (+) the calculated $\delta^{18}O$ values of the soil-phytolith assemblages for each site.

produced by daily climate changes. The sandy substrates, in which most of these grasses were grown, are poor thermal conductors. High temperatures present at the soil's surface during the peak of the day drop rapidly with depth below the surface (Baldwin and Maun, 1983). Rhizomes are insulated from the warming effects of the sun and do not experience the rapid drop in temperature associated with the cooling of the night air. Rhizome silica that formed at lower average temperatures than coexisting stem silica can be expected to have a higher $\delta^{18}O$ value and such behaviour was observed for many of our samples (Fig. 3-5a).

In addition, differences between rhizome- and stem-silica $\delta^{18}O$ values may be the result of differences in age. *C. longifolia* is perennial and underground rhizomes survive several growing seasons (up to 5 years at the depth sampled for this study). However, new shoots are produced each year. Consequently, the rhizome and root tissues are unlikely to exhibit the increase in silica content at the end of each growing season observed in the senescent above-ground tissues. Silica formed in the above-ground tissues will form a record only of the most recent growing season temperatures, weighted towards late growing-season conditions. However, rhizome-silica $\delta^{18}O$ values will reflect several years of growth with silica deposition having occurred over the entire growing season, during which temperatures and soil-water $\delta^{18}O$ values would have fluctuated.

Within individual plant tissues, silica is deposited over a range of temperatures, which vary diurnally as well as seasonally. Any temperature signal recorded by the silica $\delta^{18}O$ value will therefore be a weighted average of these temperatures. Figure 3-6 shows the variation of $\delta^{18}O_{silica}$ values for the stems, sheaths, leaves and inflorescence with average daily (May to August) temperatures. Correlations between the average temperatures for the July to August period and the $\delta^{18}O_{silica}$ values for above-ground tissues are similar to the relationships shown in Figure 3-6.

Equilibrium fractionation between plant water and silica during phytolith formation will act to reduce the $\delta^{18}O$ values of the silica as temperatures increase. Hence, the

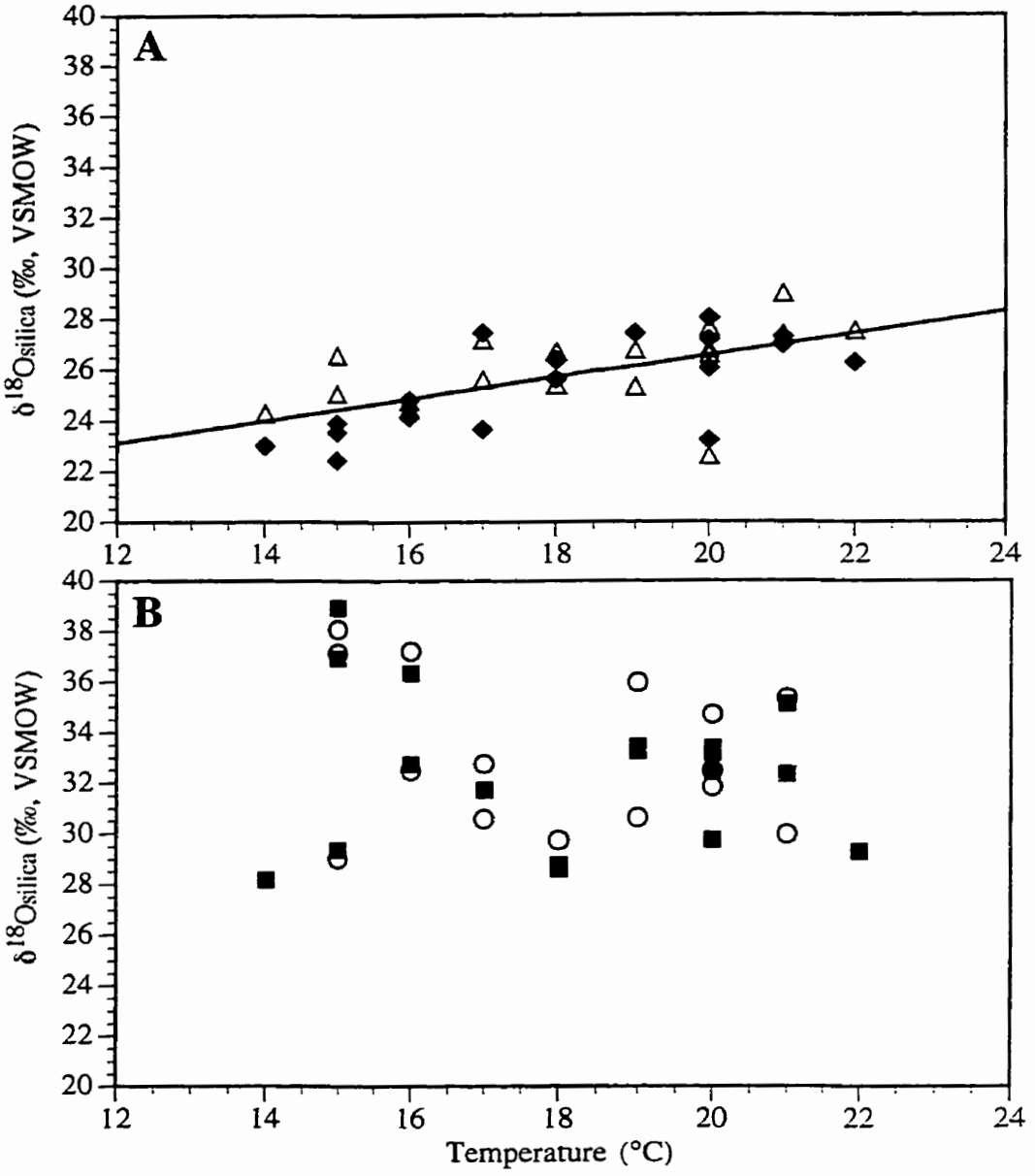

Figure 3-6. Correlation between $\delta^{18}O_{silica}$ values and average daily growing temperatures (May to August) for: A) non- or weakly transpiring *C. longifolia* tissues: sheaths (Δ) and stems (◆); and B) transpiring *C. longifolia* tissues: inflorescence (O) and leaves (■). The solid line describes the correlation between temperature and silica from sheath and stem tissues: $\delta^{18}O_{silica} = 0.43$ x temperature (°C) + 18.0 ($R^2 = 0.4$, $P = 0.0001$).

positive correlation shown in Figure 3-6a between the $\delta^{18}O$ values of stem and sheath silica on one hand, and temperature on the other hand, cannot be related directly to temperature-induced equilibrium isotope-fractionation between plant silica and plant water. Instead, this relationship most likely reflects higher $\delta^{18}O_{soil\ water}$ values at locations that experience higher average temperatures. Higher temperatures are often associated with low-latitude areas where precipitation is generally enriched in ^{18}O relative to more northern areas. Elevated temperatures are also characteristic of arid regions in which soil waters are most likely to be enriched in ^{18}O through evaporation. The $\delta^{18}O$ values of leaf and inflorescence silica do not correlate with temperature (Fig. 3-6b). In these tissues, it is evident that the variable ^{18}O-enrichment of plant water during transpiration under differing climatic conditions obscures any potential correlation between silica $\delta^{18}O$ values on one hand, and their temperature of formation and/or soil-water $\delta^{18}O$ values on the other.

Although silica uptake and deposition in grasses is passive, transpiration-, photosynthesis- and growth-rates are metabolic processes controlled in part by temperature. If the growth rate of *C. longifolia* is temperature dependent, we can assume that this grass has an increased productivity within a certain temperature range. In this case, most phytoliths may form preferentially over a relatively narrow temperature range, regardless of the climatic variation at a given site. In general, C4 plants have an advantage over C3 plants under conditions of high temperatures, light intensities and water stress. The relative distribution of C4 versus C3 grasses across the Great Plains can be positively correlated with July minimum temperatures (Teeri and Stowe, 1976). In climates where the average maximum daytime temperatures equals 32°C, the canopies of C3 and C4 grasses are equally productive, with higher temperatures favouring the growth of C4 plants and lower temperatures favouring C3 plants (Ehleringer, 1978). In addition, C4 grasses have been shown to germinate more readily at temperatures of 27°C, with maximum rates of photosynthesis and dry-matter accumulation generally occurring

between 30 and 35°C (Black, 1971; Harper, 1977; McWilliam, 1978). In contrast, the production of C4 grasses is limited by cooler temperatures under which C3 plants compete more successfully by assimilating CO_2 more efficiently than C4 plants (Ode et al., 1980). The growth of C4 grasses appears to be inhibited when July minimum temperatures are less than 10°C (Teeri and Stowe, 1976). Temperature dependency also produces a temporal separation in the primary production of different grass types. C3 plants dominate growth in the spring and fall when temperatures are lower, while C4 plants dominate growth in the mid-summer during the warmest periods (Ode et al., 1980).

Not only is the distribution of different species dependent on temperature but separate ecotypes of the same species will also vary in their growth requirements. The growing season is extended in more southern regions and, as a result, southern ecotypes mature later in the season than their northern counterparts (Madakadze et al., 1998). It follows then, that ecotypes of C4 grasses will likely be the most productive during the most favourable conditions of the growing season at any one location. This period may vary from site to site and from year to year, and is not necessarily correlated with the entire growing-season temperatures recorded in Table 3-1. Hence our estimate of growing-season temperatures may not reflect the temperature over the period in which the majority of silica is precipitated. Nevertheless, they should still provide upper and lower temperature limits for phytolith formation at any site.

3.4.4. Meteoric water

The $\delta^{18}O$ and δD values of precipitation vary predominantly with temperature and latitude and to a lesser degree, with altitude and amount of precipitation (Dansgaard, 1964; Rozanski et al., 1993). Locations at high latitudes, which have cooler temperatures, generally have precipitation with lower $\delta^{18}O$ values. As precipitation accumulates in the terrestrial environment, these variations are reflected on a continental scale in reservoirs that include ground water, surface water and soil water. Hence, the stable isotopic

composition of meteoric water can be used as a general indicator of climate. The meteoric water $\delta^{18}O$ values that we have obtained for each sampling locality reflect the typical trend, with the most ^{18}O-poor waters occurring in the coolest and most northern sites (Table 3-1 and Appendix B).

The isotopic composition of ground water represents an accumulated average of yearly precipitation, including ^{18}O-enriched summer precipitation events and winter precipitation (and associated spring run-off) that is generally depleted of ^{18}O relative to the summer rain (Fritz et al., 1987; Krabbenhoft et al., 1990). Individual precipitation events, which collectively form the majority of soil water, can be highly variable in their oxygen- and hydrogen-isotope compositions. Temperature changes create seasonal fluctuations in the isotopic composition of precipitation at a given location, resulting in summer-rain events that can be highly enriched in ^{18}O relative to most winter precipitation. In continental regions, the ^{18}O-enrichment of summer precipitation has been shown to depend not only on temperature, but also on the amount of precipitation and the nature of the atmospheric circulation patterns (Lawrence and White, 1991). However, mixing in the unsaturated zone of precipitation, which has highly variable $\delta^{18}O$ values, with ground water, which generally exhibits only a limited range of $\delta^{18}O$ values, will buffer significant variations in the average $\delta^{18}O$ values of the soil water reservoir (Krabbenhoft et al., 1990).

Grasses have shallow rooting systems that preferentially utilize summer precipitation contained in the upper layers of the soil. This behaviour commonly prevents much of this water from reaching the water table (Gupta, 1979; Darling and Bath, 1988; Berndtsson et al., 1996). However, some winter precipitation, which is generally depleted of ^{18}O relative to summer rain, can also be retained in the soil-water compartment throughout the year where it is available for plant uptake (Maulé et al., 1994). In addition, the $\delta^{18}O$ values of precipitation can be altered in the soil-water reservoir as a result of daily cycles of evaporation and condensation in the unsaturated

zone (Allison et al., 1984; Walker and Brunel, 1990). Since it is likely that the silica phytoliths are precipitated in varying amounts throughout the growing season, it will therefore have formed from soil water that had a reasonably wide range of $\delta^{18}O$ values.

The estimates provided for surface-water $\delta^{18}O$ values at each sampling locality describe the net product of mixing between residual winter precipitation and accumulated summer precipitation, plus evaporation. Whether these values can be used to approximate average soil-water $\delta^{18}O$ values, however, is an issue to be discussed further (below). The relationship between $\delta^{18}O_{silica}$ values and $\delta^{18}O_{surface\text{-}water}$ values is shown in Figure 3-7. There is a positive correlation between stem-, rhizome- and sheath-silica values on one hand, and the $\delta^{18}O$ values of surface waters on the other hand (Fig. 3-7a). Stem-silica $\delta^{18}O$ values can be most directly related to present climatic conditions, with the correlation line between the stem-silica and surface-water $\delta^{18}O$ values being particularly good (Fig. 3-7a; R^2=0.8, P=1.7x10^{-7}). The positive correlation here and also in Figure 3-6a suggests that the variation in the $\delta^{18}O$ values of soil water across the study area strongly overshadows any change in silica $\delta^{18}O$ values that arises directly from differences in growing-season temperatures.

By comparison, the oxygen-isotope compositions of leaf and inflorescence silica are quite scattered and exhibit a weak, apparent negative relationship with surface-water compositions (Fig. 3-7b). This situation arises from the variable degree of leaf-water ^{18}O-enrichment in the grasses at each site. Sites on the western boundary of the Great Plains lie in the rain-shadow of the Rocky Mountains (Fig. 3-1, Sites 2, 5, 10, 14, 15 and 17). The reduced amount of precipitation, increased elevation and corresponding lower temperatures at these locations results in precipitation with lower $\delta^{18}O$ values than elsewhere in the study area. However, the enhanced xeric conditions and decreased average relative humidity at these locations resulted in more extreme leaf-water ^{18}O-enrichment, and consequently, higher leaf and inflorescence $\delta^{18}O_{silica}$ values.

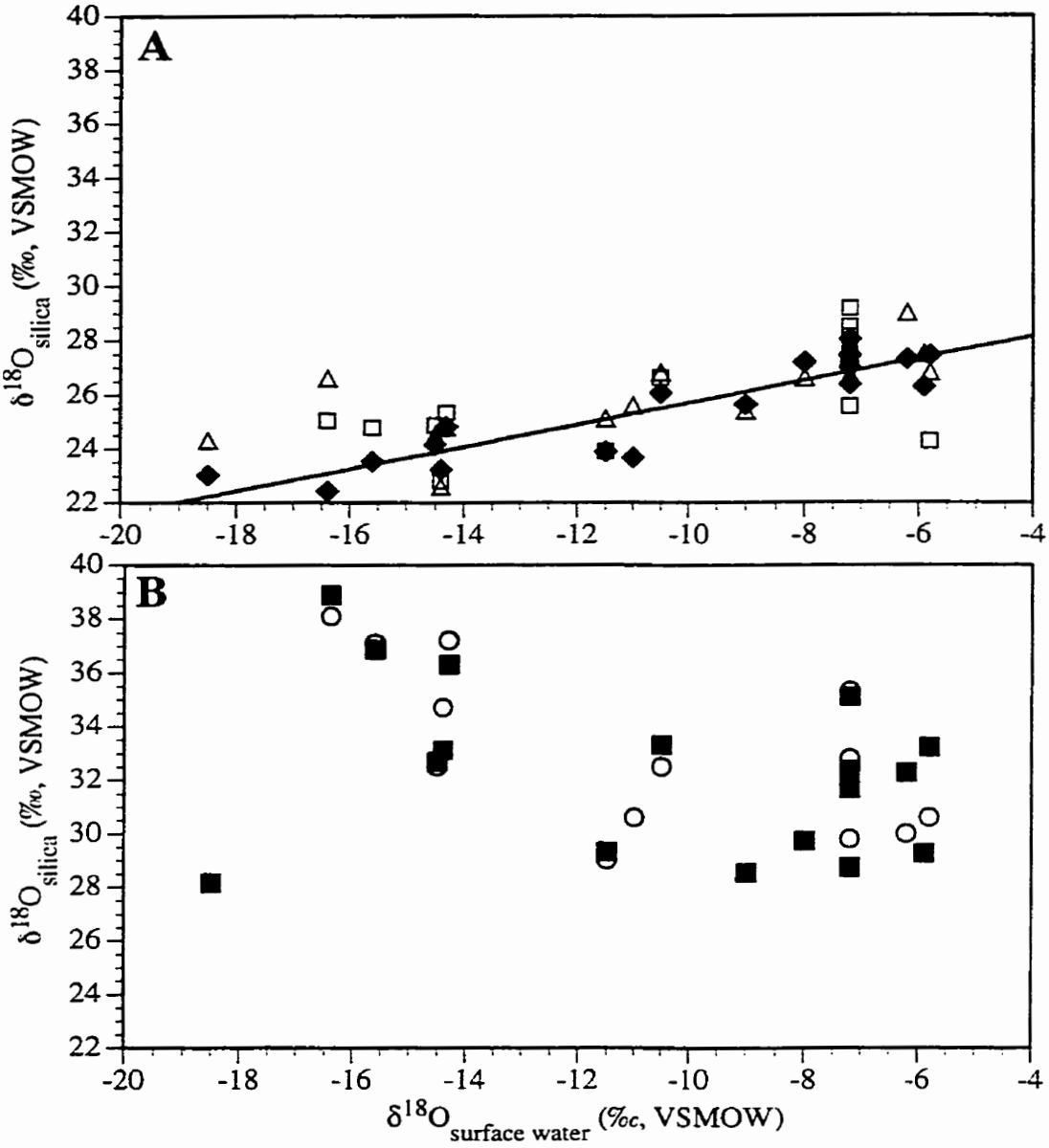

Figure 3-7. Correlation between the $\delta^{18}O$ values of silica from *C. longifolia* and the best estimates of local surface water $\delta^{18}O$ values for A) non- or weakly transpiring plant tissues: sheath (Δ), stem (◆), and rhizome (□); and B) transpiring tissues: inflorescence (O) and leaf (■). Most silica from the non- and weakly transpiring tissues plots close to the relationship between stem silica and water (solid line):

$$\delta^{18}O_{stem\ silica} = 0.40\ (\delta^{18}O_{surface\ water}) + 29.8\ (R^2 = 0.8,\ P = 1.7 \times 10^{-7}).$$

Figure 3-8 illustrates the relationship observed in our data between temperature on one hand and $\Delta^{18}O_{stem\ silica\text{-}water}$ on the other hand for (a) surface water, (b) ground water and (c) summer precipitation. The $\delta^{18}O_{stem\ silica}$ values have been used to represent the $\delta^{18}O$ values of silica extracted from the tissues that we have classified as non- or weakly transpiring (sheath, stem and rhizome). This assumption eliminates both the concern that transpiration may have an effect on sheath-silica $\delta^{18}O$ values, however minimal, and the need to consider temperature changes that may have affected the $\delta^{18}O_{rhizome\ silica}$ values over several years of growth. The paleothermometer equation:

$$t\ (°C) = 5.8 - 2.8\ (\Delta^{18}O_{stem\ silica\text{-}soil\ water} - 40) \qquad \text{(Eqn. 3-8)}$$

determined by Shahack-Gross et al. (1996), is also shown on Figure 3-8. Equation (3-8) was determined for silica phytoliths grown under known climatic conditions and $\delta^{18}O_{water}$ values and is similar in its slope to other reports of the temperature-dependent fractionation between amorphous silica and water (Kita et al., 1985; Shemesh et al., 1992). In a previous study of phytoliths extracted from living samples of *C. longifolia* and a C3 grass, *Ammophila breviligulata*, where $\delta^{18}O_{silica}$ and $\delta^{18}O_{water}$ values from corresponding plant parts were known, temperature predictions made using Equation (3-8) were identical to the measured temperatures (Webb and Longstaffe, 2000).

Our relationship between growing-season temperature (May to August) and $\Delta^{18}O_{silica\text{-}surface\ water}$ has a much shallower slope than Equation (3-8) (Fig. 3-8a):

$$t\ (°C) = 15.3 - 0.68\ (\Delta^{18}O_{stem\ silica\text{-}surface\ water} - 40);\ (R^2 = 0.5,\ P = 0.0008). \qquad \text{(Eqn. 3-9)}$$

This difference in slope suggests that average summer surface-water $\delta^{18}O$ values do not adequately reflect the oxygen isotopic composition of the soil waters that feed the grasses. The soil-water $\delta^{18}O$ values that can be predicted using May to August temperatures and Equation (3-8) are listed in the final column of Table 3-1.

The $\Delta^{18}O_{stem\ silica\text{-}water}$ values calculated using surface-water $\delta^{18}O$ values plot more or less on either side of the equilibrium relationship described by Equation (3-8) (dashed line, Fig. 3-8a). The $\Delta^{18}O_{stem\ silica\text{-}surface\ water}$ values that plot above this line must

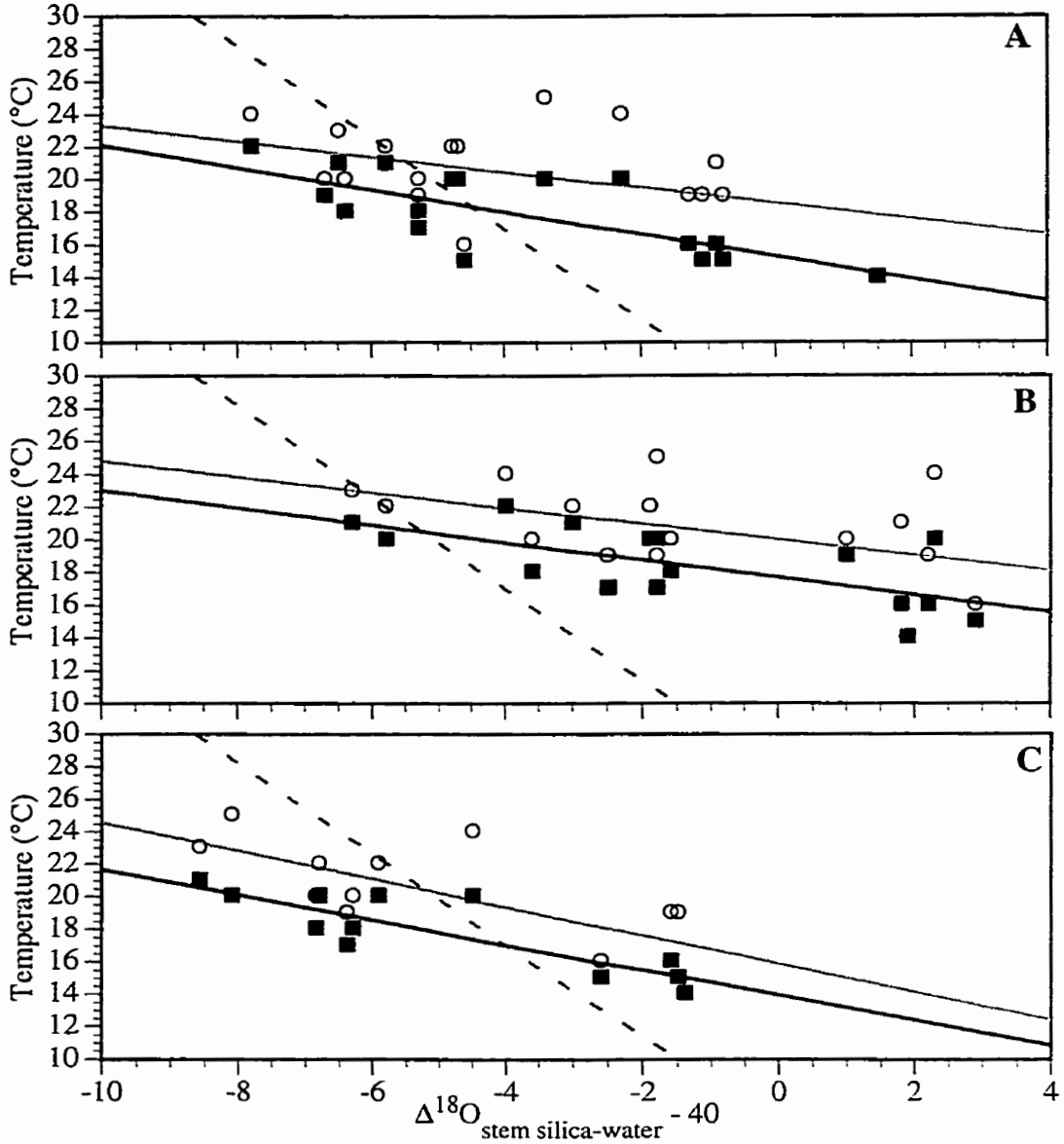

Figure 3-8. Correlation between growing-season temperatures (May to August (■) and July to August (O)) and $\Delta^{18}O_{stem\ silica\text{-}water}$ for: A) surface water, B) ground water and C) summer precipitation. The dashed line represents the temperature-dependant equilibrium fractionation between phytoliths and water defined by Equation (3-8). Equations for the lines of best fit are:

t °C (May-Aug) = 15.3 - 0.68 ($\Delta^{18}O_{stem\ silica\text{-}surface\ water}$ - 40), ($R^2 = 0.5$, $P = 0.0008$);

t °C (July-Aug) = 18.5 - 0.47 ($\Delta^{18}O_{stem\ silica\text{-}surface\ water}$ - 40), ($R^2 = 0.2$, $P = 0.7$);

t °C (May-Aug) = 17.7 - 0.53 ($\Delta^{18}O_{stem\ silica\text{-}ground\ water}$ - 40), ($R^2 = 0.4$, $P = 0.004$);

t °C (July-Aug) = 20.0 - 0.47 ($\Delta^{18}O_{stem\ silica\text{-}ground\ water}$ - 40), ($R^2 = 0.3$, $P = 0.5$);

t °C (May-Aug) = 13.9 - 0.77 ($\Delta^{18}O_{stem\ silica\text{-}precipitation}$ - 40), ($R^2 = 0.7$, $P = 0.0005$);

t °C (July-Aug) = 15.9 - 0.87 ($\Delta^{18}O_{stem\ silica\text{-}precipitation}$ - 40), ($R^2 = 0.5$, $P = 0.008$).

have been calculated using water compositions that were depleted of ^{18}O relative to the true value of soil water. Our surface water samples were collected at the end of the growing season, which ensured that the surface waters had been recharged by ^{18}O-enriched summer rains and further enriched in ^{18}O by evaporation under summer climatic conditions. However, the extent of evaporative ^{18}O-enrichment of surface water differs from that of soil waters even under the same climatic conditions. Water transport during evaporation within the unsaturated zone occurs mainly as vapour diffusion through a relatively thick, stable boundary layer formed between the liquid component of the soil-water reservoir and the turbulent flow of the open atmosphere (Allison et al., 1983). Boundary layers associated with typical surface-waters are only a few microns thick and, as a result, the kinetic fractionation associated with molecular diffusion normally has only a limited role in determining the $\delta^{18}O$ values of surface waters that have undergone evaporation. Increased molecular diffusion in the soil-water reservoir amplifies the effects of ^{18}O-enrichment via kinetic fractionation (Allison et al., 1983). As relative humidity decreases in arid regions, soil water is increasingly enriched in ^{18}O by evaporation, and estimates of $\Delta^{18}O_{\text{stem silica-soil water}}$ made using $\Delta^{18}O_{\text{stem silica-surface water}}$ become increasingly inaccurate. By comparison, $\Delta^{18}O_{\text{stem water-surface water}}$ values that plot below the line of Equation (3-8) represent calculations in which soil-water $\delta^{18}O$ values have been overestimated through the use of the summer surface-water compositions. To summarize, surface water $\delta^{18}O$ values can, at best provide only a crude approximation of the soil water compositions that feed the grasses.

Almost all $\Delta^{18}O_{\text{stem silica-water}}$ values calculated using ground-water compositions plot above the line described by Equation (3-8) (Fig. 3-8b). This behaviour confirms that shallow ground waters are generally depleted of ^{18}O relative to the soil water from which the phytoliths precipitated (Table 3-1). Not only do the shallow ground waters contain a large fraction of ^{18}O-depleted winter precipitation, but they also have been largely unaffected by the evaporative enrichment of ^{18}O that occurs in the upper layers of the

soil. A negative correlation between $\Delta^{18}O_{soil\ water\text{-}ground\ water}$ and relative humidity (R= -0.7 to -0.6 for average May to August and June to August relative humidities, respectively) is likely indicative of the role that evaporation plays in determining $\delta^{18}O_{soil\ water}$ values in the active rooting zone. To summarize, the oxygen isotopic composition of ground water cannot be used to estimate the $\delta^{18}O$ values of the soil-water reservoir that grasses utilize during phytolith formation.

In contrast to the situation for ground water, Figure 3-8c and Table 3-1 show that the majority of summer-precipitation $\delta^{18}O$ values are higher than the average seasonal isotopic composition calculated for the soil water using Equation (3-8). This observation confirms that a lower-^{18}O fraction of water stored in the soil-water reservoir, such as winter precipitation, or spring runoff, is available to mix with summer precipitation events before the moisture is taken up into the roots.

Although most of the silica is expected to have been deposited late in the growing season, the deviation from the equilibrium fractionation relationship described by Equation (3-8) is greater for calculations made using temperatures for the July to August period than for the May to August period. The scope of this study is insufficient to permit conclusions about the seasonal deposition of silica. However, we suggest that this difference arises more from poor estimates of soil-water $\delta^{18}O$ values than from a lower than expected (e.g. May to August versus July to August) temperature of formation for most phytoliths in these grasses.

Studies of the isotopic composition of soil water at Pinery Provincial Park are underway (Gage, in progress). Measured soil-water $\delta^{18}O$ values for unvegetated sands at Pinery Provincial Park during the 1997 growing season are very similar to the 1997 soil-water values that we predicted for this location ($\delta^{18}O_{soil\ water}$ = -8.5‰; Table 3-1). At the end of July, soil-water $\delta^{18}O$ values ranged from –4.1‰ at the surface to –10.0‰ at a depth of 95 cm, yielding a weighted average of -7.4‰. At the end of August, soil-water $\delta^{18}O$ values ranged from -5.1‰ at the surface to -10.3‰ at 120 cm, for a weighted average

of -8.6‰ (Gage, in progress). This enrichment pattern is typical of an evaporation front that occurs just below the surface of the soil (Allison et al., 1984). At increasing depths, the $\delta^{18}O$ values gradually become lower than measured for summer precipitation (unweighted $\delta^{18}O_{summer\ precipitation}$ = -6.1‰; Table 3-1). By a depth of one meter, soil-water $\delta^{18}O$ values begin to approach those of shallow ground water (annual average $\delta^{18}O_{ground\ water}$ = -11.1 ±0.1‰, n = 11; Gage, in progress) likely because of mixing between precipitation and a low-^{18}O fraction of water stored in the unsaturated zone of the soil-water reservoir.

A similar pattern of ^{18}O-enrichment was observed for soil-water profiles at Ellerslie, Alberta, 140 kilometers west of Kinsella, Alberta (Maulé et al., 1994). There, the unweighted $\delta^{18}O_{soil\ water}$ value over a depth of one meter was –16.8‰ and –16.2‰ for July 1986 and July 1987, respectively. Values ranged from approximately –14‰ at the surface to ~–18‰ at a depth of one meter (approaching ground-water values of $\delta^{18}O$ = -19.8‰). Allowing for differences in climate and soil texture between Ellerslie and Kinsella, the soil-water $\delta^{18}O$ values measured by Maulé et al. (1994) are similar to our calculated soil-water composition for Kinsella, Alberta ($\delta^{18}O_{soil\ water}$ = -14.0‰, Table 3-1).

The calculated soil-water $\delta^{18}O$ values for Kinsella, Alberta are more enriched in ^{18}O than summer precipitation ($\delta^{18}O_{summer\ precipitation}$ = -15.6‰). This behaviour contrasts with the majority of sites in this study, including Pinery Provincial Park, where the $\delta^{18}O_{soil\text{-}water}$ values predicted from Equation (3-8) are lower than determined for average summer precipitation. This behaviour likely reflects mixing of waters in the unsaturated zone, which is facilitated by the high infiltration rates characteristic of sandy soils. At sites where the predicted $\delta^{18}O_{soil\text{-}water}$ values are higher than summer-precipitation values, the effects of evaporative ^{18}O-enrichment have exceeded the effects of mixing in the unsaturated zone prior to root water uptake. The roots *C. longifolia* penetrate the soil to up to a depth of three meters and may utilize soil waters over this entire range. Soil waters at varying depths have experienced differing amounts of evaporation and mixing.

The soil water that enters the plant, and from which silica in non-transpiring plant tissues is precipitated, has an oxygen-isotope composition that is a composite of evaporated waters near the surface and the mixture of winter and summer precipitation stored in the soil-water reservoir at greater depths. The ultimate balance among these contributions depends not only on the distribution of soil water through the profile, but also on the (not unrelated) distribution of active root and rhizome biomass.

It is conceivable that soil-water $\delta^{18}O$ values could be determined independently, if phytolith studies were combined with the isotopic study of an additional soil component that is formed in the unsaturated zone, such as soil carbonates. Investigations into the hydrogen isotopic composition of the hydrous components of opal-A phytoliths may also provide information concerning the original plant-water $\delta^{18}O$ values. Water exists in diatom silica as loosely bound surface hydroxyls, structural water and perhaps as water trapped within pores of the silica structure. A small fraction of this water may be unavailable to hydrogen-isotope exchange under natural conditions (Knauth and Epstein, 1982). If water is bound in a similar manner to phytolith silica, it should be possible to extract original plant waters trapped in the silica structure, which have the hydrogen isotopic signature of the plant water from which the silica precipitated.

3.4.5. Potential as a paleoclimatic indicator

Extracting quantitative paleoclimatic information from soil-phytolith assemblages is complicated by several factors. The $\delta^{18}O_{soil\ water}$, relative humidity and temperature signals preserved in the $\delta^{18}O$ values of phytoliths from living plants may become obscured in the soil-phytolith record through the mixing of phytoliths from different plants as well as by the mixing of phytoliths from transpiring and non-transpiring tissues. Phytolith assemblages present in the soil are also not exclusively representative of the overlying vegetation (Fredlund and Tieszen, 1994; Alexandre, 1996). Physical disturbances arising from grazing, fire, aeolian transport and vertical mixing

caused by bioturbation and physical translocation by downward drainage in the soil can disrupt the initial distribution of phytoliths. Likewise, the dissolution rate of phytoliths will vary depending on the size and shape of the silica bodies, soil-water chemistry and pH. Nevertheless, phytoliths in the soil generally are representative of native vegetation on an extra-local to regional scale in the North American prairies (Fredlund and Tieszen, 1994).

Phytoliths that have accumulated in soils over tens to thousands of years will have formed over a wide variety of temperatures, relative humidities and soil-water $\delta^{18}O$ values. Not only will the temperature of silica precipitation have varied diurnally as well as seasonally during the life of the grass but average temperatures commonly vary from one growing season to the next. For example, over the four-year period that silica was extracted from *C. longifolia* at Pinery Provincial Park, Ontario, average growing-season temperatures varied by 3°C and summer precipitation $\delta^{18}O$ values varied by almost 1‰ (Table 3-1). These differences caused the oxygen-isotope compositions of the stem silica to vary by 1.7‰ (Table 3-3). Likewise, a change in average growing-season relative humidity (July to August) of 5% at Pinery Provincial Park between 1995 and 1996 produced an almost 2‰ change in the value of $\Delta^{18}O_{\text{leaf silica-stem silica}}$ (Table 3-3).

The mixing of phytoliths from both transpiring and non-transpiring plant parts will further obscure climatic signals recorded in the $\delta^{18}O$ values of soil-phytolith assemblages. For the purpose of discussion, we shall assume that phytoliths from all plant parts are transferred directly to the underlying soil on an annual basis with minimal disturbance and that they are equally well preserved. In addition, we note again, that where the absolute abundance of silica is higher in leaves and sheaths from areas characterized by lower relative humidity, the relative contribution of silica to the soil from transpiring versus non-transpiring parts of the grass is independent of arid or humid conditions. For such a scenario, Table 3-2 shows that the underground portions (roots and rhizomes) of *C. longifolia* contribute only ~16% of the total phytolith assemblage.

which serves to minimize contamination of underlying soils from silica formed in the root systems of modern plants (Table 3-2). Table 3-2 also shows that ~59% of the total phytolith assemblage will be produced by plant tissues that are essentially non-transpiring (sheath, stem, rhizome and root). This amount, however, will be reduced if rhizome and root silica is not returned to the soil on an annual basis. Strongly transpiring tissues provide ~41% of the phytoliths.

The $\delta^{18}O$ value of this hypothetical phytolith assemblage in the soil at each site can be calculated using the average silica concentrations obtained for each plant tissue (Table 3-2), and the measured $\delta^{18}O$ values for each plant part from each site (Table 3-3):

$$\delta^{18}O_{soil\ phytolith} = [4.2\ (\delta^{18}O_{inflorescence}) + 36.5\ (\delta^{18}O_{leaves}) + 36.0\ (\delta^{18}O_{sheath}) + 7.3\ (\delta^{18}O_{stem}) + 16.0\ (\delta^{18}O_{rhizomes})]/\ 100. \quad \text{(Eqn. 3-10)}$$

The results of this calculation are recorded in the final column of Table 3-3. The average relative contribution of silica from each tissue to total plant silica (Table 3-2c) was used in Equation (3-10), as the silica concentration of at least one plant tissue for the majority of samples, was unavailable. For plant parts whose $\delta^{18}O_{silica}$ values were not available, results for a comparable plant part were substituted. For example, where $\delta^{18}O_{inflorescence}$ values were missing, the values of $\delta^{18}O_{leaves}$ were substituted instead and $\delta^{18}O_{rhizome}$ values were substituted for $\delta^{18}O_{roots}$ values in all cases.

Direct correlations were sought between the climatic information presented in Table 3-1 and the $\delta^{18}O$ values calculated for the soil-phytolith assemblages, but none were found (Fig. 3-9). For our samples, such a result is not entirely surprising. While paleoclimate studies commonly assume that warmer conditions are accompanied by arid climates (Yapp, 1979; Fredlund, 1993), our sample localities do not follow such a trend. The enhanced ^{18}O-enrichment of leaf and inflorescence silica that arises from increased transpiration rates at the xeric-western boundary of the prairies is offset, more or less, by the lower $\delta^{18}O$ values of silica from non-transpiring tissues, whose compositions reflect

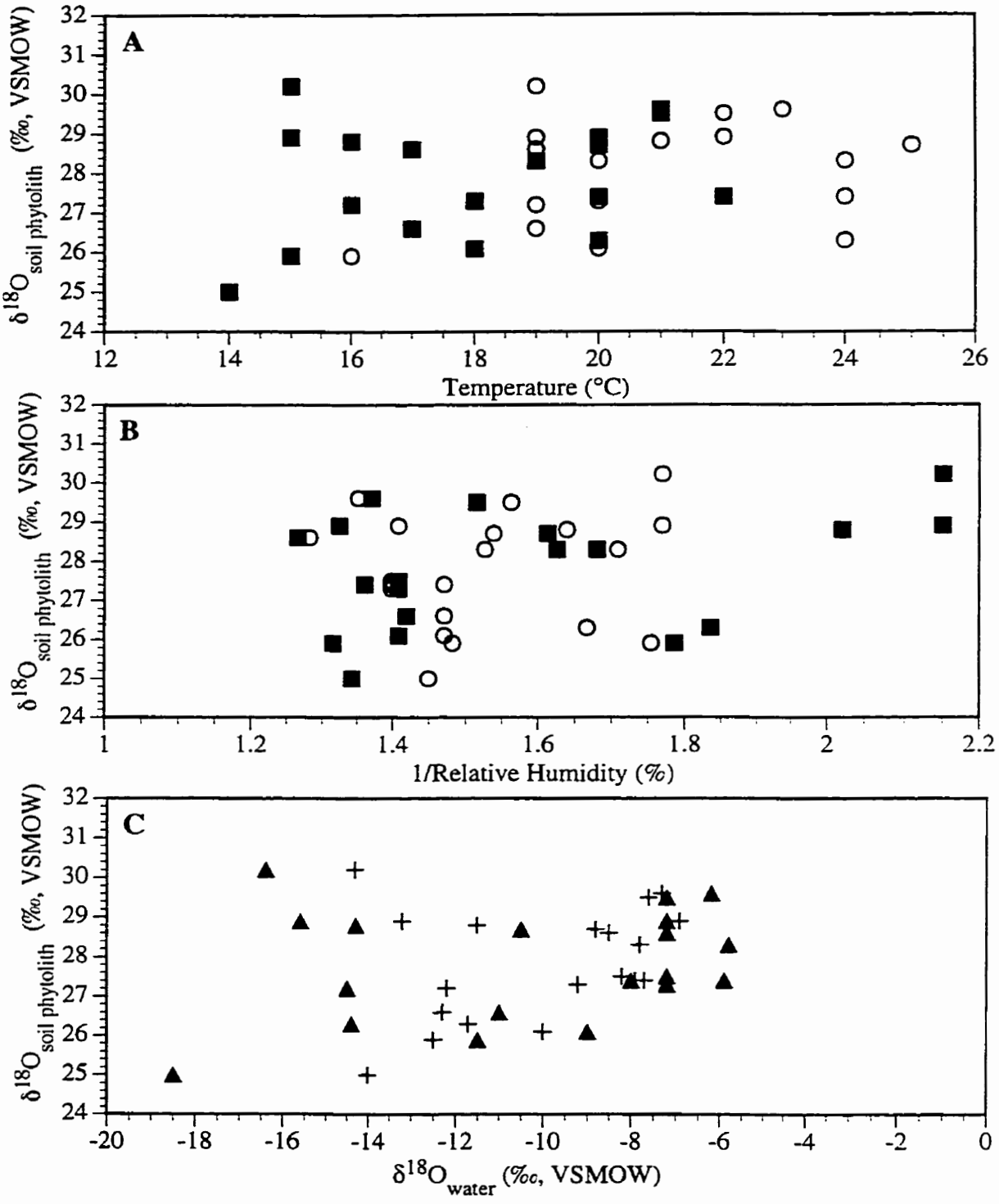

Figure 3-9. Calculated soil-phytolith $\delta^{18}O$ values versus: A) temperature and B) relative humidity (■ May to August; O July to August); and C) surface-water (▲) and calculated soil-water (+) $\delta^{18}O$ values.

more directly the greater ^{18}O-depletion of meteoric water in these cooler localities (Fig. 3-5b, Table 3-3).

There is an empirical relationship between values of $\Delta^{18}O_{soil\ phytoliths\text{-}surface\ water}$ and temperature (Fig. 3-10a). The difference between $\delta^{18}O_{soil\ phytolith}$ values and the calculated $\delta^{18}O$ values of soil water can also be negatively correlated with temperature (Fig. 3-10b). The contribution of leaf silica causes $\delta^{18}O_{soil\ phytolith}$ values to plot above the line formed by Equation (3-8) which describes the equilibrium relationship between plant silica and water. The amount of this enrichment ($\Delta^{18}O_{stem\ silica\text{-}soil\ phytolith}$; Fig 3-10b) is negatively correlated with average relative humidity (R= -0.7 and -0.8 for May to August and July to August relative humidity values, respectively). The values of $\Delta^{18}O_{soil\ phytoliths\text{-}surface\ water}$ and $\Delta^{18}O_{soil\ phytoliths\text{-}soil\ water}$ are calculated from a composite of $\delta^{18}O_{silica}$ values and have no direct causal relationship to temperature. However, the correlations in Figure 3-10 indicate that $\delta^{18}O$ values of soil-phytolith assemblages may still have value as an indicator of climate. Rough temperature estimates, based on the cumulative $\delta^{18}O$ value of phytoliths in the soil record, are possible if soil water $\delta^{18}O$ values can be ascertained.

Fredlund (1993) reports that an increase in the $\delta^{18}O$ values of soil-phytolith assemblages corresponds with a decrease in xeric conditions. He interpreted the increase in $\delta^{18}O$ values to indicate an increase in phytolith production from C3 plants and consequently cooler, wetter growing conditions. His study, however, did not consider the variation of silica $\delta^{18}O$ values within a grass. We have shown previously that there is no difference in the $\delta^{18}O$ values of comparable phytolith silica from C3 and C4 grasses grown at the same location (Webb and Longstaffe, 2000). The equilibrium relationship between plant silica and water implies that a decrease in temperature will result in soil-phytolith assemblages that are more enriched in ^{18}O. However, an increase in the $\delta^{18}O$ values of soil-phytolith assemblages over time can also indicate an increase in the rate of transpiration and/or enrichment in ^{18}O of soil water, which in turn will cause the $\delta^{18}O_{silica}$

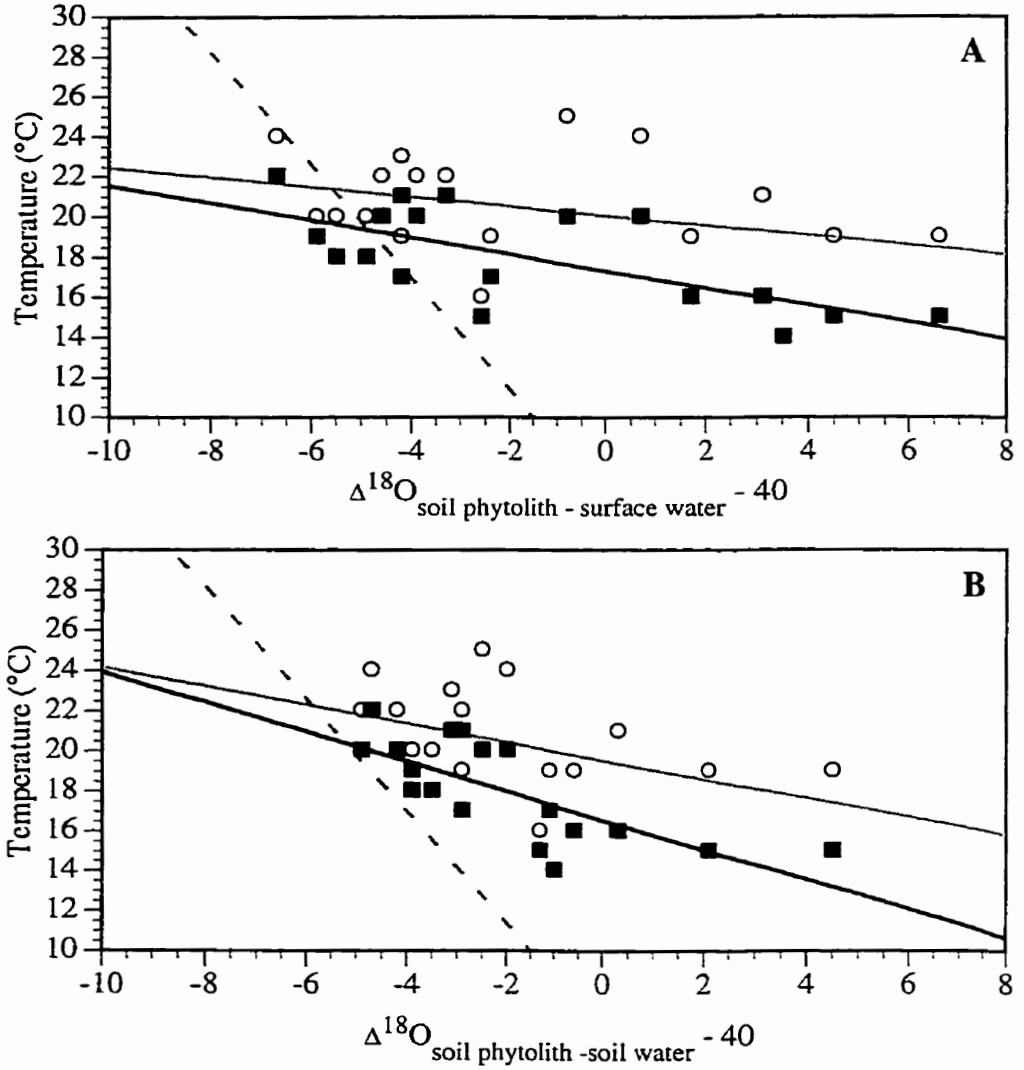

Figure 3-10. Correlation between growing-season temperatures (May to August (■) and July to August (O)) and the difference between the $\delta^{18}O$ values of soil-phytolith assemblages and A) suface waters, and B) calculated soil water. The dashed line represents the temperature-dependent equilibrium fractionation between phytoliths and water defined by Equation (3-8). The lines of best fit through the data are:

t °C (May to August) = 17.3 - 0.42 ($\Delta^{18}O_{soil\ phytolith\ -\ surface\ water}$ - 40); ($R^2 = 0.5$, $P = 0.002$);

t °C (July to August) = 20.1 - 0.24 ($\Delta^{18}O_{soil\ phytolith\ -\ surface\ water}$ - 40); ($R^2 = 0.1$, $P = 0.2$);

t °C (May to August) = 16.5 - 0.73 ($\Delta^{18}O_{soil\ phytolith\ -\ soil\ water}$ - 40); ($R^2 = 0.5$, $P = 0.0007$);

t °C (July to August) = 19.5 - 0.45 ($\Delta^{18}O_{soil\ phytolith\ -\ soil\ water}$ - 40); ($R^2 = 0.2$, $P = 0.1$).

values of phytoliths of transpiring and non-transpiring plant parts respectively, to rise.

3.5. CONCLUSIONS

The oxygen-isotope compositions of silica phytoliths from C. longifolia record valuable climatic data, including information about the $\delta^{18}O$ values of soil water. Non- or weakly transpiring parts of this grass (sheath, stem and rhizome) precipitate silica that is in oxygen isotopic equilibrium with plant water, which in turn, is unfractionated in oxygen isotopes from soil water. The $\delta^{18}O$ values of silica from these tissues are controlled by the original soil-water composition and its temperature-dependent equilibrium fractionation with the silica. Silica deposited in the leaves and inflorescence is also formed in isotopic equilibrium with plant water. However, the water in these tissues generally has been enriched in ^{18}O during transpiration. The amount of this enrichment is dependent on the transpiration rate, which is directly related to relative humidity.

The variation of $\delta^{18}O_{silica}$ values among *C. longifolia* grown under varying natural conditions is highly dependent on soil-water $\delta^{18}O$ values. Accurate calculation of the temperature of silica precipitation is only possible when plant-water $\delta^{18}O$ values for that tissue are known. For non-transpiring tissues, this $\delta^{18}O$ value corresponds to soil-water at the average rooting depth.

Soil-phytolith assemblages are composed of phytoliths from both transpiring and non-transpiring plant parts. Temperature and soil-water $\delta^{18}O$ signals carried by phytoliths from the stems, sheaths and rhizomes may be masked by $\delta^{18}O$ values of the leaves and inflorescence, which are variably further enriched in ^{18}O, depending on relative humidity. Accurate reconstruction of temperature, $\delta^{18}O_{soil\ water}$ values, and relative humidity for ancient phytolith assemblages would require, as a first prerequisite, the recognition and physical separation of phytoliths produced in transpiring and non-transpiring tissues.

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Chapter 4. The oxygen and hydrogen isotopic variation of water in grasses: Implications for phytolith $\delta^{18}O$ values

4.1. INTRODUCTION

Webb and Longstaffe (2000) have shown that the oxygen-isotope compositions of silica phytoliths in grasses from Pinery Provincial Park, southwestern Ontario, Canada provide a useful record of the climatic conditions under which the plants grew. However, this study also illustrated in a preliminary way that the extreme variations observed for plant-water $\delta^{18}O$ values were not reflected by the (much smaller) range of $\delta^{18}O$ values obtained for silica phytoliths from the same grass tissues. In this chapter, we examine more closely the diurnal, seasonal and interspecies variations in grass-water δD and $\delta^{18}O$ values that occur in this ecosystem. Understanding these variations in plant-water δ-values and in particular which portion of this isotopic signal is preserved in grass silica is essential if the phytolith isotopic proxy for paleoclimate is ever to realize its potential.

Aqueous silicic acid ($Si(OH)_4$) enters plants through their root systems and is polymerized as amorphous opal-A in cells and intercellular spaces along the transpiration stream as discrete bodies known as phytoliths (Raven, 1983). After the plant organic matter has decayed, the phytoliths remain as a relatively stable component of the underlying soils and provide a readily available record of continental paleoclimate (Rovner, 1971; Bartoli and Wilding, 1980; Fredlund and Tieszen, 1994). Morphological studies of soil-phytolith assemblages are widely used in archaeology to distinguish vegetation types and estimate climate changes over the period of soil formation (Twiss et al., 1969; Piperno, 1984; Mulholland, 1989; Powers et al., 1989; Alexandre et al., 1997; Fredlund and Tieszen, 1997).

The isotopic compositions of phytoliths can provide more detailed information regarding temperature, $\delta^{18}O_{soil\ water}$ values and relative humidity conditions during plant growth. Phytoliths form in oxygen isotopic equilibrium with plant water (Shahack-Gross

et al., 1996; Webb and Longstaffe, 2000). Consequently, the $\delta^{18}O$ values of plant silica are dependent on the cumulative effects of diurnal and seasonal changes in temperature and plant-water $\delta^{18}O$ values over the growing season. In addition, the oxygen isotopic composition of phytoliths is influenced by factors that determine the $\delta^{18}O$ values of plant water, such as relative humidity, transpiration rate and the isotopic composition of the soil water that is supplied to the plant. To understand the range in isotopic compositions of phytoliths throughout a plant, it is necessary to examine the variability in $\delta^{18}O$ and δD values of the water from which biogenic silica precipitates.

Precipitation supplied to the soil may be changed in isotopic composition prior to phytolith crystallization by evaporation from the soil surface and by transpiration through the plant's leaves. Individual precipitation events, which collectively form the majority of soil water, can be highly variable in their oxygen- and hydrogen-isotope compositions. Temperature changes create seasonal fluctuations in the isotopic composition of precipitation at a given location, resulting in summer-rain events that are typically enriched in ^{18}O relative to most winter precipitation (Dansgaard, 1964). Grasses have shallow rooting systems that preferentially utilize summer precipitation in the upper layers of the soil, commonly preventing much of this water from reaching the water table (Gupta, 1979; Darling and Bath, 1988; Berndtsson et al., 1996). However, some winter precipitation, which is generally depleted of ^{18}O relative to summer rain, can also be retained in the soil-water compartment throughout the year where it is available for plant uptake (Maulé et al., 1994). In addition, the $\delta^{18}O$ values of precipitation can be modified once in the soil-water reservoir as a result of daily cycles of evaporation and condensation in the unsaturated zone (Allison et al., 1984; Walker and Brunel, 1990).

There is no known isotopic fractionation associated with the uptake of soil water by plant roots, or its subsequent movement up the stem (Allison et al., 1984; White et al., 1985). Consequently, water in the non-transpiring tissues of the grass should have an isotopic composition similar to that of soil water. Variations in $\delta^{18}O_{stem\ water}$ values of up

to 9‰ have been observed for plants supplied with the same soil water; this behaviour has been attributed to species-related effects (Wang et al., 1998). The active rooting depth can vary from species to species, resulting in preferential uptake of soil water from different depths. Deeper root systems will likely take up a smaller fraction of the highly evaporated soil waters found near the surface. In addition, the active depth of water uptake can vary with the seasonal availability of soil moisture (Federer, 1979; Barnes and Harrison, 1982). Hence, the soil water entering a plant, from which silica in non-transpiring plant tissues is precipitated, may have an oxygen isotopic signature that is a composite of varying amounts of highly enriched, evaporated waters near the surface and a mixture of winter and summer precipitation stored in the soil-water reservoir at greater depths. Since it is likely that at least some silica is precipitated at all times throughout the growing season, the phytolith assemblage will have formed from water that had a range of $\delta^{18}O$ values.

Once soil water has been taken up by the plant through its roots and enters the leaf tissues, it may become enriched in D and ^{18}O as a consequence of both equilibrium and kinetic isotope-fractionation effects associated with transpiration. The extent of isotopic enrichment of water in transpiring tissues (e.g., leaf and inflorescence) depends on plant physiology, temperature, relative humidity and the isotopic composition of atmospheric water vapour, with isotopic enrichment increasing as relative humidity decreases (Leaney et al., 1985; Flanagan and Ehleringer, 1991a; Walker and Lance, 1991; Farquhar and Lloyd, 1993). Equilibrium fractionation between the liquid and vapour phases of water is temperature dependent (Majoube, 1971). Kinetic fractionation is dependent on the diffusion coefficients associated with the transport of $H_2{}^{16}O$, $DH^{16}O$ and $H_2{}^{18}O$ molecules through the stomatal pores (Merlivat, 1978). Kinetic fractionation effects are larger for $H_2{}^{18}O$ than $DH^{16}O$ with respect to $H_2{}^{16}O$. Consequently, as water vapour is removed the residual liquid becomes enriched in ^{18}O at a faster rate than D. The preferential enrichment of ^{18}O causes leaf water to plot on a 'transpiration line' to the

right of the Meteoric Water Line (MWL) on a δD versus $\delta^{18}O$ plot. The relative importance of equilibrium versus kinetic fractionation determines the slope of the transpiration line. As the thickness of the boundary layer increases, the importance of diffusional vapour transport increases, intensifying the effects of kinetic fractionation and causing leaf water to plot on transpiration lines with progressively lower slopes. A decrease in relative humidity will increase the rate of transpiration (Walker and Lance, 1991). This will create a net diffusional flux of water vapour, preferentially enriched in $H_2{}^{16}O$ molecules, from the boundary layer to the atmosphere and influence leaves to close a greater number of stomatal pores, creating more stagnant boundary-layer conditions. Both processes increase the effect of kinetic fractionation and result in transpiration lines with lower slopes. Typical slopes observed for the residual water in transpiring leaves range from 1.5 to 4 (e.g., Allison et al., 1985; Cooper and DeNiro, 1989; Flanagan et al., 1991a; Walker and Lance, 1991).

In theory, the isotopic enrichment of leaf water proceeds until steady-state conditions are reached. The steady-state isotopic value of leaf water has been widely described by the Craig and Gordon (1965) model for evaporation of a body of water:

$$\delta_{modelled} = \delta_s + \varepsilon^* + \varepsilon_k + h\,(\delta_a - \varepsilon_k - \delta_s) \qquad \text{(Eqn. 4-1)}$$

where $\varepsilon^* = (\alpha\text{-}1) \times 1000$ is the equilibrium fractionation factor (Majoube, 1971) and ε_k is the kinetic fractionation factor (Merlivat, 1978; Buhay et al., 1996). The values of $\delta_{modelled}$, δ_a and δ_s represent the $\delta^{18}O$ or δD values of the water in the leaf, atmospheric vapour and stem respectively, and h is relative humidity. The isotopic composition of modelled steady-state leaf water is based primarily on climatic data and does not account for differences in isotopic composition observed for leaf water of plants grown under identical climatic conditions.

Measured δ-values of bulk leaf water are commonly lower than the $\delta^{18}O$ and δD values predicted by Equation (4-1) (e.g., Allison et al., 1985; Leaney et al., 1985; Walker et al., 1989; Walker and Brunel, 1990; Flanagan and Ehleringer, 1991a; Flanagan et al.,

1991a, b; Walker and Lance, 1991; Wang et al., 1998). Farquhar and Lloyd (1993) suggest that Equation (4-1) describes the isotopic behaviour of water at the sites of evaporation only. The discrepancy between modelled and measured leaf-water δ-values is attributed to mixing between an influx of non-fractionated water from the veins and ^{18}O- and D-enriched molecules diffusing back from sites of evaporation at the surface of the leaf (Flanagan et al., 1991b; Farquhar and Lloyd, 1993). As the pathlength of water movement through a leaf increases, the relative influence of the enriched steady-state waters on the isotopic composition of the bulk leaf water diminishes with respect to unfractionated vein waters. Likewise, as transpiration rates increase, the influx of unfractionated stem water rises to replenish the water lost from leaves through evaporation. Thus, the discrepancy between the isotopic compositions of measured and modelled leaf water increases (Walker and Brunel, 1990).

It is assumed in Equation (4-1) that the leaf behaves as a single pool of evaporating water. However, spatial heterogeneity in the $\delta^{18}O$ and δD values of water within the veins and tissues of individual leaves has been documented even after isotopic steady-state has been attained (Yakir et al., 1989; Luo and Sternberg, 1992; Yakir et al., 1994). Water in the veins of leaves becomes more enriched in ^{18}O and D towards the tip of the leaf (Yakir, 1991; Luo and Sternberg, 1992; Yakir et al., 1994). This behaviour is thought to be the result of lateral exchange between ^{18}O- and D-enriched water from the mesophyll cells adjacent to the veins and water poorer in ^{18}O and D from the stems, which feed the leaf veins (Luo and Sternberg, 1992). This pattern of vein-water enrichment is pronounced in simple leaves with a large number of parallel veins, which is typical of grasses (Yakir, 1991). The gradient in water isotopic composition within leaves has also been attributed to further evaporative enrichment of already ^{18}O- and D-enriched water supplied via successive leaf-water pools (Wang and Yakir, 1995). Water that enters a leaf can be continually subjected to evaporation as it moves towards the tip of the leaf. Each consecutive "pool" of water is fed by water more enriched in ^{18}O and D

than the preceding one. As a result, the $\delta^{18}O$ and δD values of these progressively evaporated water pools may exceed those predicted using Equation (4-1) by some maximum value that is determined by ambient conditions (Gat and Bowser, 1991). Such extreme leaf-water values have been observed previously (Walker and Lance, 1991; Flanagan et al., 1993; Wang and Yakir, 1995; Wang et al., 1998).

The persistence of isotopic heterogeneity in leaf-tissue water under steady-state conditions suggests that mixing of water in the leaf is restricted and indicates some sort of leaf-water compartmentation (Yakir et al., 1994; Wang and Yakir, 1995). Many studies have implied that metabolic water within leaf cells may be isolated and exchange slowly with the ^{18}O- and D-enriched water at the sites of evaporation. For example, water extracted from sunflower and ivy leaves by applying stepwise pressure revealed that three distinct water pools exist within the leaf, which can be distinguished both on their isotopic composition and through ion analysis (Yakir et al., 1989). Intercellular water is directly involved in transpiration and is expected to reach the steady-state isotopic composition predicted by Equation (4-1) rapidly, in response to changes in climate. Vein water in the leaves is supplied directly by the stem water and is unfractionated with respect to the soil water taken up by the plant. Intracellular water involved in metabolic processes experiences only restricted mixing with the transpired water- and vein water-pools and, as a result, slows the isotopic response of the whole leaf to environmental changes (Yakir et al., 1989; Yakir, 1991). Other studies also support the idea that some mechanism of restricted mixing or isotopic compartmentation of leaf water exists. In response to changes in climatic conditions leaf water δ-values initially proceed rapidly towards steady-state δ-values. However, after the initial rapid response, the rate of approach to steady state becomes asymptotic (Wang and Yakir, 1995). The isotopic composition of water within cells that are active in photosynthesis is lower than measured bulk leaf-water $\delta^{18}O$ values and closer in composition to stem water than modelled steady-state values (Yakir et al., 1994). These effects are unlikely to be related

to fractionation during water movement; no fractionation during water transport through membranes has been observed (Allison et al., 1985).

Interpretation of the variations in $\delta^{18}O$ values of silica formed in different tissues of the same plant and between different species has led to several postulations regarding the isotopic behaviour of plant water. We have previously demonstrated that the $\delta^{18}O$ values of silica deposited in *Ammophila breviligulata*, a C3 grass and *Calamovilfa longifolia*, a C4 grass, were equivalent under relatively humid conditions (Webb and Longstaffe, 2000). It follows then that the isotopic composition of the plant water must vary in a similar manner between these two species. The $\delta^{18}O$ values of silica phytoliths formed in non-transpiring tissues (roots, rhizomes and stem) indicate that water in these plant parts is unfractionated from soil-water $\delta^{18}O$ values (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000; see also Chapter 3). In addition, despite the potential for evaporative ^{18}O-enrichment of water in the sheath tissues, silica deposited there appears to have precipitated from water with $\delta^{18}O$ values very similar to stem water (Chapter 3). Furthermore, the $\delta^{18}O$ values of leaf phytoliths are much lower than expected for formation in equilibrium with the highly ^{18}O- and D-enriched water typical of leaves under mid-day climatic conditions (Shahack-Gross et al., 1996; Webb and Longstaffe, 2000; Chapter 3). In this chapter we examine the diurnal and seasonal fluctuations in the isotopic composition of plant water from several species of grass to define the relationships between the δ-values of soil water and water in non-transpiring plant tissues as well as the variations in leaf water δ-values that arise from transpiration. The isotopic composition of water from which phytoliths precipitate can only be understood once the isotopic variations of plant water have been fully characterized.

4.2. METHODS

4.2.1. Study area

Pinery Provincial Park is located on the southeastern shore of Lake Huron (43°15' N, 81°50' W). The park encloses a series of discrete parabolic sand dunes ranging in age from 100 to 4800 years. Grass samples were collected within the dune slack between the first and second dune ridge and the area behind the second dune ridge referred to as the transition zone by Baldwin and Maun (1983). The majority of sand (40 to 65%) is medium-grained while a large proportion of the remaining soil is composed of coarse-grained sands in the slack and fine-grained sands in the transition zone (Baldwin and Maun, 1983). The vegetation in the slack is dominated by *Calamovilfa longifolia*, with scattered patches of *Ammophila breviligulata* and *Andropogon scoparius*. The coarse-grained nature of the soil at this site allows for rapid drainage of precipitation to the water table, which is more than 2 meters below the surface of the soil (Steinbachs, 1999). Almost 60% of the substrate in the slack area is bare sand and therefore susceptible to high diurnal thermal gradients, as well as physical shifting as a result of wind erosion (Baldwin and Maun, 1983). By comparison, only 5% of the soil is bare in the transition zone (Baldwin and Maun, 1983). The higher stability of the substrate in the transition zone is accompanied by an increase in the number of species present at this site, which supports vegetation typical of an oak-savanna ecosystem. The limited samplings of *Andropogon gerardi*, *Sorghastrum nutans* and *Stipa spartea* presented here were made in this area. The increased stability and development of the soil profile in the transition zone results in a soil with lower bulk density, smaller pore sizes and an increased aggregate structure, organic matter, nutrient content and field capacity relative to soils in the slack (Baldwin and Maun, 1983). The diurnal and seasonal micrometeorological changes are similar for both the slack and transition zone; however there is a reduced

effect from the prevailing westerly winds in the transition zone, which is in the lee of the second dune ridge (Baldwin and Maun, 1983).

4.2.2. Sample collection

Samples of six grass species, four C4 (*C. longifolia, A. scoparius, A. gerardi* and *S. nutans*) and two C3 (*A. breviligulata* and *S. spartea*), were collected throughout the growing seasons of 1997 and 1998. For each species, a sample was collected approximately one half hour before dawn when transpiration rates are minimal and during the more extreme climatic conditions at mid-day when transpiration rates are generally the highest. Because our plant-water sampling method consumes the grass, adjacent individuals were used to obtain data for pre-dawn versus mid-day conditions. Plant waters from *C. longifolia* and *A. breviligulata* were systematically collected at approximately one-month intervals throughout the growing season to assess the isotopic variations of plant waters from C3 and C4 grasses under field conditions. The isotopic data obtained for the plant waters of these species is also pertinent to the evaluation of the variations in oxygen isotopic composition of silica phytoliths extracted previously from these two species (Webb and Longstaffe, 2000; Chapter 3). Other grass species were collected more sporadically as a means to compare the variation in plant-water $\delta^{18}O$ and δD values between different C3 and C4 species as well as between grass species that varied in their leaf morphology and plant physiology (for descriptions of these grass species see Chapter 1).

To learn how the oxygen- and hydrogen-isotope compositions of the water varied within each grass sample, each plant was subdivided into six parts: root, rhizome, stem, sheath, leaf and inflorescence. Plants were measured and then rapidly harvested and dissected in the field. Tissue segments of approximately five centimeters in length were sealed into test tubes and immediately frozen until the water was extracted. At each sampling site, temperature was measured at an average height of 30 centimeters above the

surface of the soil, within the canopy of grass foliage. Relative humidity measurements were taken at the soil surface.

4.2.3. Isotopic analysis

All stable isotope results are expressed in the standard δ-notation, relative to VSMOW for oxygen and hydrogen (Coplen, 1994) where

$$\delta = [(R_{sample} / R_{standard}) - 1] \times 1000 \ (‰) \qquad \text{(Eqn. 4-2)}$$

and R represents D/H or $^{18}O / ^{16}O$. Dual-inlet, triple-collecting, gas-source Optima or Prism II mass-spectrometers were used for all measurements. Isotopic enrichment between two phases (a and b) is expressed as:

$$\Delta^{18}O_{a\text{-}b} = \delta^{18}O_a - \delta^{18}O_b. \qquad \text{(Eqn. 4-3)}$$

Water was extracted from grass samples under vacuum for forty-five minutes while being heated at 100°C. Three microlitres of the extracted water were equilibrated with a known amount of CO_2 for 5 days at 25°C (Epstein and Mayeda, 1953, as modified by Kishima and Sakai, 1980). Reproducibility of $\delta^{18}O_{plant\ water}$ averaged ±0.4‰. The same three microlitres were reacted with metallic zinc to produce H_2 gas for hydrogen-isotope analysis (Coleman et al., 1982). Reproducibility of $\delta D_{plant\ water}$ averaged ±2‰.

4.2.4. Soil water and precipitation

Gage (in progress) has performed concurrent measurements of the oxygen and hydrogen isotopic compositions of meteoric water and soil water at the study sites in Pinery Provincial Park. Precipitation was collected in an automated rain collector and sampled on a monthly basis. On the same day of grass sampling, soil cores were collected at two locations in the dune slack to an average depth of 1.5 meters. Soil water was subsequently extracted from the preserved core by azeotropic distallation for stable isotopic analysis (Gage, in progress).

4.2.5. Isotopic analysis of silica

The oxygen isotopic compositions of silica extracted from *C. longifolia* and *A. breviligulata* at this site have been reported previously (Webb and Longstaffe, 2000; Chapter 3). Plant samples were collected at Pinery Provincial Park at the end of the growing season for the years 1994 through 1997. The 1994 sample of *A. breviligulata* was collected approximately 40 km west of Pinery Provincial Park. Silica was extracted from dozens of individual plants from the same area in order to obtain sufficient quantities for analysis. This material represents an average for opal-A deposited in each grass species over its lifetime (one to five growing seasons, depending on the plant part). Samples were washed in distilled water to remove detrital minerals and dried at 65°C for a minimum of 36 hours. Dried grass samples were digested in 99% sulfuric acid for an average of two hours. The organic matter was then reacted with 30% hydrogen peroxide until only silica remained (Geis, 1973).

With the exception of the 1994 samples, the isotopic exchange procedure for opal-A described by Labeyrie and Juillet (1982) and Juillet-Leclerc and Labeyrie (1987) was employed in order to account for oxygen-isotope exchange between unstable Si-O bonds and hydroxyl groups in the opal-A during dehydration prior to oxygen-isotope analysis (Webb and Longstaffe, 2000). Oxygen was liberated from silica by reaction with bromine pentafluoride at 600°C (Clayton and Mayeda, 1963) and prepared for mass spectrometric analysis by conversion to CO_2 gas by reaction with incandescent graphite. During the course of these experiments, the $\delta^{18}O$ value of the laboratory's standard quartz corresponded to a value of +9.7±0.2‰ for standard silica sand (NBS-28). Using the exchange procedure, the standard deviation of the measured $\delta^{18}O_{silica}$ values averaged ±0.2‰. By comparison, reproducibility of oxygen-isotope values for the 1994 phytolith samples that were not subjected to the exchange procedure was substantially worse, averaging ±1.2‰ (Webb and Longstaffe, 2000).

4.3. RESULTS

The temperature and relative humidity during the collection of grasses at Pinery Provincial Park are listed in Table 4-1. Measurements made adjacent to each plant sampled varied by less than 2°C for temperature and less than 5% for relative humidity over the course of each day's mid-day or pre-dawn sampling. In general, the difference in climate between the slack and the transition zone was negligible. However, on two occasions (mid-day samplings in June and July 1998) plants sampled from the transition zone were subject to distinctly higher temperatures and lower relative humidities than grasses in the slack. Wind-speed measurements were not taken. However, even when strong winds were present atop the dune ridge, no air movement was noticeable at the sampling locations, which are generally protected from wind by the dunes. All grass sampling took place on clear, rain-free days, with the exception of a rain event (4 mm), which occurred during the evening of May 6, 1998, prior to sampling in the morning of May 7, 1998. The number of rain-free days prior to sampling is also listed in Table 4-1; rain events of <2mm have not been considered. The presence of dew and/or guttation was observed August 7, 1997 on the leaves of *A. breviligulata*, May 7 1998 on the leaves of *C. longifolia*, *A. breviligulata* and *S. nutans*, and September 23, 1998 on the leaves of *A. breviligulata.*

The $\delta^{18}O$ and δD values of the plant waters are listed in Tables 4-2, 4-3 and 4-4. Included in these tables are the calculated values for the isotopic composition of leaf water under steady-state conditions (modelled leaf). These values were calculated using Equation (4-1), with modifications to the kinetic fractionation factor (ε_k) proposed by Buhay et al. (1996). Wind-speed values were estimated at 60 cm/s for all sampling times. The value of δ_a used in the calculation was obtained by assuming the atmospheric water vapour was in equilibrium with Lake Huron water ($\delta^{18}O$ = -7.2‰, δD = -57‰). This is a reasonable approximation, given the sparse plant canopy and the close proximity of this body of water. Previously, Baldwin and Maun (1983) have observed a decrease in

Table 4-1. Climatic conditions at Pinery Provincial Park during plant water sampling.

Date			Time			Temperature °C		Relative	# days since	last rain	¥ monthly precipitation			¥ soil water	
Month	Day	Year	Start	End	Area §	Air	Soil	Humidity %	last rain*	event mm*	mm	$\delta^{18}O$	δD	$\delta^{18}O$	δD
August	6	1997	13:45	15:07	SL & TZ	23*		20*	3	14	75	-9.5	-70	-7.2	-52
August	7	1997	5:00	5:50	SL & TZ	8*		90*	4	14					
October	8	1997	6:30	7:10	SL & TZ	15.2		85	3	3	35	-5.0	-28	-6.5	-47
October	8	1997	12:36	13:15	SL & TZ	30.7		33	3	3					
May	6	1998	14:30		SL & TZ	33.0	37.0	43*	2	14	100	-5.9	-40	-8.1	-57
May	7	1998	6:15	6:50	SL & TZ	12.9		95*	0	4					
May	7	1998	13:05	13:50	SL & TZ	29.3		37*	0	4					
June	1	1998	13:00	14:00	SL	14.2	34.5	31	2	4	25	-4.6	-39	-6.1	-45
June	1	1998	13:00	14:00	TZ	24.8	46.5	25	2	4					
June	2	1998	5:50	6:25	SL & TZ	16.4		67	3	4					
June	2	1998	13:15	13:55	SL	29.0		34	3	4					
June	2	1998	13:15	13:55	TZ	36.4		25	3	4					
July	2	1998	13:40	14:55	SL	30.2	46.2	34	3	3	35	-7.1	-48	-5.7	-44
July	3	1998	5:30	6:35	SL	18.5		79	3	3					
July	3	1998	12:10	13:00	SL	31.8		19	4	3					
August	12	1998	5:35	7:15	SL	13.4		81	3	28	210	-4.8	-26	-4.4	-29
August	12	1998	13:20	14:35	SL	27.1		28	3	28					
September	23	1998	5:50	7:20	SL	8.5		77	1	6	99	-12.3	-97	-8.4	-64
September	23	1998	13:40	15:15	SL	21.5	22.8	40	1	6					

§ SL= slack behind the first dune ridge and TZ = transition zone behind the second dune ridge. * Data obtained from Environment Canada; all other climate information was measured at the time of sample collection. ¥ from Gage (in progress), isotopic values represent the monthly average of precipitation of the previous month or the weighted average isotopic composition of soil water over a one meter depth.

Table 4-2. Stable isotopic compositions of water in tissues of *C. longifolia* collected at Pinery Provincial Park.

		inflorescence		leaf	upper leaf		lower leaf		modelled leaf		sheath		stem		rhizome		root	
Date	Time	$\delta^{18}O$	δD	#	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD
August 6, 1997	PM	-7.8		5													-4.8	-48
				7														
August 7, 1997	AM	0.8	-45	6			-2.7	-53	-3.0	-32		-61	-7.3				-8.1	-71
		0.7	-49	3	0.8		-6.5	-61	-3.1	-32	-7.7	-62					-7.6	
October 8, 1997	AM			6			-3.9		-1.4	-29	-5.9	-24	-8.3		-7.5	-59	-4.1	-21
				4					-1.1	-29	-5.3	-38	-7.2					
October 8, 1997	PM			6			9.4	1	19.0	29	4.1	-5	-6.2	-33	-6.0	-42	-6.0	-45
				4			7.9		19.3	29	-0.9							
May 6, 1998	PM			1	13.6	-7	-0.2	-36	16.4	8	-5.1	-47			-6.6	-49	-5.6	-47
				3	10.6	5	0.7	-36	19.3	11	-7.7	-47						
May 6, 1998	PM			3	23.7	19	4.1	-29	15.6	8	-5.5	-41			-6.0	-47		
				2	16.0*	3*			15.6	8	-2.3	-41						
				4	17.6	8	7.0	-18	18.2	10	-5.9	-41						
May 7, 1998	AM			2			-3.2	-48	-4.9	-38	-3.6	-47			-7.2	-49	-6.6	-49
				3	-6.2	-35	-4.9	-43	-4.9	-38	-4.8	-48					-7.8	-42
May 7, 1998	AM														-6.5	-57		
May 7, 1998	PM			2	18.5	16	6.9		20.3	14	-6.9	-41			-4.7	-53	-5.0	-47
				3	4.5	-32	4.8	-33	19.8	13	-5.1	-44						
June 1, 1998	PM			3	26.2	50	4.1	-18	21.8	44	-4.0	-45	-4.2	-34	-3.7	-40	-2.6	-19
				4	20.4	22	1.7	-30	21.8	44	-4.7	-48						
June 2, 1998	AM			3	13.9		-0.6	-37	6.6	-7	-1.3	-42	-4.1	-46	-3.6	-48	-0.9	-27
				4	13.6	7	-2.9	-44	6.6	-7	-3.0	-44						
June 2, 1998	PM			4	45.7	85	5.3	-28	19.9	19	-3.6	-51	-3.5	-47	-3.3		1.6	-23
				5	42.3	80	3.1	-29	19.5	18	-4.5	-42			-1.7	-39		
July 2, 1998	PM			5	18.1	10	0.7	-48	18.9	10	-5.3	-60	-5.3	-57	-4.8	-64	-3.7	-50
				8	16.0	9	2.5	-41	19.9	11	-4.9	-56	-6.0	-63				
July 3, 1998	AM	0.7	-33	5	-0.6	-34	-4.8	-49	1.5	-23	-5.4	-52			-5.3	-50	-3.9	-48
				8	1.9	-30	-1.5	-42	1.9	-23	-4.1	-43	-5.1	-51				
July 3, 1998	PM			3	22.3	24	1.7	-35	26.1	30	-4.1	-52	-4.6	-51	-3.4	-46	-1.5	-43
				6	17.0		6.0		26.2	30	-1.9	-45	-5.1	-49				
August 12, 1998	AM	-2.6	-46	4	-4.3	-54	-5.5	-47	0.7	-22	-5.1	-43	-4.8	-49	-4.2	-36	-4.3	-32
		-3.1	-39	7	-2.7	-53	-2.6	-51	0.9	-22	-3.2	-48	-5.1	-43				
August 12, 1998	PM	0.8	-34	7	16.5	10	4.0	-20	21.1	36	-1.2	-26	-4.1	-28	-4.2	-33	-3.1	-29
		1.1	-36	11	17.8	15	8.5	-8	22.3	37	2.1	-23	-4.9	-32				
													-5.0	-40				
September 23, 1998	AM	-3.7	-57	13	1.2	-39	1.3	-41	1.3	-24	-0.6	-55	-11.8	-80	-8.0	-85	-10.1	-97
		-5.0	-59	7	-4.5	-58	-8.6	-77	0.5	-25	-11.8	-77	-12.4	-98				
	PM	-8.5	-72	9	-2.3	-1	10.0	-35	13.3	-12	0.9	-61	-11.7	-95	-8.8	-75	-8.4	-81
		-2.5	-56	5	18.2	-3	-1.3	-81	12.2	-13	-8.6	-90	-12.0	-100				

*whole leaf; ¥ leaf number counted from the base of the plant.

Table 4-3. Stable isotopic compositions of water in tissues of *A. brevifigulata* collected at Pinery Provincial Park

		inflorescence		[v]leaf	upper leaf		lower leaf		modelled leaf		sheath		stem		rhizome		root		guttation	
Date	Time	$\delta^{18}O$	δD	#	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD
August 6, 1997	PM												-7.0	-52						
	PM												-6.7	-53	-7.6	-58	-5.7	-52		
August 7, 1997	AM						-5.9	-65	-2.6	-31	-6.7	-61	-5.3		-8.4					
					12.8		-5.8	-57	-2.4	-31	-7.4				-6.6	-53				
October 8, 1997	AM				10.8	18	-6.6		-1.1	-25	-7.1		-8.0	-32	-8.8	-43				
					-1.1		-7.2	-44	-0.7	-25	-6.6									
October 8, 1997	PM			3	10.3	23	1.0	-20	19.9	24	-5.1	-41	-7.0	-41			-0.3	-13		
				2			-0.7	-29	20.4	25	-5.0	-43								
October 8, 1997	PM	-5.9	-34																	
May 6, 1998	PM			1	14.7	7	2.8	-31	15.4	5	-4.0	-44	-9.4				-2.2	-39		
				2	17.4	-2	5.1	-19	15.4	5	-2.7	-43								
May 6, 1998	PM																-2.1	-42		
May 7, 1998	AM			1	-3.4	-39	-7.1	-53	-4.9	-38	-7.1	-53			-5.8	-51	-6.1	-49	-7.0	-42
				2	-3.3	-39	-13.0	-56	-5.0	-38	-7.1	-55								
May 7, 1998	PM			1	36.4	56	4.9	-34	18.1	14	-6.7	-54	-5.6	-50	-5.6	-60	-3.1	-47		
				3	22.7	37	1.6	-42	18.4	15	-6.2	-57								
June 1, 1998	PM			3	7.0	-25	1.9	-33	24.0	41	-3.3	-42	-4.4	-42	-3.4	-40	-1.3	-34		
					16.0*	2.8*														
				4			-0.6	-28	24.7	41	-3.9	-47								
June 2, 1998	AM			4	26.5	28	-2.0	-49	7.9	-6	-3.5	-41	-3.8	-44	-5.5	-34	-1.7	-31		
				5	30.1	62	-0.1	-35	7.4	-6					-1.5	-41				
June 2, 1998	PM			4	2.1	-10	-1.5	-39	23.5	28			-4.3	-39	-1.8	-42	2.2	-10		
				3	47.5	113	34.3	35	21.9	26	-2.4	-41								
July 2, 1998	PM			4	16.2	-5	2.4	-27	20.6	19	-3.9	-45	-4.6	-48	-4.6	-42	-3.1			
				5	13.9	2	-2.3	-36	23.2	21	-5.6	-48								
July 3, 1998	AM			2	17.5	-5	-1.4	-49	2.3	-24	-3.5	-57	-3.6		-0.5	-46				
				3	12.2	-6	-0.9	-49	2.1	-24	-3.2	-52								
July 3, 1998	PM			2	21.2	12	1.6	-33	26.7	33	-3.9	-41	-4.7	-47	-4.7	-44				
				3	23.5	20			26.7	33	-5.3	-48								
August 12, 1998	AM	-4.7	-48	3	-3.8	-49	-4.1	-44	1.1	-20	-4.3	-32	-5.1	-36	-6.5	-40				
				4	-2.7	-32	-3.6	-41	1.0	-20	-3.4	-40	-5.5	-35						
August 12, 1998	PM	-2.2	-51	2	5.6	-30	0.3	-30	22.6	30	-3.7	-24	-5.3	-43	-5.7	-41	-3.7	-44		
				3			1.9	-21	23.1	30	-2.9	-28	-6.0	-45						
September 23, 1998	AM	-11.6	-88	2	7.7	-22	2.2	-40	2.2	-20	-4.4	-63	-7.9	-72	-10.1	-72			-4.7	-45
		-3.9	-59	1	-2.2	-36	-6.1	-51	2.4	-20	-8.8	-66	-8.8							
September 23, 1998	PM			2	16.3	3	-3.6	-69	16.4	-6			-10.6	-91	-10.6	-90	10.1 [§]	-71 [§]		
September 23, 1998	PM	-9.7	-77	2					15.3	-7			-7.5	-73						

*mid-leaf; [§] not used in transpiration line in Figure 4-10. [v] leaf number counted from bottom of plant

Table 4-4. Stable isotopic compositions of water from tissues of *A. scoparius* (AS), *S. nutans* (SN), *S. spartea* (SS) and *A. gerardi* (AG) collected at Pinery Provincial Park.

			inflorescence		[V]leaf	upper leaf		lower leaf		modelled leaf		sheath		stem		rhizome		root	
Species	Date	Time	$\delta^{18}O$	δD	#	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD	$\delta^{18}O$	δD
AS	August 6, 1997	PM	1.5	37	2	27.5		-0.3	-51	25.4	35	-6.1	-48	-7.2		-6.8	-54		
AS	August 7, 1997	AM	4.8	-24	1			1.3	-17	-2.7	-31			-6.9	-52			-6.5	-63
			-0.4[§]	-87[§]	2	-1.0*	-40*			-2.7	-31	-3.7	-46	-6.9	-50				
AS	August 7, 1997	AM			>2													-3.0	-38
AS	May 7, 1997	AM			3	-4.4*	-40*			-4.7	-38			-2.8	-42	-2.2	-43		
					4	-4.9*	-44*			-4.8	-38	-3.5	-46						
AS	May 7, 1997	PM																-0.5	-30
AS	June 1, 1998	PM			3							-1.1	-26			-2.8	-30		
					4							-0.8							
AS	June 1, 1998	PM																-0.9	-26
AS	June 2, 1998	PM			3							-2.0	-32			-2.4	-40	1.4	-26
SN	May 6, 1998	PM			1			9.2	-7	15.0	9	-2.1	-34			-5.4	-45		
					2	23.8	27	2.2	-26	15.4	9	-4.3	-39						
SN	May 7, 1998	AM			2	-6.0	-42	-5.7	-47	-4.9	-38	-5.4		-5.4	-41				
					1					-4.9	-40	-5.6	-48						
SN	May 7, 1998	PM			2	28.2	49	2.2	-22	18.2	19	-4.5	-38	-5.1	-42			-2.2	-35
					1	27.2	52			18.2	19								
SN	June 1, 1998	PM			2	31.5	59	1.4		26.0	44			-2.4	-32	-3.4	-31	1.1	-21
					3			7.6	14	25.3	43	-3.0	-35						
SN	June 2, 1998	AM			2	10.3	2	-1.3	-32	7.2	-6	-2.2	-38			-4.6	-43	-1.5	-36
					3	11.8	4	-3.0	-32	6.7	-6	-4.8	-42						
SN	June 2, 1998	PM			2	33.4	58	4.1	-24	25.6	32	-3.3	-46	-3.9	-37	-5.6	-49	-0.1	-13
					4	32.9	62	-1.8	-39	23.4	30	-6.1	-44						
SS	August 6, 1997	PM			2	22.3	14	-5.4	-57	26.9	32	-6.3	-54			-7.5	-61	-8.2	-63
					1	23.0	23	-9.2		28.1	33	-8.1	-65						
SS	August 6, 1997	PM	9.5	-29										4.8	-36				
														-7.1	-59				
SS	August 7, 1997	AM			2			2.0	-32	-2.8	-32	-2.5	-47	1.8	-30			-7.1	-65
					3			-1.1	-49	-2.8	-32	-6.9	-70	-7.6	-60			-8.3	-61
SS	May 6, 1998	PM			1	17.8	7			20.5	17								
					2			0.5	-27	18.3	15	-4.4	-38						
AG	August 6, 1997	PM	0.7	-31	2	31.8	62	-2.8	-35	24.5	36			-9.3	-47	-9.0	-58	-7.4	-60
			-3.5	-39	4	33.6	51	0.7	-34	23.2	35	-5.0	-46	-7.3	-56				
AG	October 8, 1997	AM	-2.5		5					-1.0	-26	-2.8	-27			-7.0	-43	-3.7	-28
					2			-6.1	-27	-1.2	-26	-6.1	-36	-7.0	-36				
AG	October 8, 1997	PM	4.2	-8	4	15.7	36	1.5	-21	18.3	20			-5.7		-7.4	-45	-4.2	-34
					2	20.3	28	-1.8	-28	19.6	21	-4.1	-37	-6.6	-45				

* whole leaf; [§] not used in transpiration line in Figure 4-10; [V] leaf number counted from bottom of the plant.

vapour-pressure deficit at this site throughout the day. They suggest a possible influx moist air from the adjacent lake surface during the afternoon. Estimations of δ_a based on equilibrium fractionation between meteoric water and atmospheric water vapour (Majoube, 1971) may be accurate within 3‰ for $\delta^{18}O$ values of atmospheric water vapour (Buhay et al., 1996). However, atmospheric water vapour is commonly enriched in D relative to values predicted from the equilibrium separation between source water and atmospheric water vapour (Buhay et al., 1996). In such a case, our modelled leaf-water δD values will underestimate the D-enrichment of leaf water. In addition, possible corrections for the underestimation of leaf temperature, which is typically higher than air temperature surrounding the plants, were not applied. Elevated leaf temperatures produce higher vapour concentration-gradients within the leaf boundary-layer, which, if not taken into account, can result in modelled leaf-water $\delta^{18}O$ and δD values that are lower than actual leaf waters (Buhay et al., 1996).

4.4. DISCUSSION

4.4.1. Isotopic variation of plant waters in non-transpiring tissues

4.4.1.1. *Upper versus lower stem-water δ-values*

The process of water uptake from the soil by roots does not alter the $\delta^{18}O$ and δD values of the water (Zimmerman et al., 1967; White et al., 1985; Brunel et al., 1995). Once the water has entered the plant it will remain isotopically unaltered until affected by transpiration processes. Active transpiration does not occur within stem tissues. Hence, the isotopic composition of water within an individual stem is not expected to vary from the isotopic composition of the soil water from which it is derived. It is somewhat surprising, therefore, that we observed variations of up to 11.9‰ and 23‰ for $\delta^{18}O$ and δD values respectively, of waters extracted from the upper and lower portions of *S. spartea* stems (Fig. 4-1). By comparison, the $\Delta^{18}O$ values for upper-lower stem-water

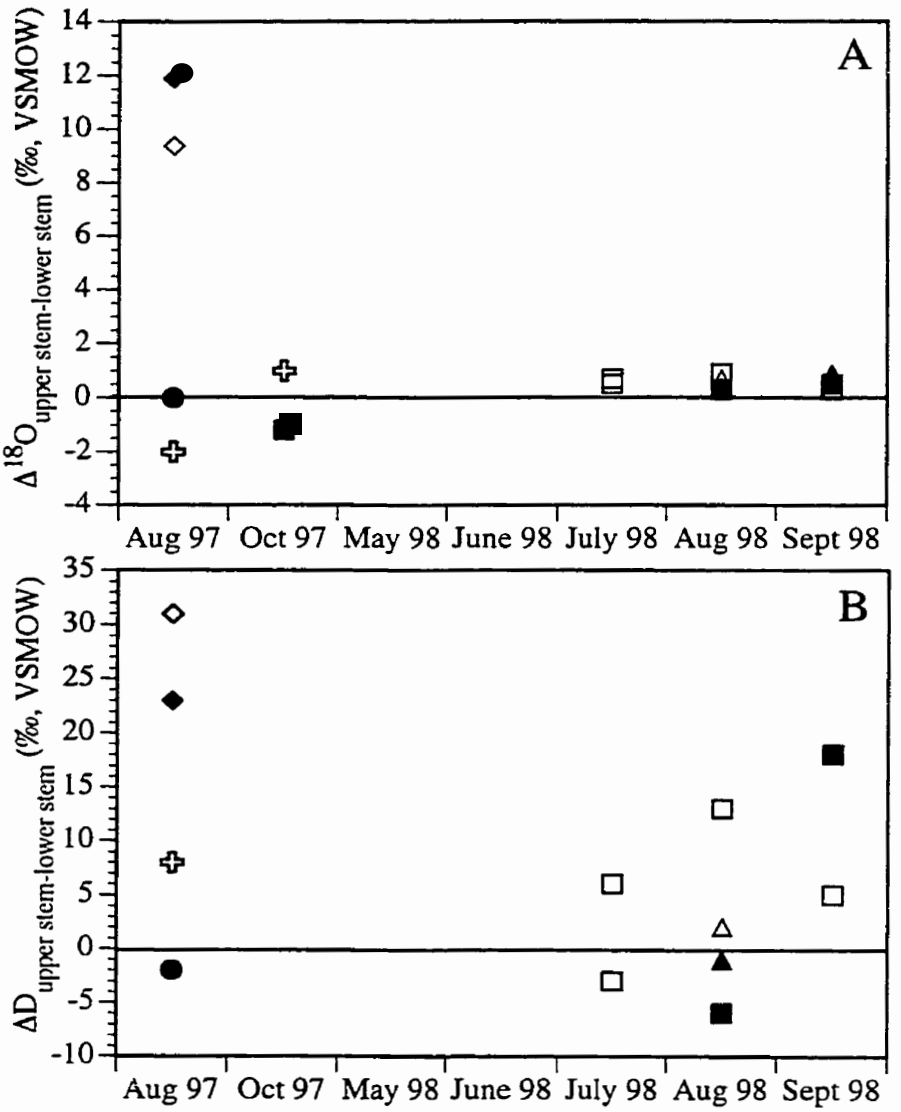

Figure 4-1. A) $\Delta^{18}O_{upper\ stem\text{-}lower\ stem}$ and B) $\Delta D_{upper\ stem\text{-}lower\ stem}$ values for (□) *C. longifolia*, (△) *A. breviligulata*, (○) *A. scoparius*, (◇) *S. spartea*, and (✣) *A. gerardi*. Filled symbols represent pre-dawn and open symbols represent mid-day stem samples.

pairs are much closer to zero for the other grass species that were sampled (Fig. 4-1). Nevertheless, this variation is often greater than the analytical error, especially for $\Delta D_{upper\ stem\text{-}lower\ stem}$ values (Fig. 4-1). Three possible causes for isotopic variation of waters within a stem are: 1) evaporative enrichment of stem waters, 2) a change in the $\delta^{18}O$ and δD values of the soil water supplied to the plant, and 3) mixing of stem water with partially evaporated leaf water.

The first possibility, evaporative enrichment, is the most feasible explanation for the extreme enrichment of upper versus lower stem portions of *S. spartea*. Evaporative loss of water directly from the stem may occur in young stem tissues in which the cell walls have not been thickened by lignin or suberin (Dawson and Ehleringer, 1993). This would cause waters in the younger, upper stems to become more highly enriched than lower stem waters, which have a more immediate influx of non-fractionated soil water. In the upper portions of the *S. spartea* stems, evaporative loss of water has caused the remaining liquid to become enriched in ^{18}O and D and plot further to the right of the local meteoric water line (Fig. 4-2). The evaporative water loss from the stem of this species is likely a physiological phenomenon related to the life cycle of this plant. *S. spartea* is a native cool-season perennial grass. Growth begins in the late fall and plants may remain green all winter. Seed production occurs in late June and the grass remains dormant for the remaining summer months. At the time of sampling, in August 1997, the *S. spartea* stems were yellow and dry, and active water movement through the stem appeared unlikely. For the remaining discussion only the lower-stem waters of *S. spartea* will be considered.

It is unlikely that isotopic differences between waters of the upper and lower stem of the other species sampled here are the result of stem-water evaporation. These species do not demonstrate a consistent enrichment of water within the upper versus lower stem and, in some cases, the lower stem waters are more enriched than the upper stem waters (Fig. 4-1). On a plot of δD versus $\delta^{18}O$ values (Fig. 4-2). all stem waters

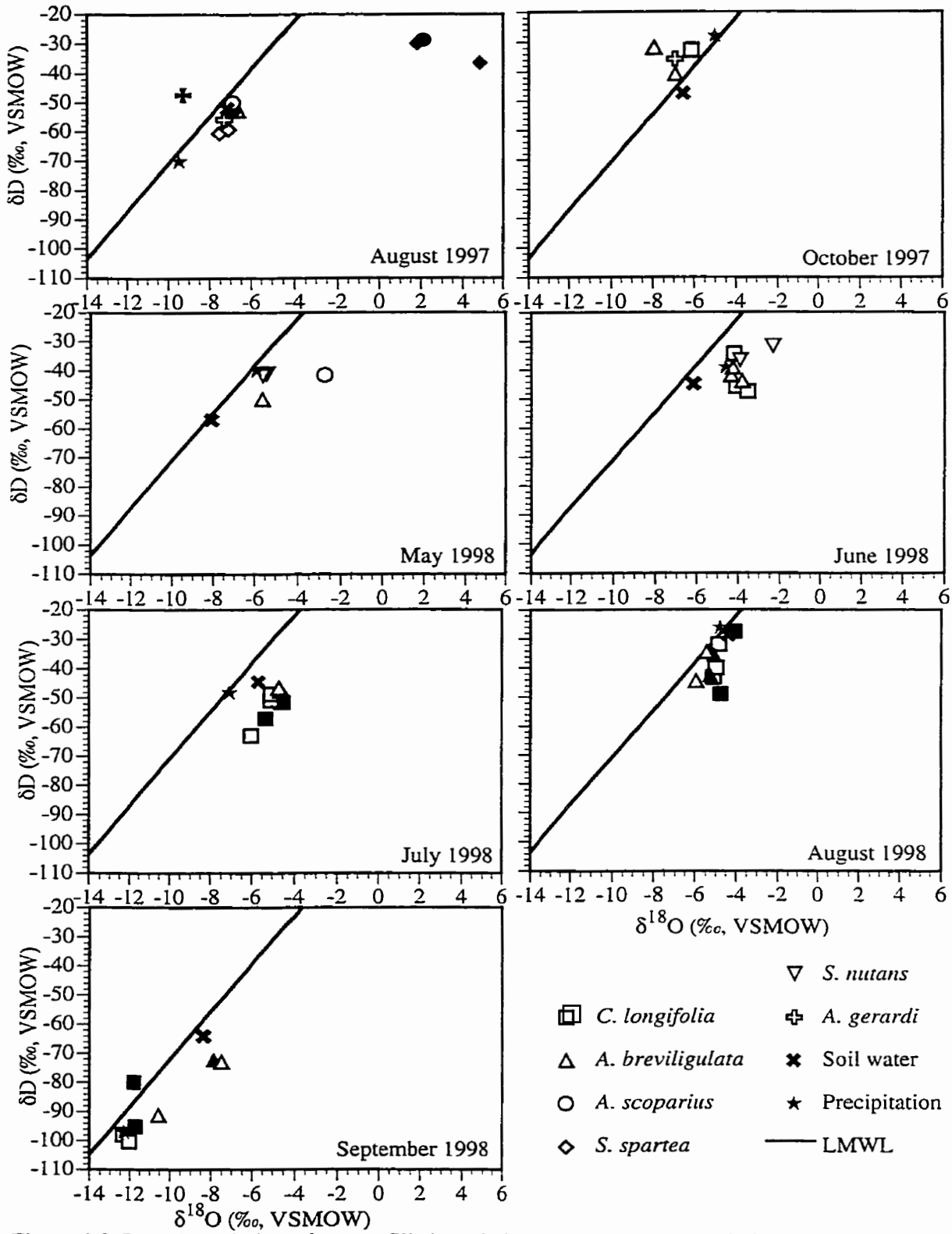

Figure 4-2. Isotopic variarion of upper (filled symbols) and lower (open symbols) stem water. The solid line is the weighted summer local meteoric water line. Soil-water δ-values are a weighted mean over a depth of one meter.

cluster near the local summer meteoric water line (LMWL; $\delta D = 8.1(\delta^{18}O) + 10.6$). The close proximity of stem-water compositions to the meteoric water line, monthly precipitation and weighted average soil-water δ-values indicate that these waters have not suffered significant evaporative enrichment subsequent to movement into the stem.

The discrepancy between the isotopic composition of upper versus lower stem waters in the other species studied may also be the result of a rapid change in the $\delta^{18}O$ and δD values of the soil water supplied to the plant. If the rate of root-water uptake and subsequent movement through the stem is slower than the rate of change in soil-water isotopic compositions, the upper stem water might represent water from an older supply of soil water. In such a scenario, the upper stem water may have been fed earlier by water that had different δD and $\delta^{18}O$ values than that which had more recently fed the lower stems. However, this is unlikely since the turnover rates of water in a plant are very rapid, on a scale of hours. If the isotopic composition of water in the soil profile changed more rapidly than the turnover rate of stem water, we would expect the spread between upper and lower stem-water δ-values to be smaller under mid-day conditions when the rate of transpiration and water movement through the plant is higher than under pre-dawn conditions. No such pattern is evident (Fig. 4-1).

Most likely, the isotopic variation of water within an individual stem results from the addition of ^{18}O- and D-enriched leaf water to the stem. This facilitates nutrient transport through the phloem from the older leaves to younger leaves and other plant tissues. Regardless, we note that these variations are minor and for the purposes of further discussion, we will refer to the average stem-water $\delta^{18}O$ and δD values.

4.4.1.2. *Diurnal variations in plant water δ-values*

The $\delta^{18}O$ and δD values of stem water display only minor variations between pre-dawn and mid-day samplings (Tables 4-2, 4-3 and 4-4), as illustrated in Figure 4-3 for *C. longifolia* and *A. breviligulata*. These diurnal variations may be the result of evaporative

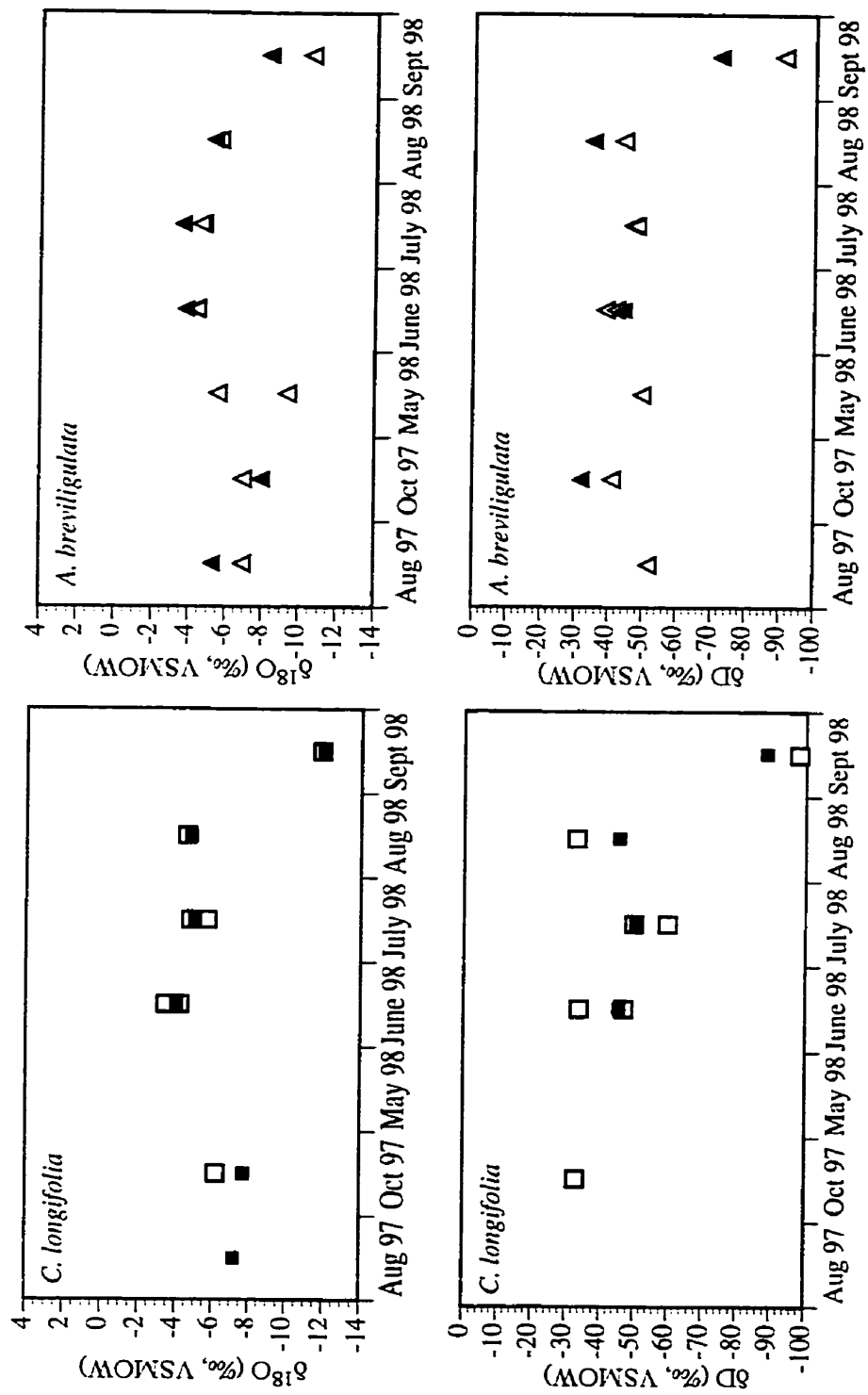

Figure 4-3. Stem-water $\delta^{18}O$ and δD values for (□) *C. longifolia* and (Δ) *A. breviligulata*. Filled symbols represent pre-dawn samplings and open symbols represent stem waters sampled at mid-day.

enrichment of water within the stem or the result of temporal and spatial variations in the isotopic composition of the soil water available for plant uptake.

It is unlikely that the diurnal variations are the result of transpiration, as all stem-water samples, including both pre-dawn and mid-day values, cluster near the meteoric water line (Fig. 4-2). In addition, there is no consistent enrichment of ^{18}O or D in stem water associated with mid-day samplings when transpiration is most active. Nor is there an increase in the spread of mid-day/pre-dawn samples in months when the relative humidity was lower (i.e. June and July 1998) and evaporation associated with transpiration should be at a maximum.

A second explanation for the observed diurnal variations of stem-water $\delta^{18}O$ and δD values are daily fluctuations in the ^{18}O- and D-composition of the soil water that feeds the plant. Soil water that feeds the grasses in the morning can differ in its $\delta^{18}O$ and δD values from soil water available to the grasses at mid-day. This has the potential to create diurnal variations in the δ-values of stem water. Evaporation from the soil surface proceeds at a higher rate during the warmest portion of the day, which can enrich near-surface soil water in both D and ^{18}O to a depth of 0.1 to 0.5 meters (Barnes and Allison, 1988; Walker and Lance 1991). In addition, the low vegetative cover and poor thermal conductance of sand generates very high temperatures in the surface layers during the day. This is followed by a rapid reversal of the subsurface heat gradient overnight as the sand surface cools, which induces wet, moist air from deeper in the soil profile to rise and condense at shallower levels (Ranwell, 1972; Baldwin and Maun, 1983; Walker and Brunel, 1990). This process can add water depleted in ^{18}O and D to the enriched water already present in the upper layers of the soil (Walker and Brunel, 1990). However, such diurnal variations in stem-water $\delta^{18}O$ and δD values are not apparent for our samples. In many cases the pre-dawn stem water is more enriched in ^{18}O and D than the mid-day stem water (Fig. 4-3). Moreover, the water provided to the stems has the isotopic composition of soil-water integrated over the depth of the active rooting depth (e.g., up to

3 meters for *C. longifolia*). Consequently, the majority of water feeding the stems of these grasses is not greatly affected by the changes in soil-water δD and $\delta^{18}O$ values that are concentrated in the uppermost portions of the soil-water profile.

By comparison, mid-day enrichment and pre-dawn depletion of ^{18}O and D in soil water are more apparent in the δD and $\delta^{18}O$ values of root and rhizome waters, particularly for *C. longifolia* (Figs. 4-4 and 4-5). The root and rhizome tissues analysed here were collected from the top 5 cm of the soil. At this depth changes in soil-water isotopic values that result from evaporation and condensation cycles are highly accentuated. That the majority of mid-day root and rhizome waters are more enriched in ^{18}O and D than their pre-dawn counterparts likely reflects the uptake of water in the upper layers of the soil that are affected by evaporation under mid-day conditions.

The distinction between the $\delta^{18}O$ and δD values of pre-dawn versus mid-day stem, rhizome and root waters may also be the result of overall spatial variations in soil water δ-values. Rain infiltration through dune sand follows complex pathways delineated by subtle changes in density, grain size and moisture content of the sand (Ritsema and Dekker, 1994; Berndtsson et al., 1996). Since our sampling technique is destructive, adjacent plants were used for mid-day and pre-dawn samplings. However, soil water sampled at two locations approximately 10 meters apart in the dune slack showed variations in the top 5 cm of up to ±2.3‰ and ±17‰ for $\delta^{18}O$ and δD values respectively (Gage, in progress; Fig. 4-6). Even larger variations in $\delta^{18}O$ and δD values occur at depth between the two profiles (e.g., September 1998, Fig. 4-6g). Hence, lateral variations in soil-water composition likely contribute to the observed differences between pre-dawn and mid-day δ-values of water in the non-transpiring tissues.

4.4.1.3. *Variations in plant-water δ-values between adjacent plants*

To determine the reliability of our sampling method, comparisons were made between the water in the corresponding non-transpiring plant tissues of adjacent plants. The stem waters extracted from adjacent plants of the same species (e.g., May 6, 1998, *C.*

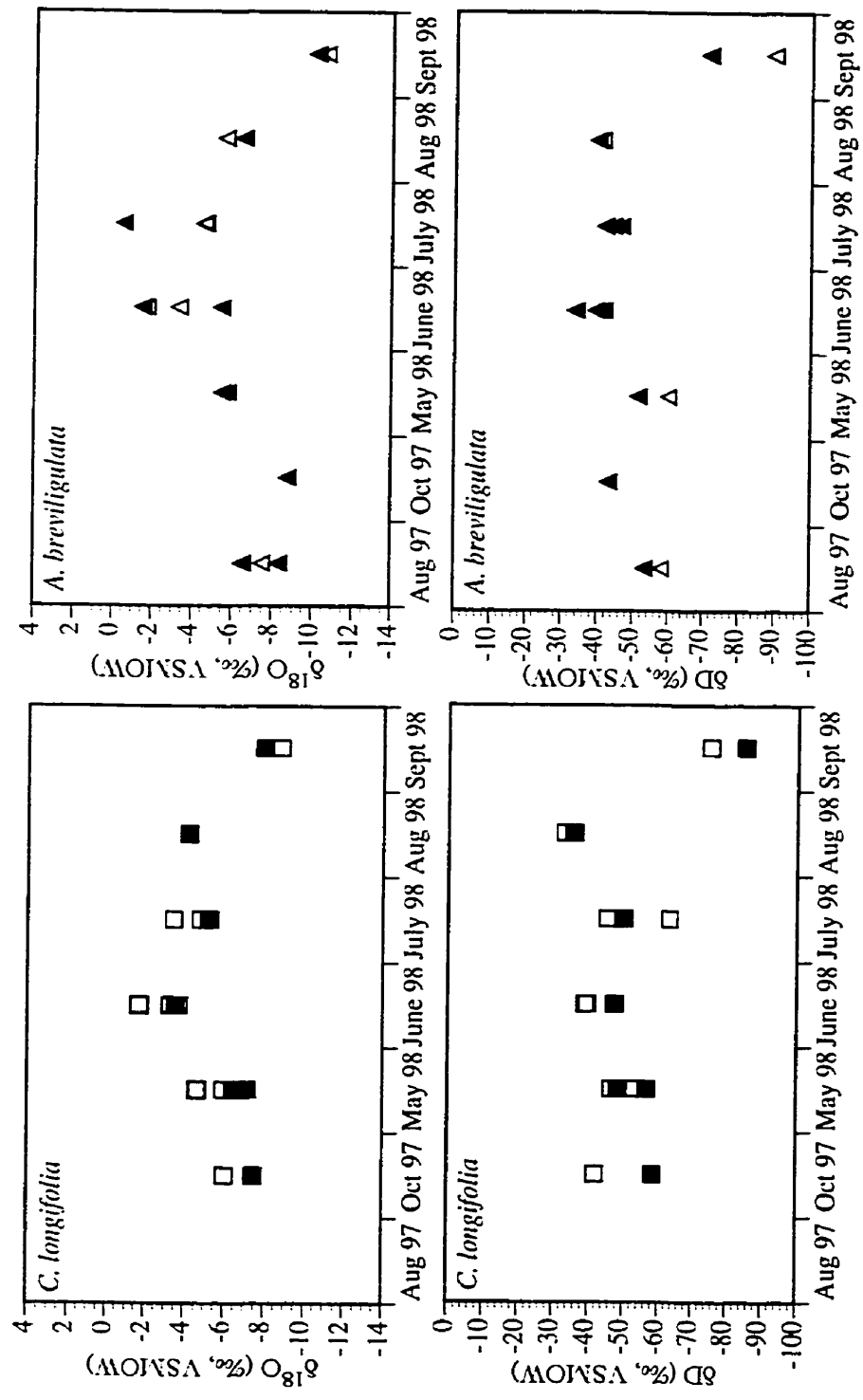

Figure 4-4. Rhizome-water $\delta^{18}O$ and δD values for (□) *C. longifolia* and (Δ) *A. breviligulata*. Filled symbols respresent pre-dawn waters and open symbols represent waters from rhizomes collected at mid-day.

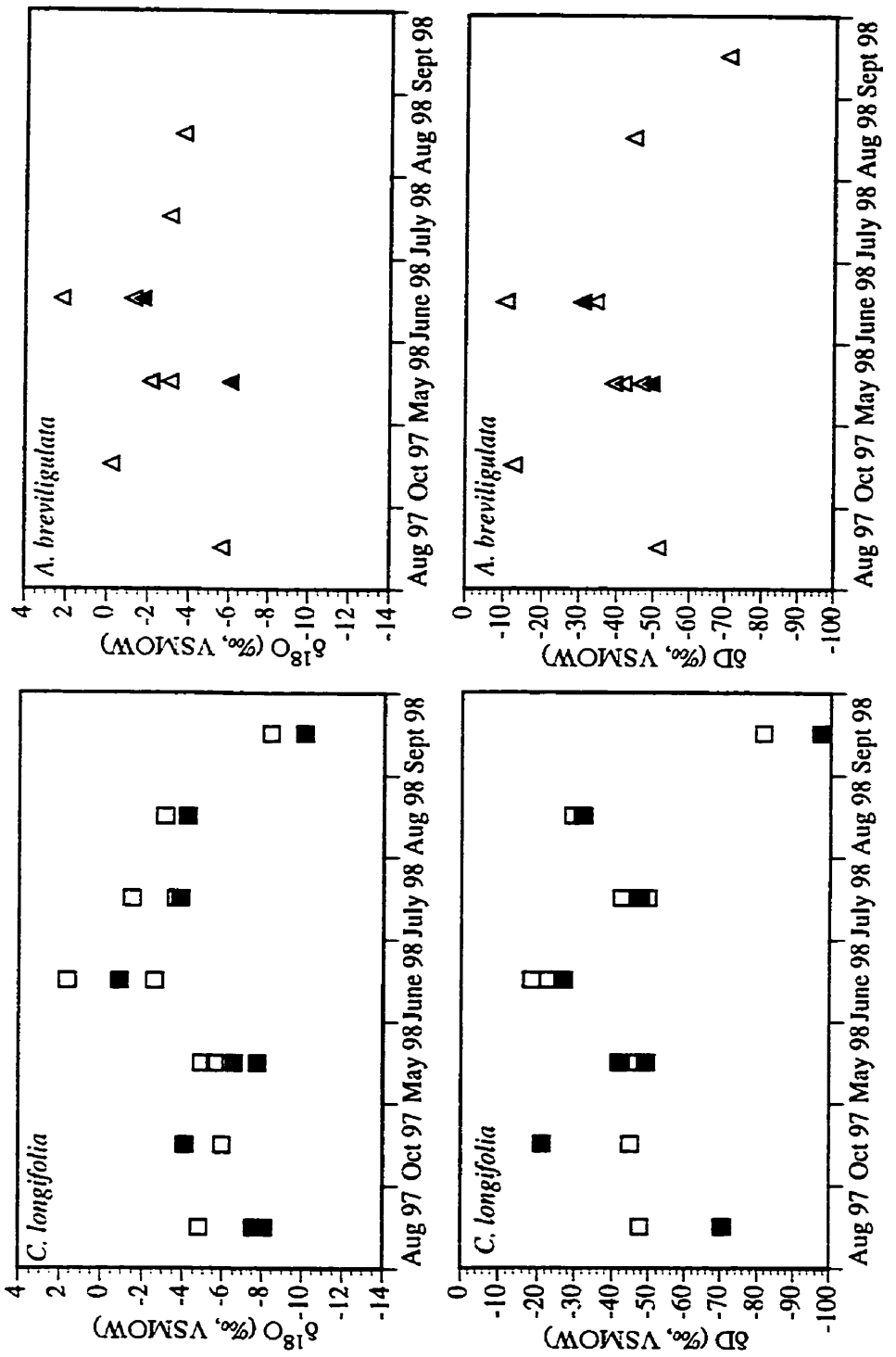

Figure 4-5. Root-water $\delta^{18}O$ and δD values for (□) *C. longifolia* and (Δ) *A. breviligulata*. Filled symbols respresent pre-dawn root waters and open symbols represent waters collected from roots at mid-day.

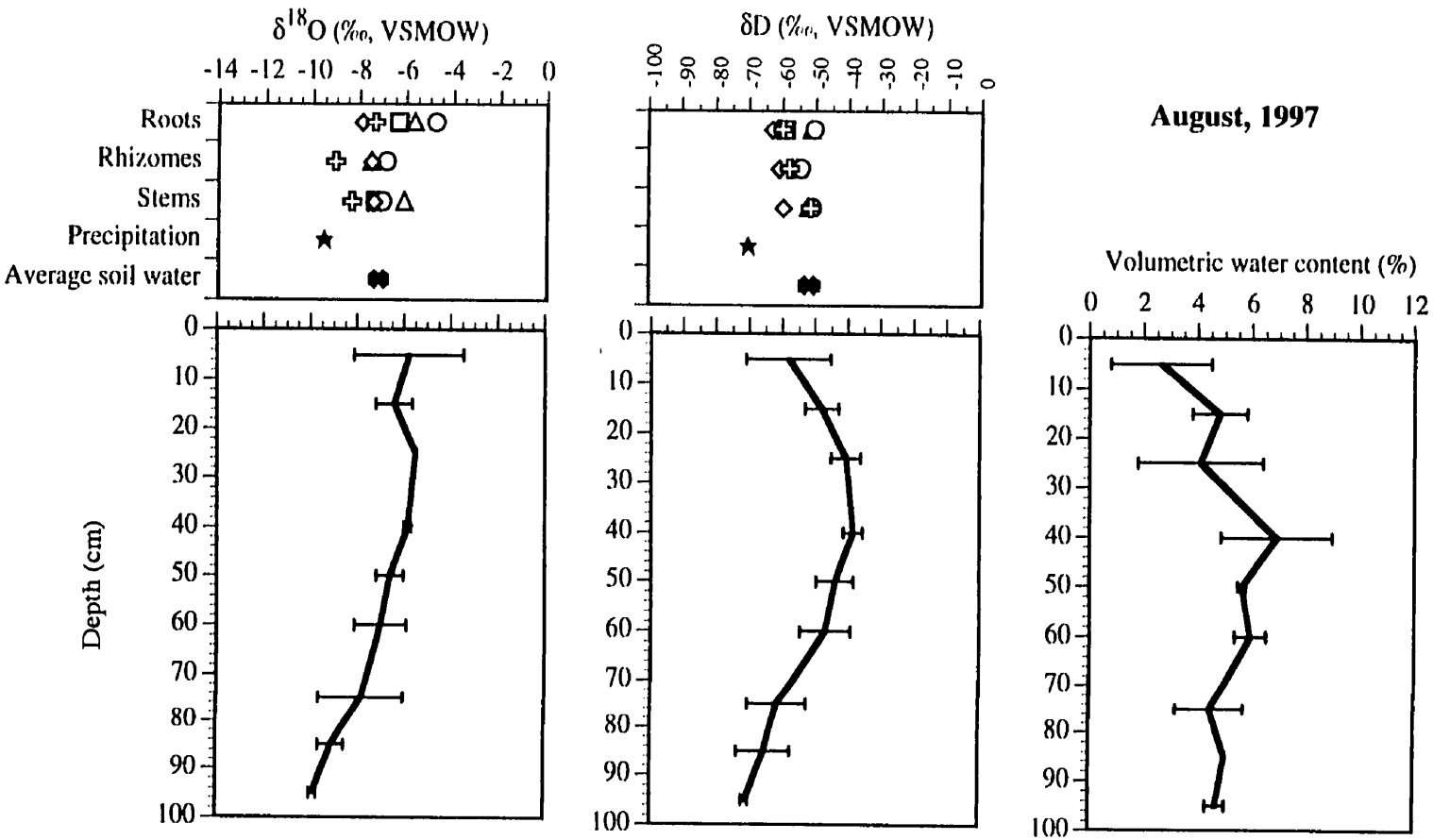

Figure 4-6a. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for August, 1997. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent the $\delta^{18}O$ and δD values for monthly precipitation (★); average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

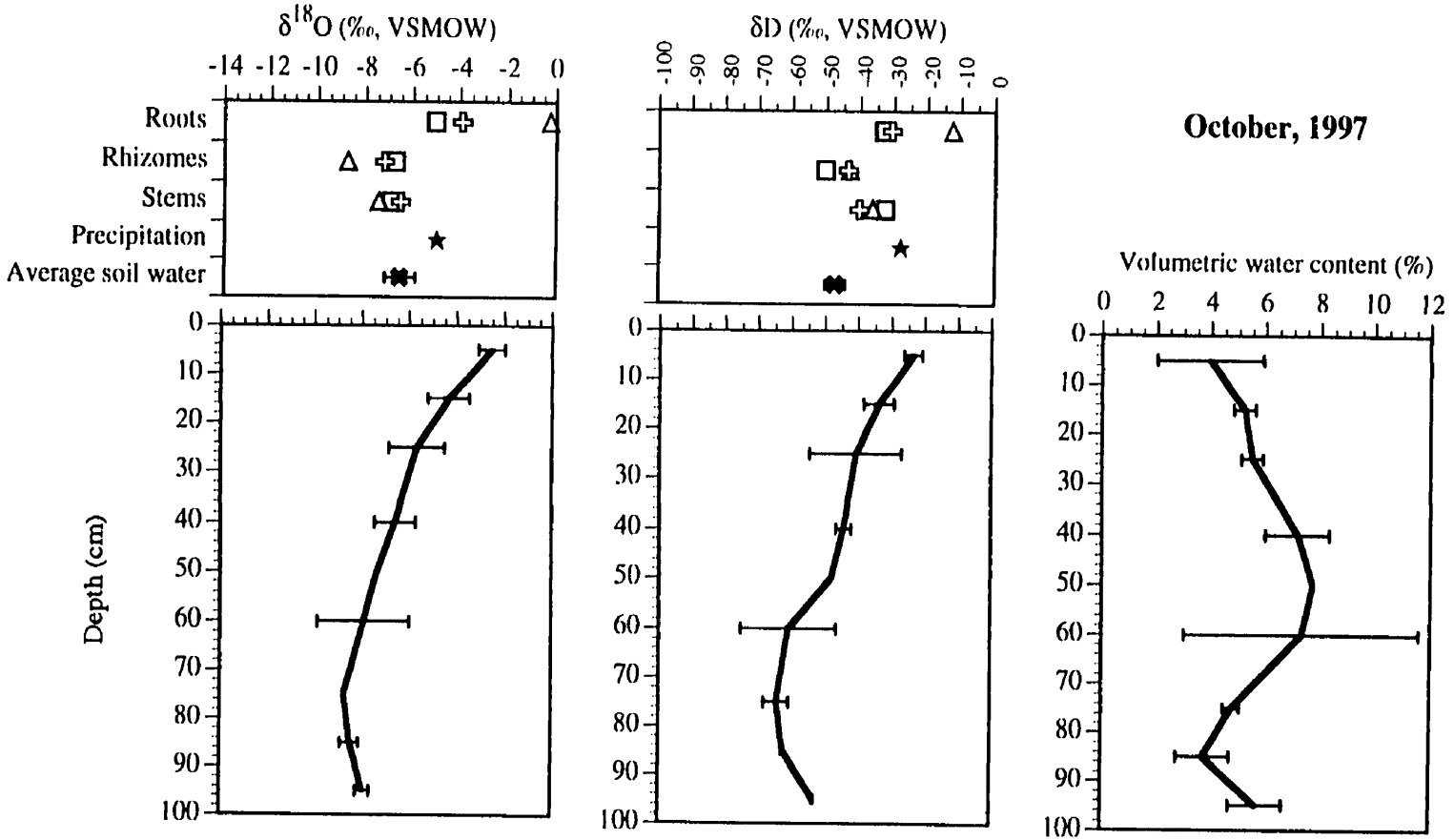

Figure 4-6b. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for October, 1997. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent the $\delta^{18}O$ and δD values for monthly precipitation (★); average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

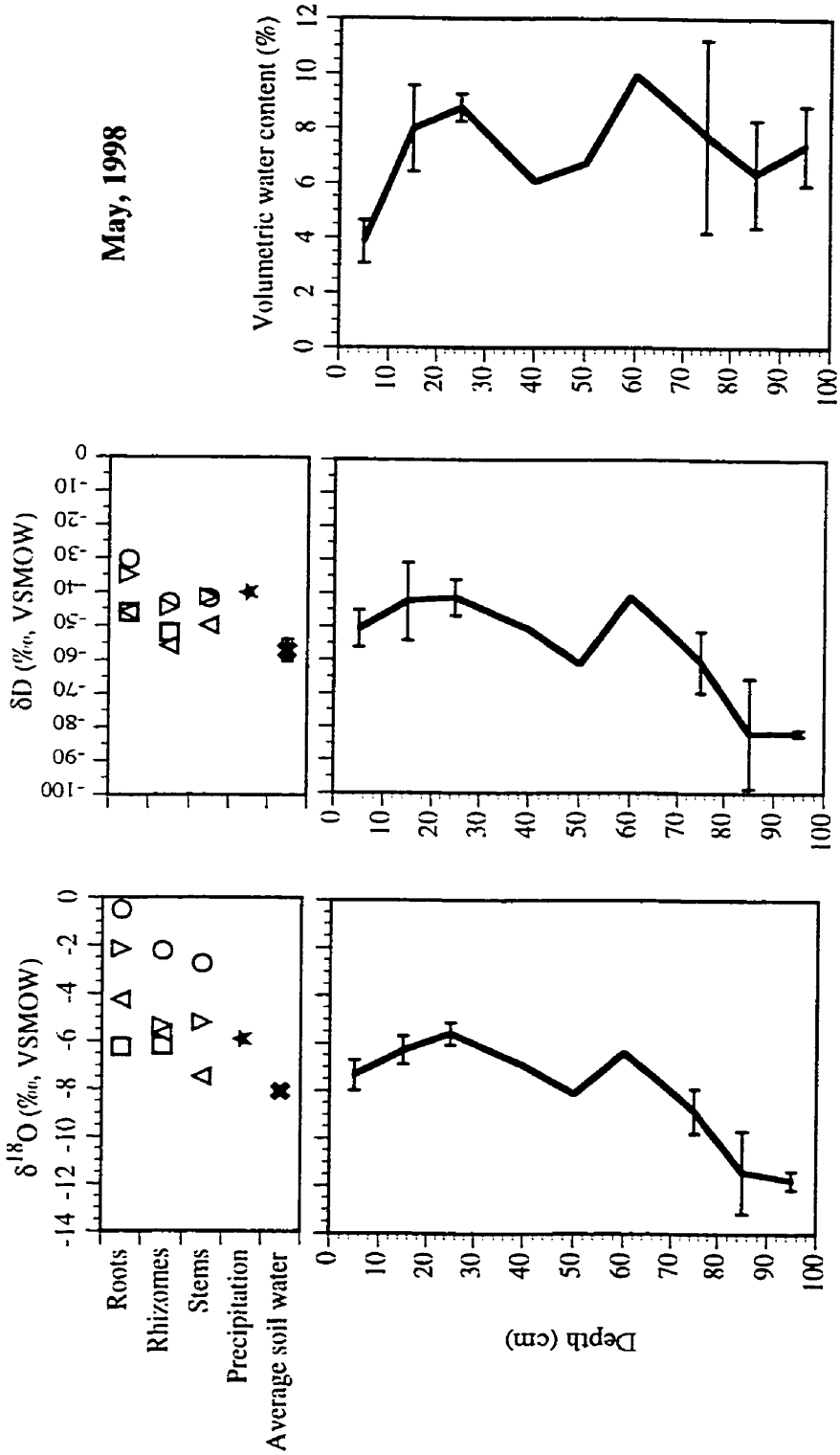

Figure 4-6c. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for May, 1998. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent $\delta^{18}O$ and δD values for monthly precipitation (★); the average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (⌀); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

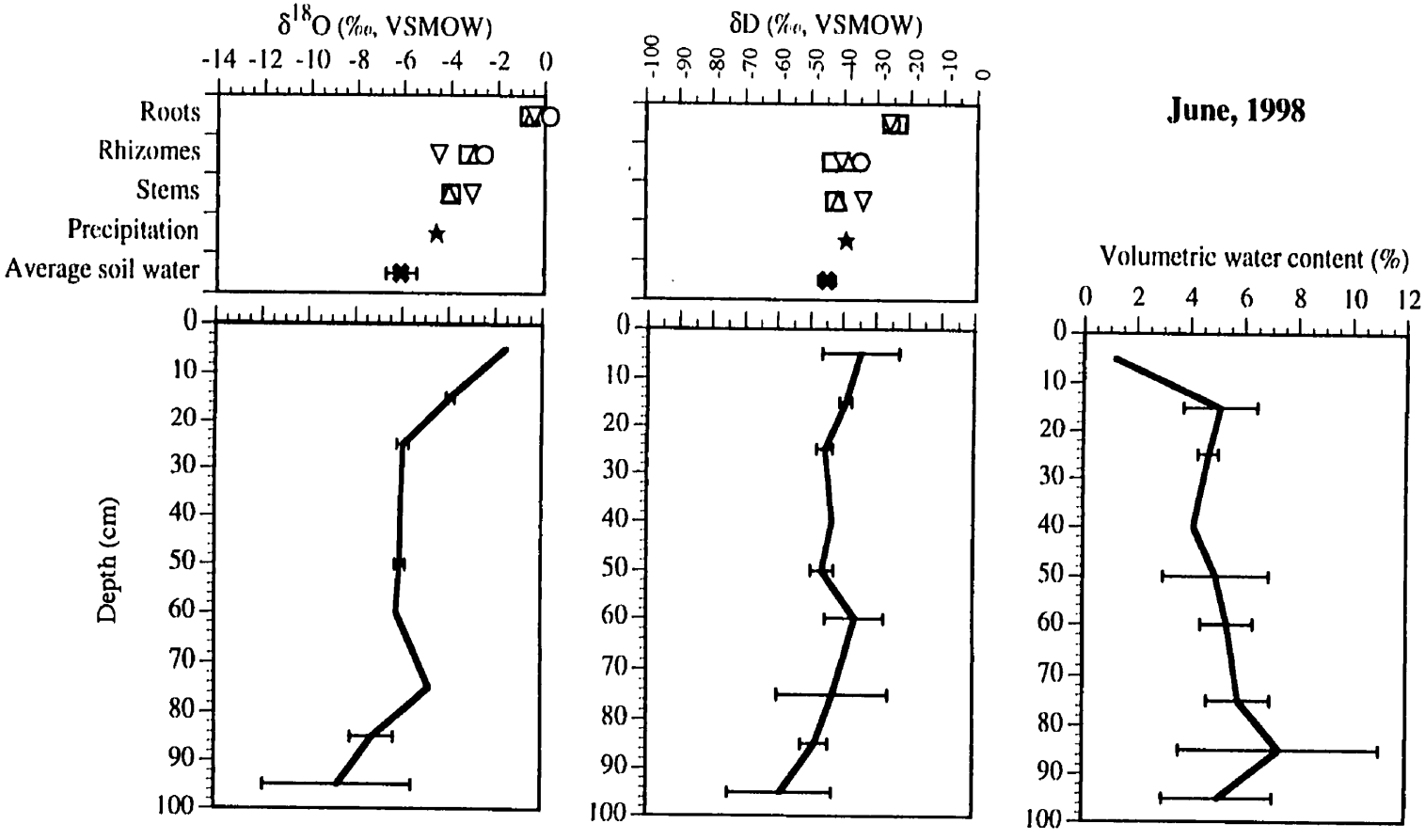

Figure 4-6d. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for June, 1998. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent $\delta^{18}O$ and δD values for monthly precipitation (★); the average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

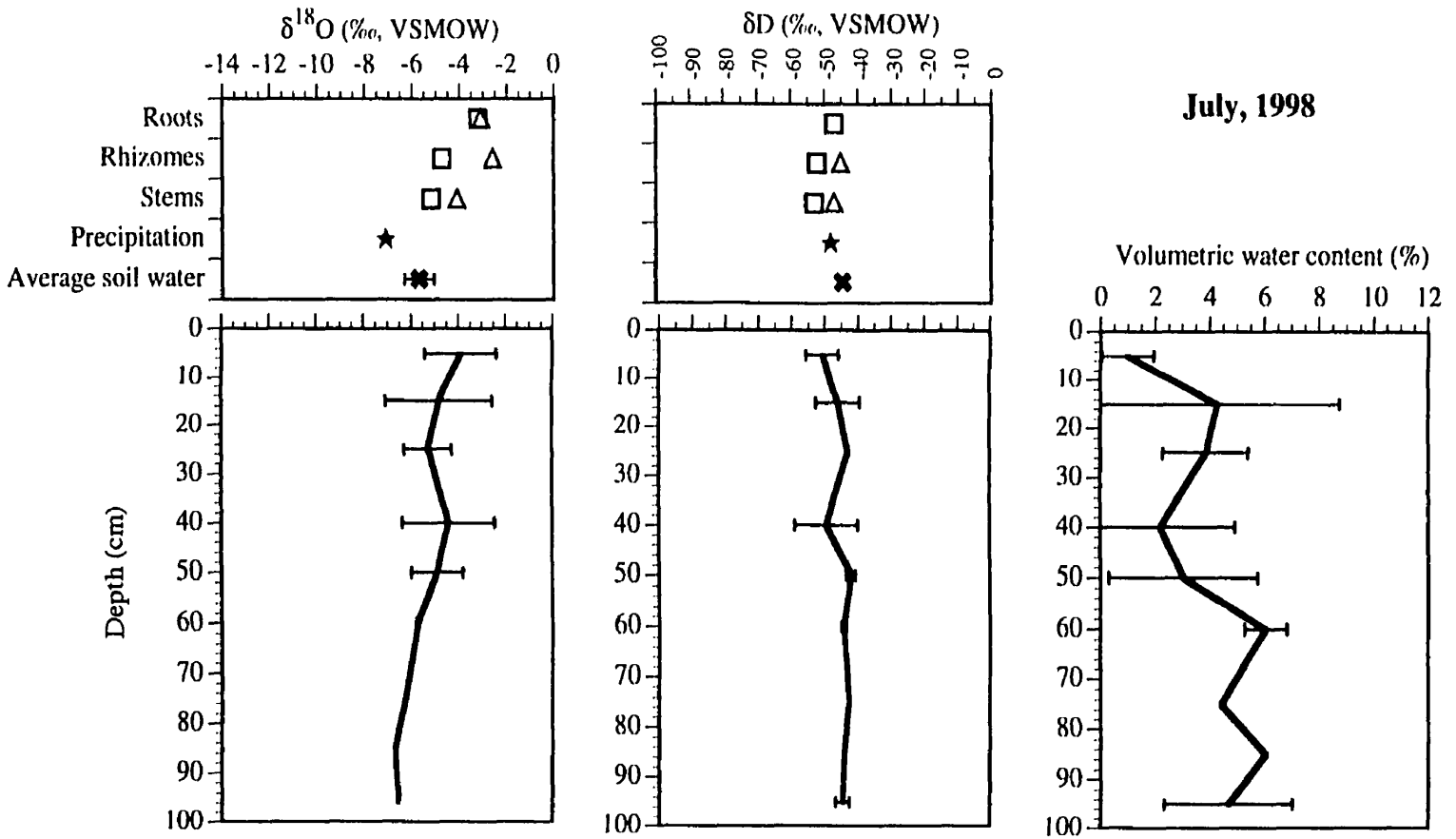

Figure 4-6e. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for July, 1998. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent $\delta^{18}O$ and δD values for monthly precipitation (★); the average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

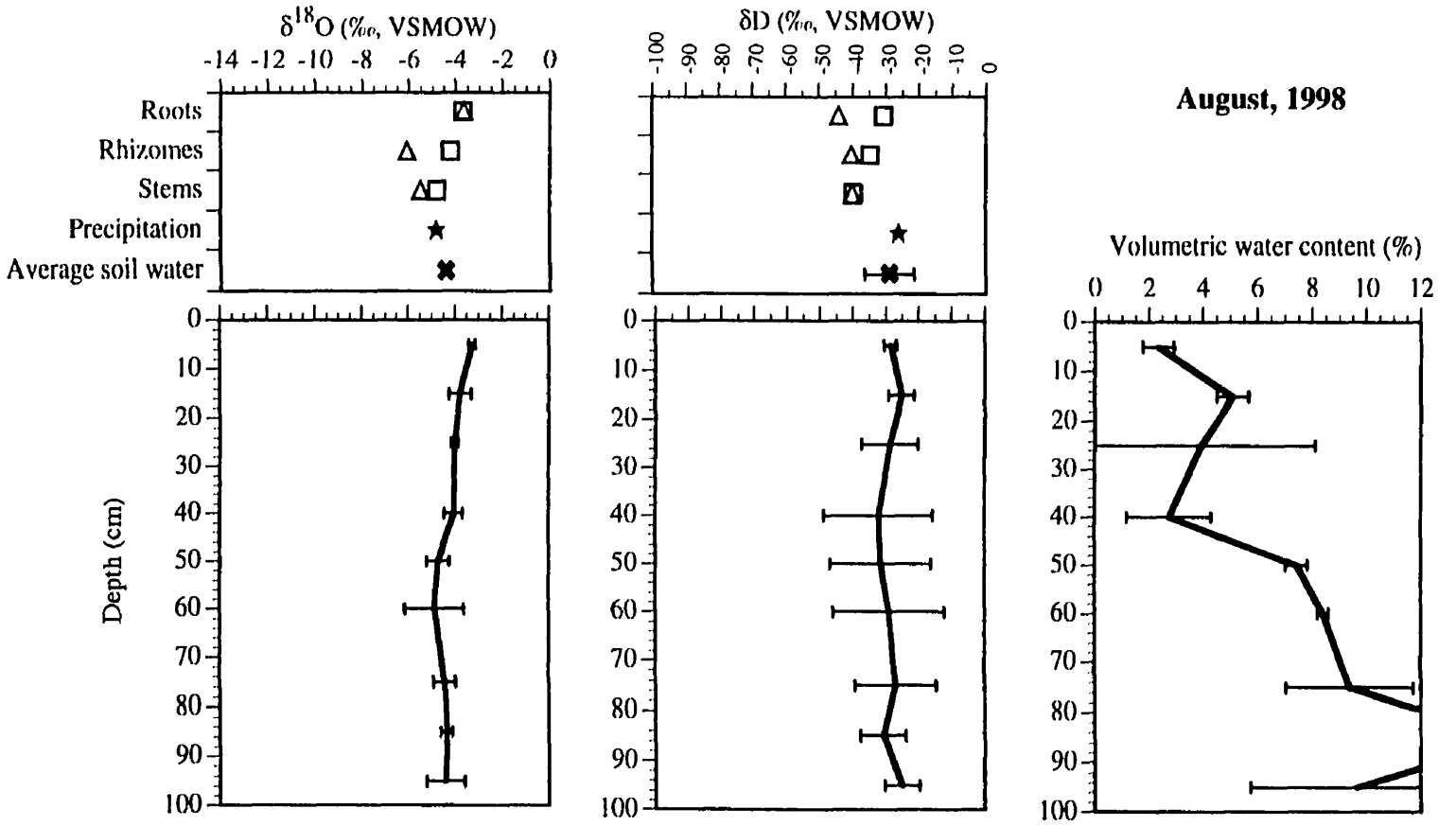

Figure 4-6f. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for August, 1998. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent $\delta^{18}O$ and δD values for monthly precipitation (★); the average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

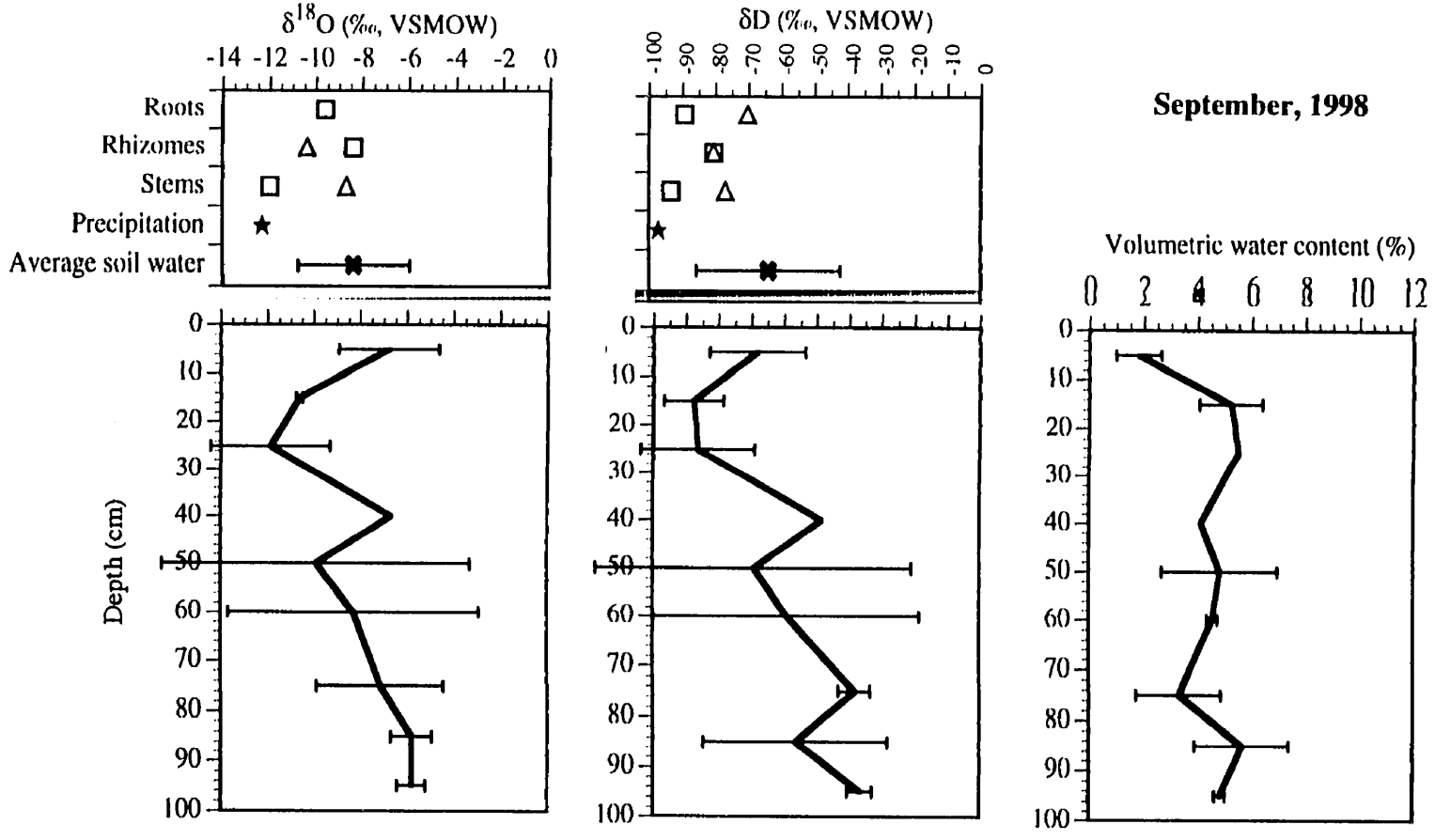

Figure 4-6g. Profiles of the $\delta^{18}O$ and δD values of water and volumetric water content of soils at Pinery Provincial Park, for September, 1998. Error bars represent the variations between two adjacent soil cores, approximately 10 meters apart. Where no error bars are shown data was only available from one soil core. Symbols represent $\delta^{18}O$ and δD values for monthly precipitation (★); the average $\delta^{18}O$ and δD values of water from the non-transpiring tissues of *C. longifolia* (□), *A. breviligulata* (△), *A. scoparius* (○), *S. nutans* (▽), *S. spartea* (◇), and *A. gerardi* (✣); and the weighted average soil-water (✖) $\delta^{18}O$ and δD values.

longifolia) and samples of the same species collected on consecutive days (e.g., mid-day samples of *C. longifolia* and *A. breviligulata* collected in June and July 1998 and mid-day samples of *S. nutans* collected in June 1998) do not vary greatly in their δ-values (Fig. 4-3). The consistency of stem-water $\delta^{18}O$ and δD values at each sampling interval implies that silica in the stem may be precipitated from a water reservoir that has a fairly stable isotopic composition on a day-to-day basis. However, the uptake of recent rain events can cause stem water δ-values to vary over short periods of time. For example, the mid-day samplings on May 6th and May 7th were separated by an overnight rain event ($\delta^{18}O$ = -3.3‰, δD = -36‰; Gage, in progress). The higher δ-values of stem water from *A. scoparius* and *A. breviligulata* sampled on May 7th ($\delta^{18}O$ = -5.6‰, δD = -50‰ for *A. breviligulata*; $\delta^{18}O$ = -2.8‰, δD = -42‰ for *A. scoparius*) relative to stem waters collected the previous day ($\delta^{18}O$ = -9.4‰ for *A. breviligulata*) reflect the isotopic composition of the new precipitation and implies a rapid uptake of this water from the soil-water reservoir (Tables 4-3 and 4-4; Fig. 4-7).

By comparison, variations in the $\delta^{18}O$ and δD values of plant water between adjacent rhizome and root tissues are greater than for stem waters (Figs. 4-4 and 4-5). As discussed previously, these variations likely reflect the increased spatial and temporal heterogeneity in the isotopic composition of shallow soil water. In addition, roots and rhizome tissues are more difficult to sample. When in place, the underground tissues are protected from surface climatic conditions, but when exhumed, they have a greater potential to suffer evaporative enrichment even during the short time needed for dissection of the grass in the field. Moreover, it is unknown whether the particular roots and rhizomes sampled were active in water uptake as they were located at a depth that has, in general, a lower water content (Fig. 4-6). If the underground tissues in the upper layers of the soil were dormant at the time of sampling, they may have contained waters that were enriched in ^{18}O and D because of successive periods of evaporation.

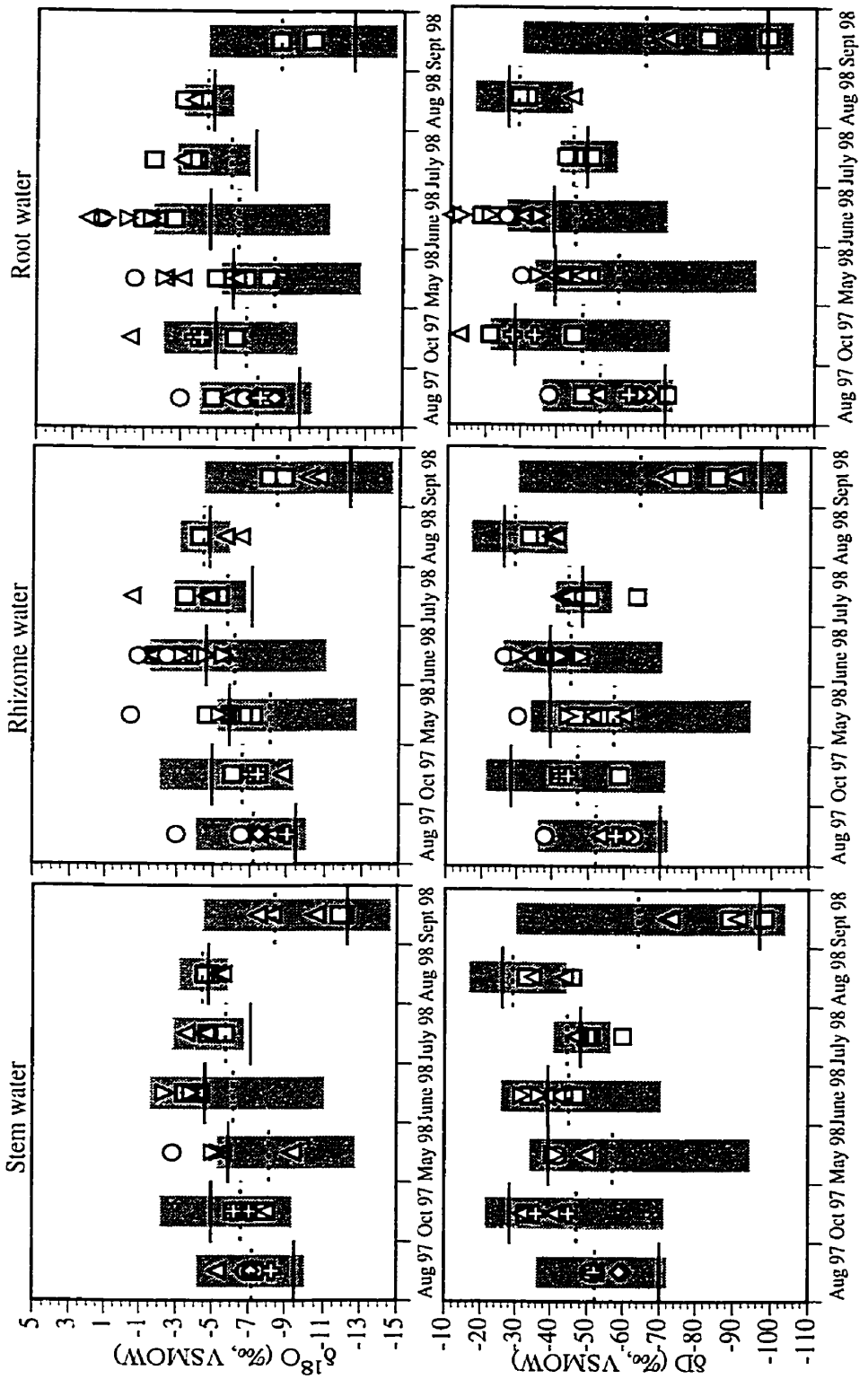

Figure 4-7. Stable isotopic compositions of water from the stems, rhizomes and roots of (□) *C. longifolia*, (△) *A. brevifiligulata*, (○) *A. scoparius*, (▽) *S. nutans*, (◇) *S. spartea* and (⊕) *A. gerardi*. The shaded areas represent the range in the stable isotopic compositions of soil water to a depth of one meter. The dashed lines are the weighted average soil-water stable isotopic compositions, and the solid lines represent the stable isotopic compositions of monthly precipitation.

4.4.1.4. *Synopsis*

With the exception of *S. spartea*, the stem water of most grasses studied here does not exhibit evaporative enrichment in ^{18}O and D. In the non-transpiring tissues (stems, rhizomes and roots), the variations in δ-values between pre-dawn and mid-day, day to day or adjacent plant samplings are minimal and most likely the result of spatial heterogeneity or temporal changes in soil-water isotopic compositions. Variations among rhizome and root waters of the same species are more variable than stem waters. However, these differences can be explained by the increased variation of soil-water δ-values within the very shallow depths at which these underground tissues were sampled.

4.4.2. Implications for soil-water uptake

Figure 4-7 compares (i) the $\delta^{18}O$ and δD values of waters extracted from non-transpiring tissues (stems, rhizomes and roots) of each grass species, (ii) $\delta^{18}O$ and δD values of monthly precipitation, (iii) the weighted average soil-water $\delta^{18}O$ and δD values, and (iv) the range of soil-water $\delta^{18}O$ and δD values to a depth of one meter on the day of plant-water sampling. A depth interval of one meter was deemed adequate to define the isotopic composition of the soil-water reservoir from which the grasses may uptake water. While it is true that the roots of *C. longifolia* and *A. gerardi* may extend to a depth of three to four meters, respectively (Phillips, 1963; Weaver, 1968), the majority of root mass for these species occurs in the top 60 cm. In addition, 85% of the root mass of *A. breviligulata* occurs in the top 40 cm depth (Hansen, 1976) and excavations performed by Maun (1985) found that the majority of underground biomass of *C. longifolia* and *A. breviligulata* was situated in the top 40 centimeters of the soil profile at Pinery Provincial Park.

Water is supplied to the stems from roots present over the entire active rooting depth. Hence, the isotopic composition of stem water is the most indicative of soil water that is collectively available for plant uptake. The variation in δ-values of stem waters

between different grass species at Pinery Provincial Park is minimal. Stem-water $\delta^{18}O$ and δD values vary by less than 3‰ and 15‰, respectively, for all months with the exception of May, 1998 and September 1998. For those two months, preferential uptake of recent rain events is evident (see Tables 4-2, 4-3 and 4-4). Varying rooting patterns of different grass species provide the potential to extract water from different depths of the soil-water profile. For example, the roots of *A. gerardi* may extend to a depth of 4 meters, whereas those of *S. spartea* reach a maximum depth of 1.2 meters (Phillips, 1963; see Chapter 1). However, within each monthly sampling period, individual stem-water $\delta^{18}O$ and δD values of any one species are not consistently higher or lower relative to the other species (Fig. 4-7). This suggests that individual plants of the same species display as much variation in their pattern of soil-water uptake as grasses from different species.

Root and rhizome δ-values are more enriched in ^{18}O and D than stem water (Fig. 4-7) and display a greater spread in $\delta^{18}O$ and δD values between species. These larger variations are a result of their collection from the top 5 centimeters of the soil profile. At this depth soil waters have a more variable isotopic composition (Fig. 4-6). The variations are not likely related to species effects as there is no associated fractionation with the uptake of water into roots, and the roots were all collected from approximately the same depth. Within each month there is as much isotopic variation between the waters from root and rhizome tissues of the same species as between the corresponding tissues of different species (Fig. 4-7). This suggests that the variations are as much the result of spatial variations in the isotopic composition of soil water as the consequence of species-dependent effects.

Some root and rhizome waters are enriched in ^{18}O and D beyond the measured range of soil-water δ-values (Fig. 4-7). In these cases, the roots and rhizomes sampled must have been growing in portions of the soil containing waters that are locally more enriched in ^{18}O and D than the average water present over the entire top 5 centimeter interval. The additional enrichment of root waters above the δ-values of soil water is

most prominent in the hottest, driest, summer months when evaporation is more extensive. Root waters may be more enriched than rhizome waters because root tissues hold less water than rhizomes. The δ-values of this smaller pool of water will be more sensitive to changes in climate, evaporation rates or even changes in environment during field dissection.

In general, soil waters are most enriched in ^{18}O and D near the surface as a result of evaporation. However, the isotopic composition of the soil profile is very dynamic from month to month. The relative depth of soil-water uptake by any species cannot be determined without knowledge of the variation in isotopic composition of the soil water with depth. Figure 4-6 (a through g) displays the stable isotopic composition and volumetric water content of the soil profiles, along with the average $\delta^{18}O$ and δD values for the water in the transpiring plant parts each month. These representations allow several useful comparisons to be made. In most of the profiles, the soil water is enriched in ^{18}O and D at the surface and becomes increasingly depleted at greater depths. This pattern is typical of an evaporation front just below the surface of the soil (Allison et al., 1984). At increasing depths, soil waters gradually become more depleted of ^{18}O and D relative to monthly precipitation. By a depth of one meter, soil-water $\delta^{18}O$ values begin to approach those of shallow ground water (annual average $\delta^{18}O_{ground\ water}$ =-11.1±0.1‰, n=11; Gage, in progress), likely because of mixing between precipitation and a low-^{18}O fraction of water stored in the unsaturated zone of the soil-water reservoir.

For most months, the average $\delta^{18}O$ and δD values of stem water collected over each sampling period are very similar to the weighted average soil-water composition. Excluding stem-water δ-values of *C. longifolia* for September 1998 and *A. scoparius* for May 1998, the standard deviation of the difference between average stem water and average weighted soil water is less than ±2.1‰ and ±10‰ and averages ±0.7‰ and ±5‰ for $\delta^{18}O$ and δD, respectively. Individual stem water samples that are depleted of ^{18}O and D relative to the weighted average soil-water composition, for example the August

1997 *A. gerardi* sample (Fig. 4-7), have drawn water from the deeper, wetter portions of the profile rather than the highly enriched, but meager water supply near the surface (Fig. 4-6a). Likewise, in September 1998, *C. longifolia* stem water is depleted of ^{18}O and D relative to the weighted average soil-water composition. At that time, there was a high volumetric water content at a depth of 15 to 30 centimeters in the soil profile (Fig. 4-6g). The soil water at this depth had an isotopic composition similar to the monthly precipitation and is likely the residual water from recent rain events that had not yet drained from the soil. The match between the δ-values of *C. longifolia* stem water and soil water at this depth strongly suggests that its roots were preferentially taking up water from this depth where moisture was most abundant. However, an adjacent sample of *A. breviligulata* appears to have drawn water over the entire soil profile. One month earlier, in August 1998, the isotopic composition of water fluctuates very little over the entire depth of the soil profile (Fig. 4-6f). The isotopic composition of water in the soil and hence the non-transpiring plant tissues corresponds to the abundant precipitation that fell during the previous month and saturated the soil profile (210 mm, Table 4-1).

In the warmer months, stem water is more enriched in ^{18}O (June and July, 1998) and D (June, 1998) than the average soil-water compositions (Figs. 4-6d and 4-6e). In June 1998, stem-water isotopic compositions are more similar to the monthly precipitation δ-values (Fig. 4-6d). During the warmest, driest months, when the monthly amount of precipitation is minimal (see Table 4-1) and soil profiles are marginally drier (Fig. 4-6), the grasses may rely more heavily on precipitation for a direct water supply, thus intercepting much of this water before it can reach the deeper layers of the soil. In other words, some of the active water uptake appears to have shifted to the upper layers of the soil. In arid regions the water potential and leaf-growth rate of grasses increases in direct response to large rainfall events (Golluscio et al., 1998). More specifically, the high concentration of water at the 15 centimeter depth in July 1998 has a similar isotopic

composition to that of average stem water, which suggests that the roots indeed were actively drawing water from this depth.

In summary, stem water appears to be derived from soil water over the entire soil profile to at least a depth of one meter. However in warmer, drier months, stems appear to take up water preferentially from the upper, generally more ^{18}O- and D-enriched portions of the profile, perhaps to utilize more efficiently incoming precipitation. The isotopic composition of stem water is very sensitive to rain events. Water with an isotopic composition similar to precipitation appears in stems within a day (e.g., *A. scoparius* stem waters in May 1998). Large rain events that increase the water content in a portion of the soil may also be preferentially utilized by the grasses (e.g., *C. longifolia* stem waters in September 1998). Overall, the average stem-water δ-values for each species each month varied by less than 3‰ and 15‰ for $\delta^{18}O$ and δD values, respectively (with the exception of May, 1998 and September, 1998). The average of stem-water $\delta^{18}O$ and δD values for *C. longifolia* and *A. breviligulata* throughout the 1998 growing season were almost identical ($\delta^{18}O$ = -6.4‰, δD = -56‰ and $\delta^{18}O$ = -6.0‰, δD = -51‰, respectively).

Although data are not available over the entire growing season for the four other species, we note the similarity in stem-water isotopic compositions between all species for the months they were collected. The silica phytoliths from the stems likely have $\delta^{18}O$ values that closely reflect the average isotopic composition of stem water over the entire growing season. Therefore, it is encouraging to note that average stem-water δ-values are similar for these grasses, which are common species of the North American Great Plains.

4.4.3. Stable isotopic variation of waters in transpiring tissues

The $\delta^{18}O$ values of sheath, upper leaf, lower leaf and inflorescence waters of *C. longifolia* and *A. breviligulata* are shown in Figure 4-8 together with the $\delta^{18}O$ values of stem water. For each species, upper and lower leaf waters collected at mid-day are

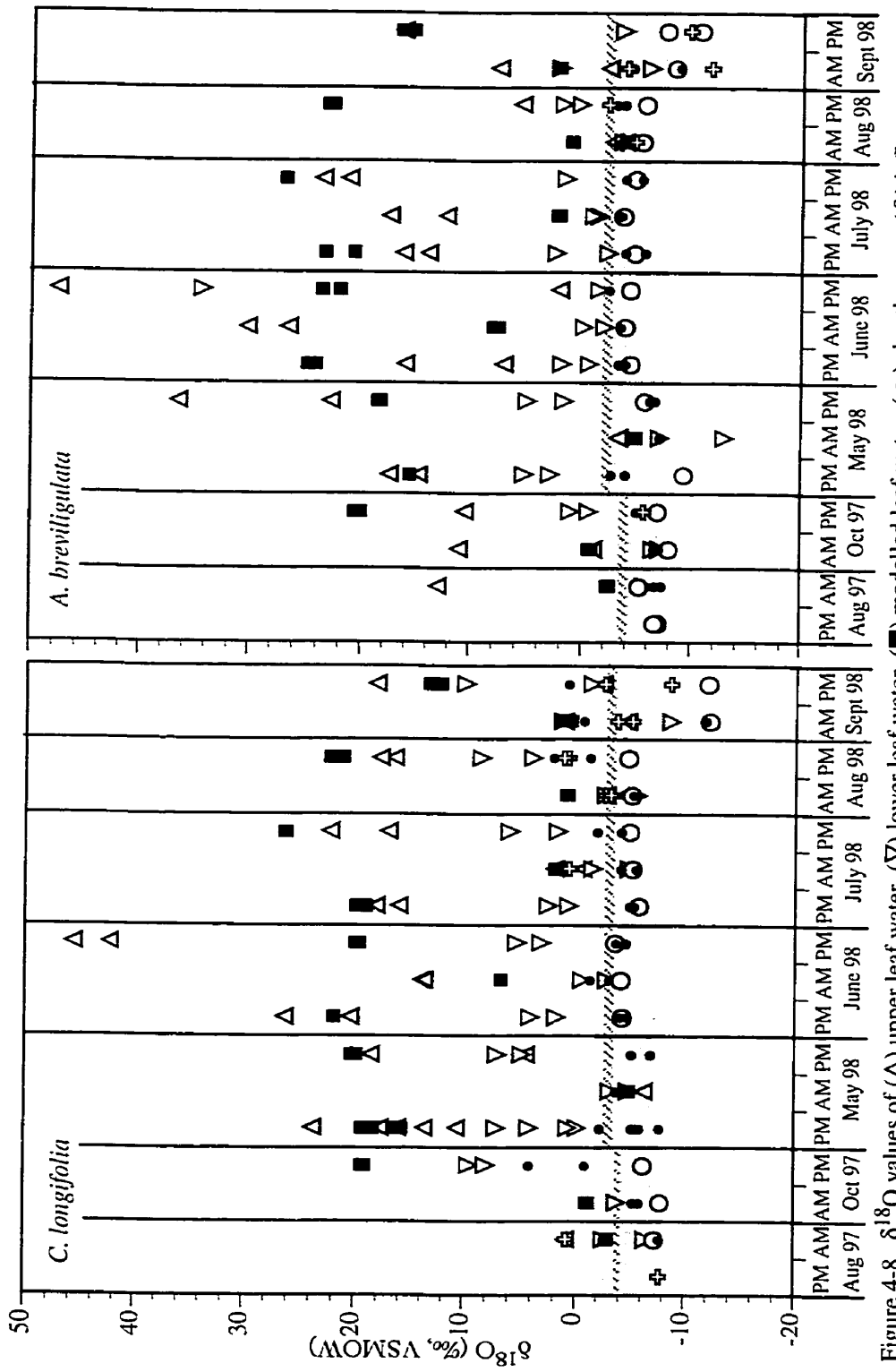

Figure 4-8. $\delta^{18}O$ values of (Δ) upper leaf water, (∇) lower leaf water, (■) modelled leaf water, (•) sheath water, (⊕) inflorescence water and (O) stem water of *C. longifolia* and *A. brevilligulata* at Pinery Provincial Park. The shaded area represents the range of $\delta^{18}O$ values of leaf water from which leaf silica was precipitated from 1994-1997 at Pinery Provincial Park, as calculated using Equation (4-4). The hatched areas are the $\delta^{18}O$ values predicted for leaf water, based on measured leaf-silica $\delta^{18}O$ values and temperatures for 1997, and on average stem-water $\delta^{18}O$ values and Equation (4-5) for 1998.

enriched in ^{18}O and D relative to the pre-dawn waters from the corresponding leaf tissue (Tables 4-2, 4-3 and 4-4; Fig. 4-8). As well, upper and lower leaf waters are more enriched in ^{18}O and D during the months with the lowest relative humidity (e.g., June 1998; Fig. 4-8). These diurnal and seasonal fluctuations are the result of an increase in evaporation of water from the leaf in response to lower relative humidity conditions that exist at mid-day and through the warmest months of the growing season when transpiration rates are highest (Allison et al., 1985; Leaney et al., 1985; Walker and Lance. 1991).

4.4.3.1. *Sheath-water δ-values*

Figure 4-9 displays the variation of sheath-water $\delta^{18}O$ and δD values between mid-day and pre-dawn collections for *C. longifolia* and *A. breviligulata*. Other species behave in a similar fashion (see Table 4-4). Relative to leaf water, sheath water does not exhibit a strong diurnal variation in $\delta^{18}O$ and δD values. The mid-day sheath values are, in general, only slightly higher than the pre-dawn values. The variation between average pre-dawn and average mid-day sheath-water $\delta^{18}O$ and δD values is less than ±1.6‰ and ±7‰, respectively for *A. breviligulata* and ±5‰ and ±18‰ for *C. longifolia*. The larger range of δ-values for sheath waters from *C. longifolia* relative to *A. breviligulata* is likely the result of differences in the positioning of leaves along the stems of these two species. The culms of *A. breviligulata* occur in tufts that are clothed at the base of the plant by numerous overlapping sheaths. By comparison, sheaths are positioned along the entire length of the stem of *C. longifolia*, where they are less protected from the environmental conditions that encourage evaporation.

4.4.3.2. *δ-values of older versus younger leaves*

It has been demonstrated for bulk leaf water of wheat and barley that older leaves are less enriched in ^{18}O and D than younger leaves, which are positioned further up the stem (Walker et al., 1989; Walker and Lance, 1991). This enrichment has been attributed to more turbulent boundary-layer conditions surrounding younger leaves near the top of

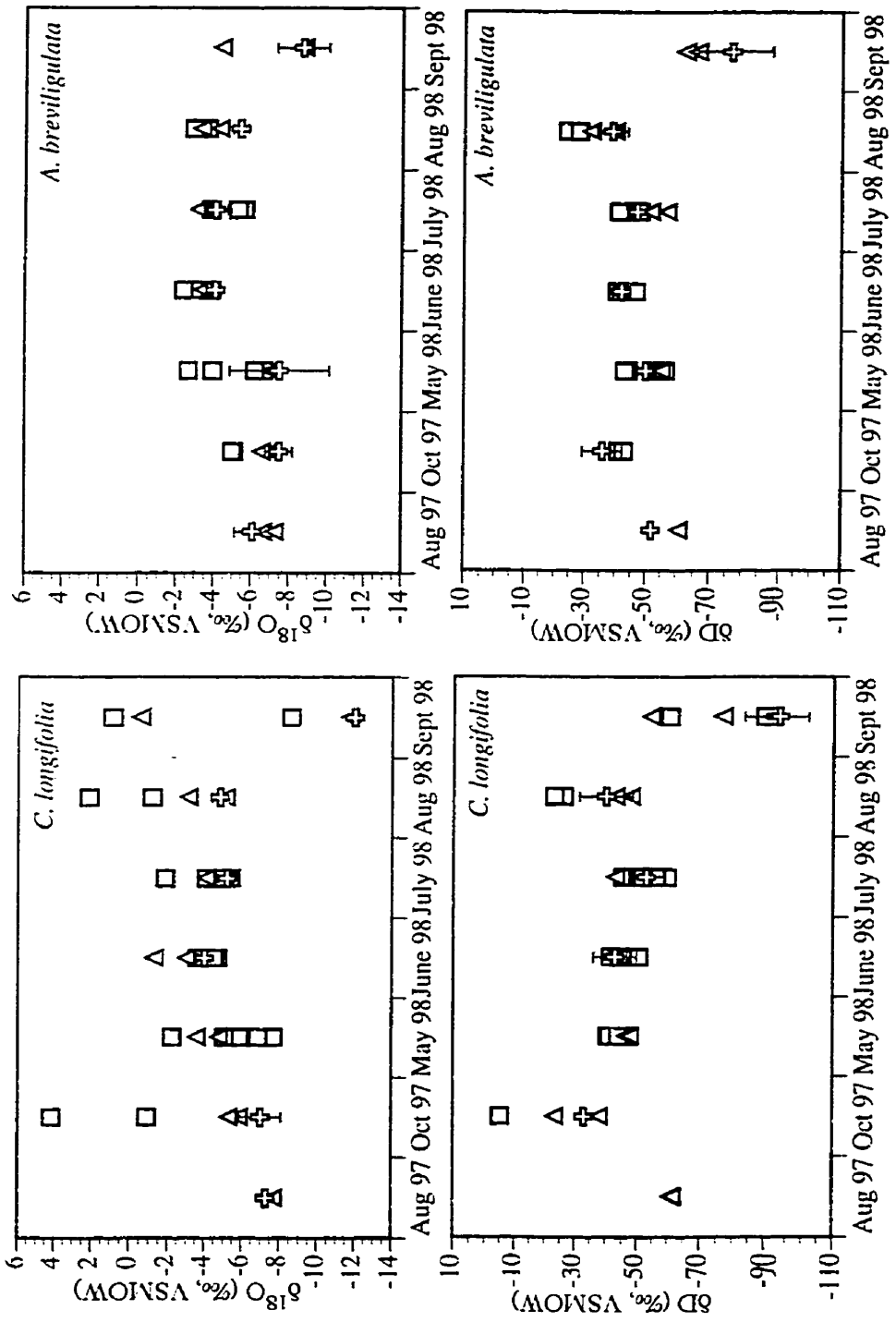

Figure 4-9. The $\delta^{18}O$ and δD values of sheath water from *C. longifolia* and *A. breviligulata*, for pre-dawn (Δ) and mid-day (□) samplings. Average stem-water $\delta^{18}O$ and δD values (✠) are included for comparison.

the canopy, higher relative humidity conditions at lower positions within the leaf canopy, a higher degree of stomatal closer in older leaves, and (or) the supply of nutrient-rich, mildly evaporated waters from mature leaves to younger leaves near the top of the plant (Walker et al., 1989; Walker and Lance, 1991). Our results, which consist of the isotopic values of water from upper- and lower-leaf sections rather than bulk-leaf water, are less consistent (Tables 4-2, 4-3 and 4-4). In the majority of cases, the upper leaf water of older leaves is more enriched than younger leaves. Conversely, the lower leaf water of younger leaves (higher on the stem) is more enriched in ^{18}O and D than water in the lower portion of older leaves.

The reasons for this pattern are unknown. If lower leaf water is more enriched towards the top of the plant because the younger leaves are supplied by ^{18}O- and D-enriched waters from leaves further down the stem, then the stem water involved in nutrient transport should a show similar enrichment. Yet we observe only minimal ^{18}O and D enrichment in upper stem water relative to lower stem water (Fig. 4-1). Although the extent of stem-water enrichment is much smaller than the observed isotopic enrichment between two lower leaf samples of the same plant, upper stem water may be a mixture of unfractionated stem water and mildly enriched water that is transported from older to younger leaves. Nevertheless, the sheath water, which directly feeds the lower leaves, is not D- or ^{18}O-enriched consistently towards the top of the plant. On average, two sheaths sampled at higher and lower positions on the same plant have δ-values that differ only marginally (average standard deviation = ±1.6‰ for $\delta^{18}O$ and ±4‰ for δD values) regardless of species or sampling time (Fig. 4-9). In two cases, however, water extracted from older versus younger sheaths collected from *A. breviligulata* and *C. longifolia* in September 1998 exhibit considerable enrichment ($\Delta^{18}O_{\text{younger-older sheath}}$ = 11.2‰ for *C. longifolia* and $\Delta^{18}O_{\text{younger-older sheath}}$ = 6.6‰ for *A. breviligulata*). Such enrichment may be more pronounced in more mature plants when older leaves have ceased growing and now transfer the majority of their nutrients to younger leaves.

4.4.3.3. *Intra-leaf variations in δ-values*

Upper leaf water is almost always more enriched in ^{18}O and D than the corresponding lower leaf water, which in turn is enriched relative to sheath water (Fig. 4-8). In two cases where the upper leaf water was significantly depleted relative to mid or lower leaf water (June 1, 1998, pre-dawn, *A. breviligulata* leaf #3 and September 23, 1998, mid-day *C. longifolia* leaf #9; Tables 4-2 and 4-3), the leaf tips were brown and probably not involved in active transpiration. The enrichment from the sheath to the tip of the leaf is attributed to the progressive enrichment of water in the leaf cells as it moves towards the tip (Wang and Yakir, 1995). Sheath water is at the beginning of this string of pools and therefore has an isotopic composition most similar to stem water (Fig. 4-9).

The enrichment of upper leaf water relative to lower leaf water collected from the same leaf is greatest for mid-day samplings and during months when relative humidity is the lowest and transpiration rates are highest. Similar enrichment has been observed for water in the veins of corn leaves (Yakir, 1991). Such behaviour is expected for simple leaves with numerous parallel veins, as is typical of the monocotyledons. In more complex leaves, comparable enrichment trends from the base to the tip, as well as from the vein towards the outer edge of the leaf tissue, have been observed (Luo and Sternberg, 1992; Wang and Yakir, 1995). However, irregular spatial heterogeneity of $\delta^{18}O$ and δD values is also common. These variations have been attributed to a combination of patchy stomatal openings, complex water pathways within the leaf and poor water communication between adjacent cells (Luo and Sternberg, 1992; Wang and Yakir, 1995). In our samples, there is no correlation between length of leaf and the amount of isotopic enrichment from the base to the tip of the leaf or between the upper leaf and stem water. This suggests that the water pathways are complex and that enrichment from the base to tip of the leaf does not depend solely on the length of vein through which the water must travel.

4.4.3.4. *Transpiration lines and approach to steady-state conditions*

As discussed earlier, the ^{18}O- and D- enrichment of leaf water is generally considered in terms of modelled steady-state values as described by Equation (4-1). These values are calculated primarily from climatic data and are therefore similar for all species grown under the same temperature and relative humidity conditions. The discrepancy between calculated leaf-water $\delta^{18}O$ and δD values and measured bulk leaf-water values will therefore vary among species with different water pathways and boundary-layer conditions. Previous studies have shown that measured bulk leaf waters are generally less enriched in ^{18}O and D than those calculated for steady-state conditions (e.g., Allison et al., 1985; Leaney et al., 1985; Walker et al., 1989; Walker and Brunel, 1990; Flanagan and Ehleringer, 1991a, b; Flanagan et al., 1991a, b; Walker and Lance, 1991; Wang et al., 1998). The difference between measured and modelled leaf-water δ-values increases with the length of the water pathway through the leaf (Flanagan et al., 1991a, b; Farquhar and Lloyd, 1993) and with increasing transpiration rates (Walker and Brunel, 1990; Wang et al., 1998).

As we did not measure bulk-leaf water isotopic compositions, it is difficult to compare the approach of measured leaf-water δ-values to steady-state conditions for different species. This comparison is further complicated by the fact that, for our samples, the measured δ-values of two leaves on the same plant are quite variable. Wang et al. (1998) suggest that careful sampling results in <1‰ variation in measured leaf-water $\delta^{18}O$ values of leaves on the same plant. However, the variations that we measured are much larger for water from the upper portions of two leaves from the same plant. In some cases progressive evaporative enrichment of the water as it moves through the leaves has caused the δ-values of upper leaf water to be even more enriched than calculated steady-state values. To summarize, it is difficult to reach conclusions regarding the transpiration pathways of water within the leaves of different species based solely on the approach of leaf waters to isotopic steady-state conditions. The situation is

complicated by the isotopic heterogeneity of water within one leaf as well as the isotopic variation between leaves on the same plant that in many cases is comparable to the leaf-water δ-variations between different species.

The relationships between δD and $\delta^{18}O$ values for water samples extracted from all grass tissues are shown in Figure 4-10 (a through e). The correlation between $\delta^{18}O$ and δD values of plant waters is excellent (Table 4-5). Each month for a given species, water samples from upper and lower leaves, inflorescence, sheaths, stems and rhizomes all plot on the same transpiration line (Fig. 4-10). This condition remains true regardless of whether the water originated from different leaves of the same plant, adjacent plants (May 6, 1998 mid-day *C. longifolia*), or mid-day plant samples collected on consecutive days. The covariance of the $\delta^{18}O$ and δD values of plant waters form transpiration lines with slopes ranging from 2.0 to 3.8 (average = 2.8±0.4, $P < 0.05$; Table 4-5). Slopes with these values are typical of leaves in which evaporation of water proceeds via diffusion through a relatively stable boundary layer (e.g., Allison et al., 1985; Cooper and DeNiro, 1989; Flanagan et al., 1991a; Walker and Lance, 1991). In general, the modelled steady-state leaf-water δ-values fall on these lines. This suggests that the isotopic compositions of most leaf water is the result of mixing between non-fractionated stem water and water at the sites of evaporation that is approaching steady-state values (Flanagan, 1993). In those instances where the transpiration lines do not intersect the values predicted for steady-state conditions, we suspect that the modelled δ-values are inaccurate because of the uncertainties surrounding our estimation of δ_a-values and leaf temperatures.

The slope of a transpiration line depends on relative humidity and boundary-layer conditions at the surface of the leaf. The δ-values of plant water from pre-dawn collections ought to lie along a higher slope than mid-day collections of similar species, as the evaporation has occurred across a lower vapour-pressure gradient. However, the majority of the slopes of the pre-dawn transpiration lines are, within error, identical to the mid-day slopes (transpiration lines for which there insufficient points to form a

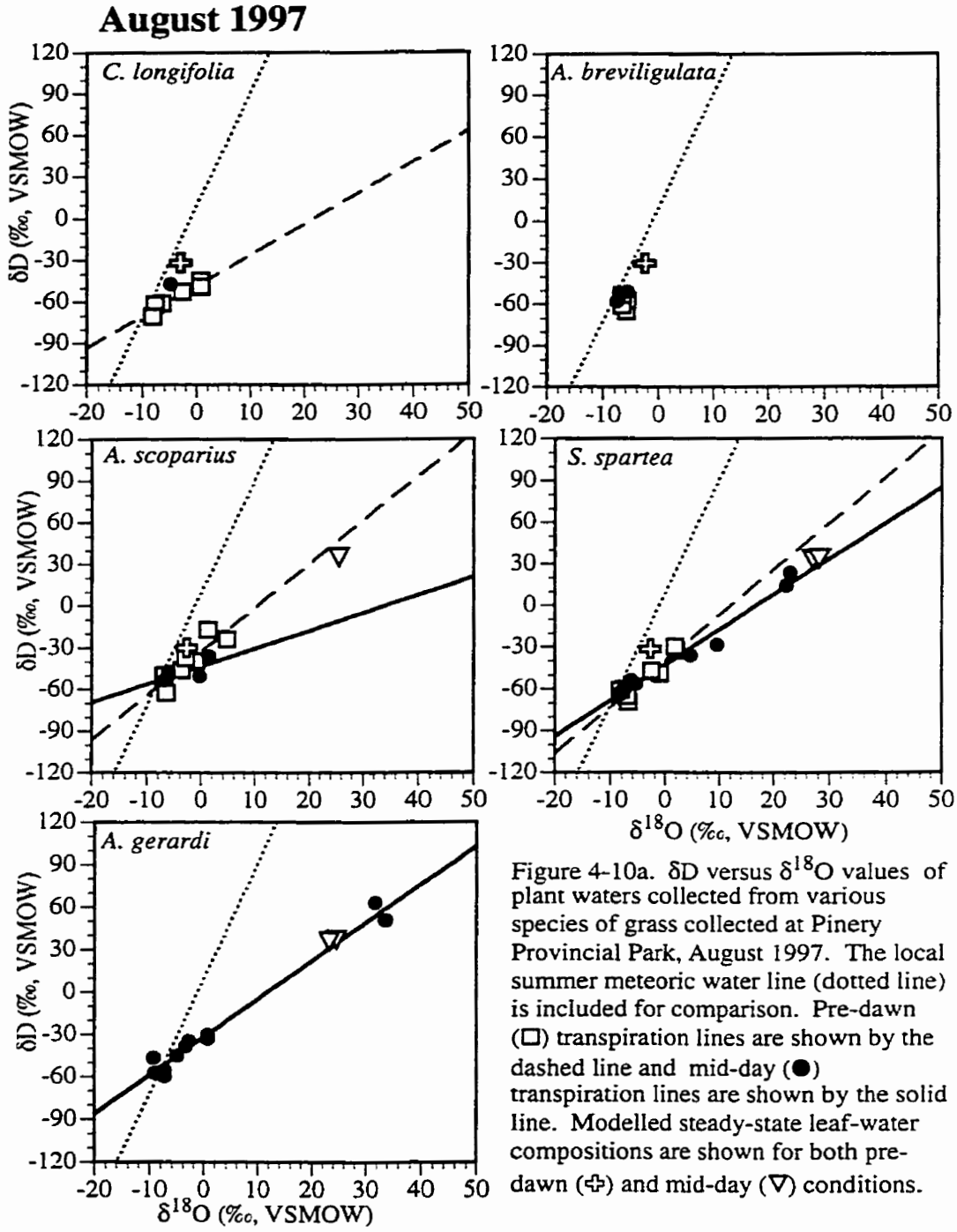

Figure 4-10a. δD versus $\delta^{18}O$ values of plant waters collected from various species of grass collected at Pinery Provincial Park, August 1997. The local summer meteoric water line (dotted line) is included for comparison. Pre-dawn (□) transpiration lines are shown by the dashed line and mid-day (●) transpiration lines are shown by the solid line. Modelled steady-state leaf-water compositions are shown for both pre-dawn (✣) and mid-day (▽) conditions.

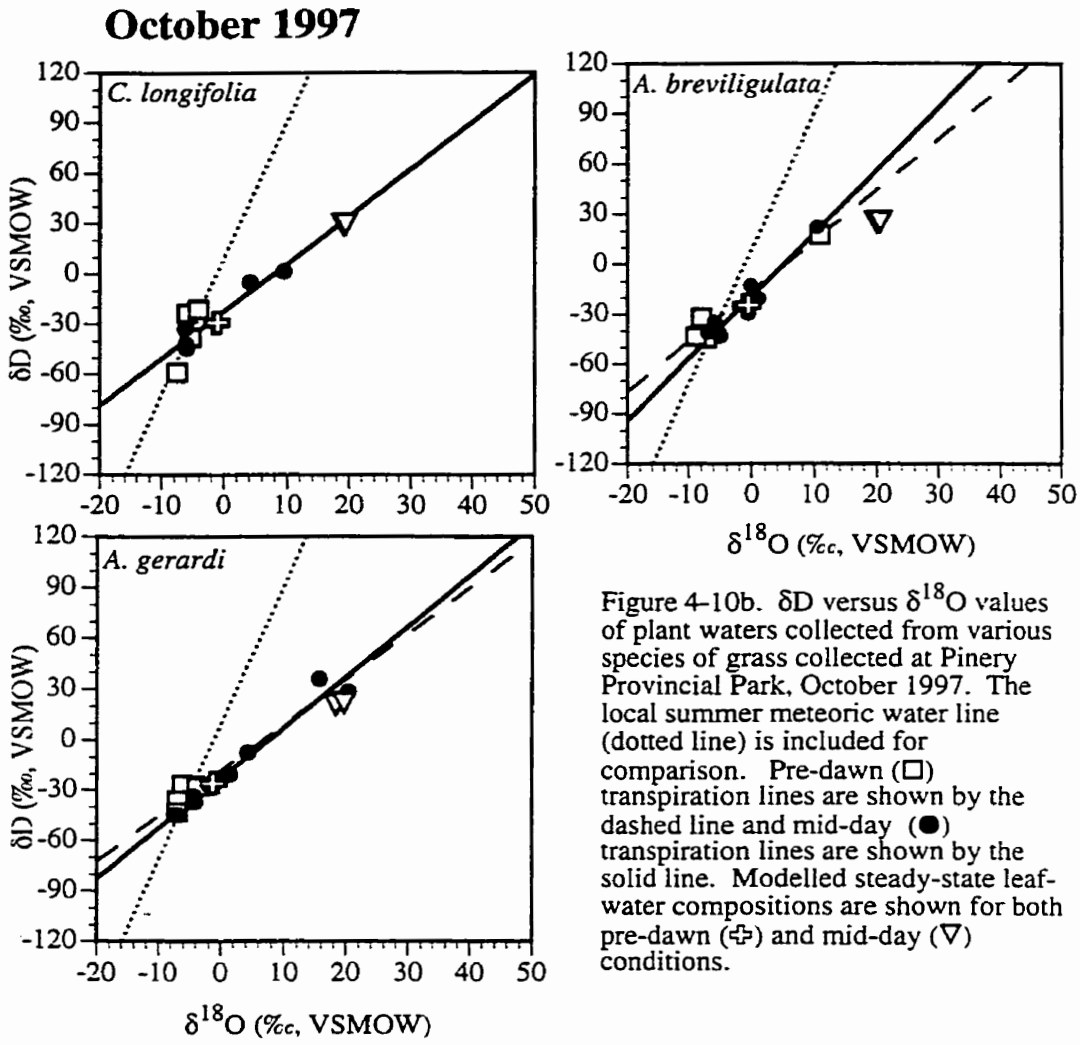

Figure 4-10b. δD versus $\delta^{18}O$ values of plant waters collected from various species of grass collected at Pinery Provincial Park, October 1997. The local summer meteoric water line (dotted line) is included for comparison. Pre-dawn (□) transpiration lines are shown by the dashed line and mid-day (●) transpiration lines are shown by the solid line. Modelled steady-state leaf-water compositions are shown for both pre-dawn (✣) and mid-day (▽) conditions.

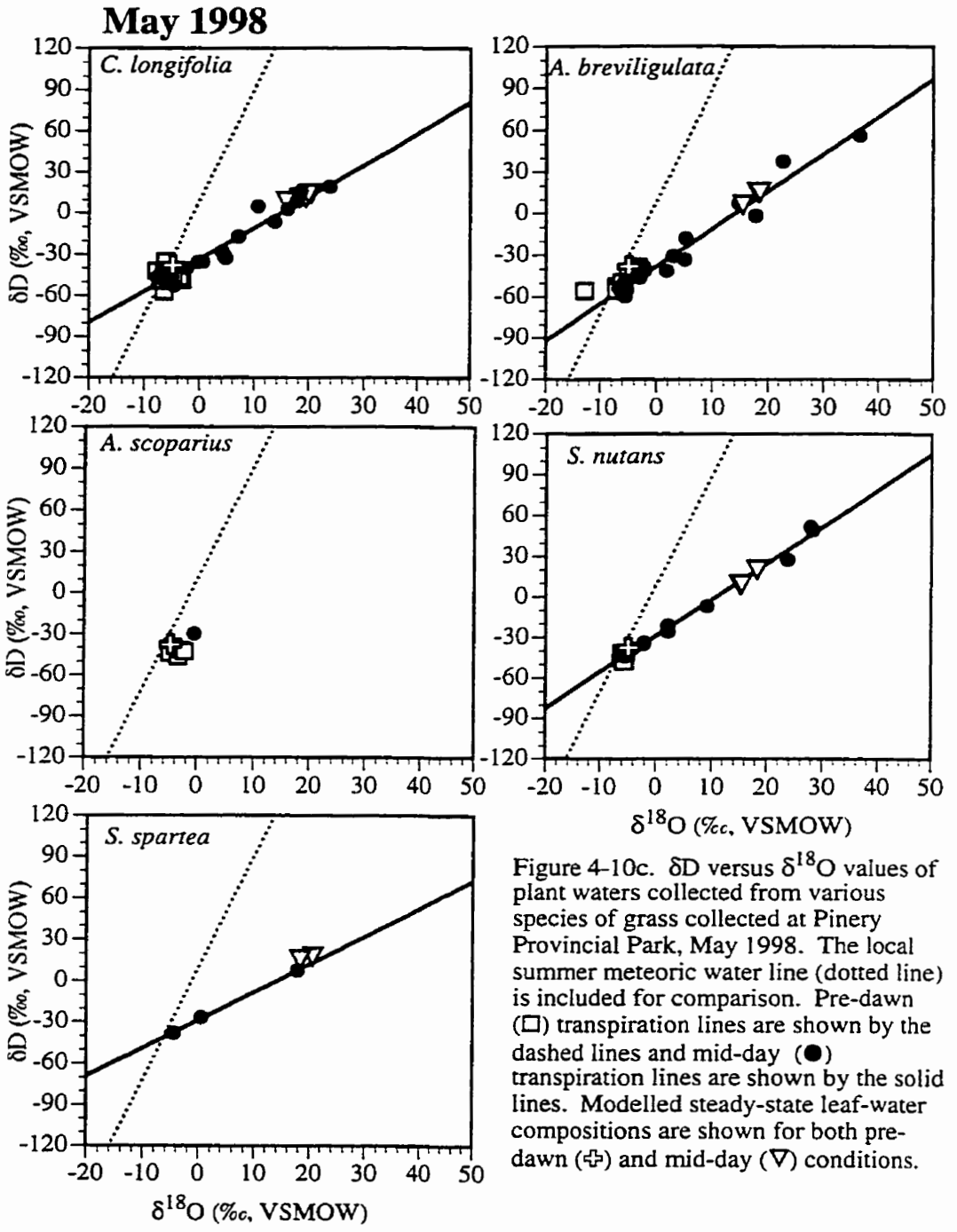

Figure 4-10c. δD versus $\delta^{18}O$ values of plant waters collected from various species of grass collected at Pinery Provincial Park, May 1998. The local summer meteoric water line (dotted line) is included for comparison. Pre-dawn (□) transpiration lines are shown by the dashed lines and mid-day (●) transpiration lines are shown by the solid lines. Modelled steady-state leaf-water compositions are shown for both pre-dawn (⌖) and mid-day (▽) conditions.

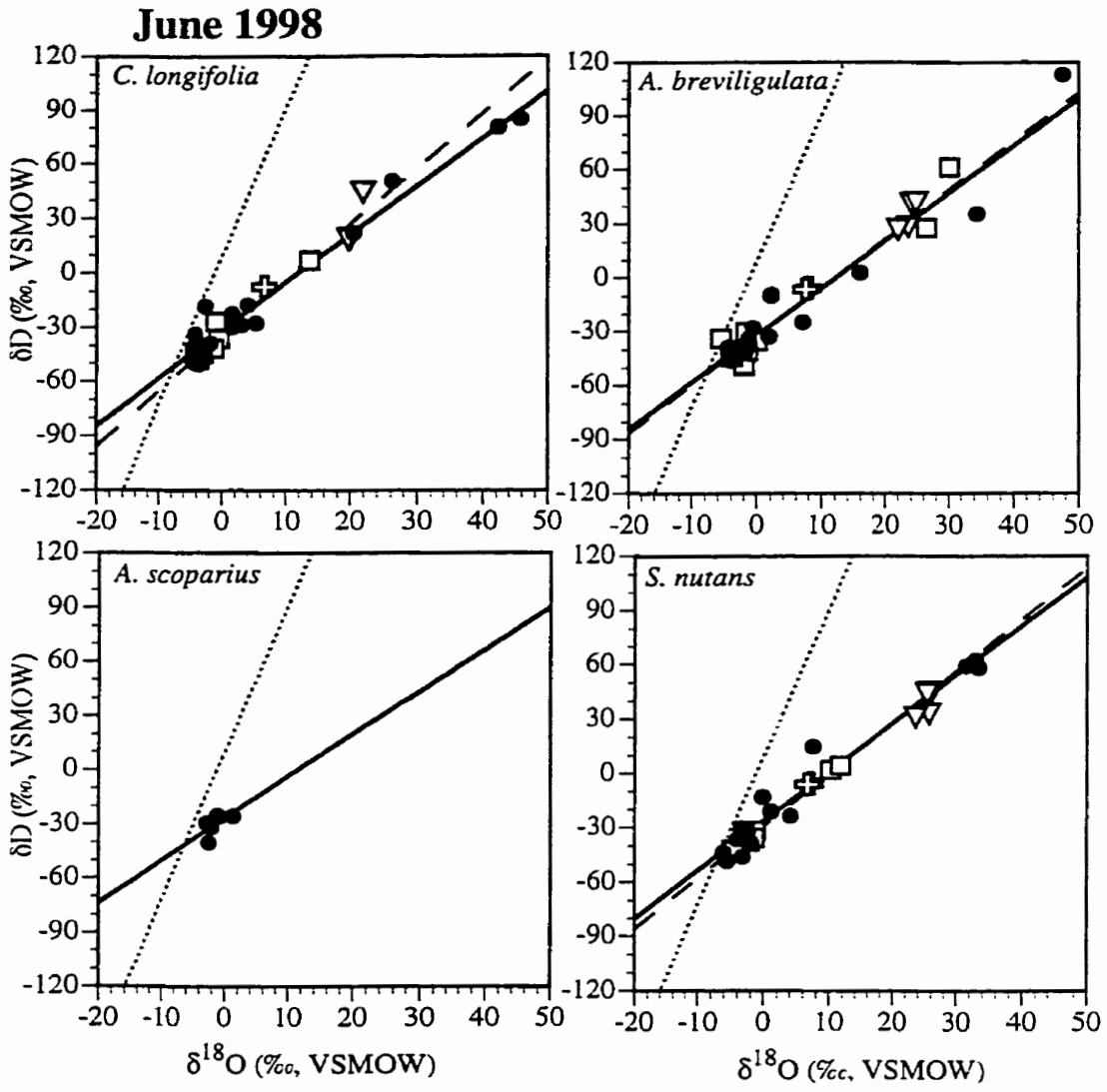

Figure 4-10d. δD versus $\delta^{18}O$ values of plant waters extracted from various species of grass collected at Pinery Provincial Park, June 1998. The local summer meteoric water line (dotted line) is included for comparison. Pre-dawn (□) transpiration lines are shown by the dashed line and mid-day (●) transpiration lines are shown by the solid lines. Modelled steady-state leaf-water compositions are shown for both pre-dawn (✣) and mid-day (▽) conditions.

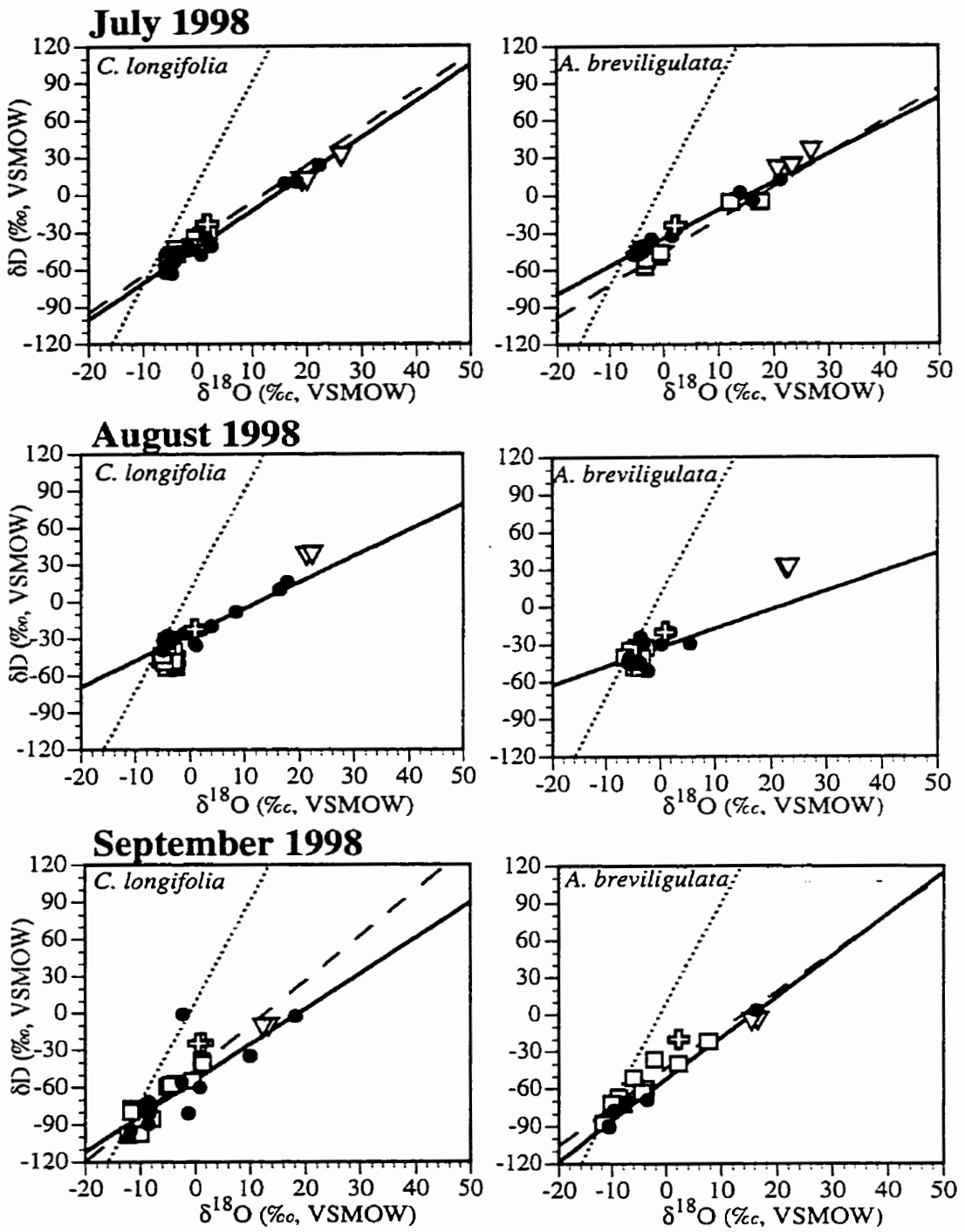

Figure 4-10e. δD versus $\delta^{18}O$ values of plant waters extracted from various species of grass collected at Pinery Provincial Park, July, August and September, 1998. The local summer meteoric water line (dotted line) is included for comparison. Pre-dawn (□) transpiration lines are shown by the dashed lines and mid-day (●) transpiration lines are shown by the solid lines. Modelled steady-state leaf-water compositions are shown for both pre-dawn (✣) and mid-day (▽) conditions.

Table 4-5. Transpiration lines describing the $\delta^{18}O$ versus δD relationships illustrated in Figure 4-10 for waters extracted from grasses at Pinery Provincial Park. Climatic conditions at the time of sampling are given in Table 4-1.

Month	¥ Time	Species	slope	intercept	R^2	*P**
Aug-97	AM	*C. longifolia*	2.2±0.4	-47.9	0.9	3.0E-03
Aug-97	AM	*A. scoparius*	3.2±0.7	-32.6	0.8	3.0E-03
Aug-97	AM	*S. spartea*	3.3±0.5	-39.6	0.9	4.0E-04
Aug-97	PM	*A. scoparius*	1.3±0.9	-43.7	0.5	*0.3
Aug-97	PM	*S. spartea*	2.6±0.1	-42.9	1.0	7.1E-08
Aug-97	PM	*A. gerardi*	2.7±0.1	-32.1	1.0	5.7E-09
Oct-97	AM	*A. breviligulata*	3.0±0.5	-15.6	1.0	2.0E-02
Oct-97	AM	*A. gerardi*	2.7±1.3	-17.9	0.5	*0.099
Oct-97	PM	*C. longifolia*	2.8±0.4	-22.2	0.9	8.0E-03
Oct-97	PM	*A. breviligulata*	3.8±0.4	-19.1	0.9	1.0E-04
Oct-97	PM	*A. gerardi*	3.0±0.2	-22.9	1.0	2.4E-06
May-98	PM	*C. longifolia*	2.3±0.1	-33.7	0.9	9.8E-15
May-98	PM	*A. breviligulata*	2.7±0.1	-38.0	1.0	4.3E-12
May-98	PM	*S. nutans*	2.7±0.1	-29.0	1.0	1.4E-11
May-98	PM	*S. spartea*	2.0±0.1	-28.7	1.0	2.0E-02
Jun-98	AM	*C. longifolia*	3.1±0.3	-34.0	0.9	4.5E-05
Jun-98	AM	*A. breviligulata*	2.7±0.2	-32.1	0.9	1.3E-05
Jun-98	AM	*S. nutans*	2.9±0.2	-28.6	1.0	1.9E-06
Jun-98	PM	*C. longifolia*	2.7±0.1	-31.6	1.0	1.7E-13
Jun-98	PM	*A. breviligulata*	2.6±0.2	-31.9	0.9	4.6E-10
Jun-98	PM	*A. scoparius*	2.3±1.5	-26.9	0.4	*0.19
Jun-98	PM	*S. nutans*	2.7±0.2	-26.3	1.0	2.7E-10
Jul-98	AM	*C. longifolia*	3.0±0.2	-34.8	1.0	1.5E-06
Jul-98	AM	*A. breviligulata*	2.6±0.2	-45.2	1.0	1.2E-05
Jul-98	PM	*C. longifolia*	2.9±0.1	-41.5	1.0	1.7E-13
Jul-98	PM	*A. breviligulata*	2.2±0.1	-34.5	1.0	6.4E-13
Aug-98	PM	*C. longifolia*	2.1±0.2	-26.5	0.9	7.4E-07
Aug-98	PM	*A. breviligulata*	1.5±0.8	-32.4	0.3	*0.1
Sep-98	AM	*C. longifolia*	3.7±0.5	-46.1	0.8	2.4E-05
Sep-98	AM	*A. breviligulata*	3.1±0.4	-42.8	0.9	1.0E-04
Sep-98	PM	*C. longifolia*	2.9±0.7	-54.2	0.6	2.0E-03
Sep-98	PM	*A. breviligulata*	3.3±0.2	-52.0	1.0	1.0E-04

¥ AM = pre-dawn and PM = mid-day sampling times; * *P* values > 5.0E-2 are statistically insignificant.

significant regression ($P>0.05$) are not considered further). In four cases, *C. longifolia* in June 1998 and September 1998, *A. breviligulata* in July 1998, and *S. spartea* in August 1997, the slopes of the pre-dawn transpiration lines were higher than mid-day waters (Fig. 4-10). Regardless, the upper leaf water of pre-dawn samples tends to lie on the mid-day transpiration line. It appears that pre-dawn, upper leaf waters lie on a mixing line between the highly enriched mid-day upper leaf waters and soil water (Fig. 4-10). Such behaviour has been explained previously as a consequence of compartmentation of leaf water (Yakir et al., 1990).

Water at the sites of evaporation is isotopically sensitive to changes in environmental conditions, and as a result, ^{18}O- and D-enriched waters from these sites diffuse back into the leaf where they mix with the advective flux of unfractionated vein water. Yakir et al. (1990) proposed that a secluded fraction of "metabolic" water exists within the leaf and that it may exchange isotopically only very slowly with water from sites of evaporation or unfractionated vein waters. This limitation of exchange with transpired water would cause "metabolic" water within the cells, which can comprise a significant portion of the bulk leaf water (up to 69%, Yakir et al., 1989), to vary on a much smaller diurnal scale than the water directly affected by transpiration.

The diurnal fluctuations in the isotopic composition of these water fractions are illustrated schematically in Figure 4-11. The diurnal variation in upper-leaf water δ-values is greater than that of lower leaf water. Overnight, the dominant isotopic change in both upper and lower leaf tissues is the slow exchange between "metabolic" water and the advective flux of non-fractionated stem water. Throughout the previous day, the "metabolic" water fraction of lower leaf tissues exchanged slowly with water from transpiration sites in the lower leaf that were less enriched ^{18}O and D than water from such sites in the upper leaf tissues. As a result, lower leaf water experiences a less dramatic change in isotopic composition during overnight mixing with unfractionated stem waters than upper leaf water.

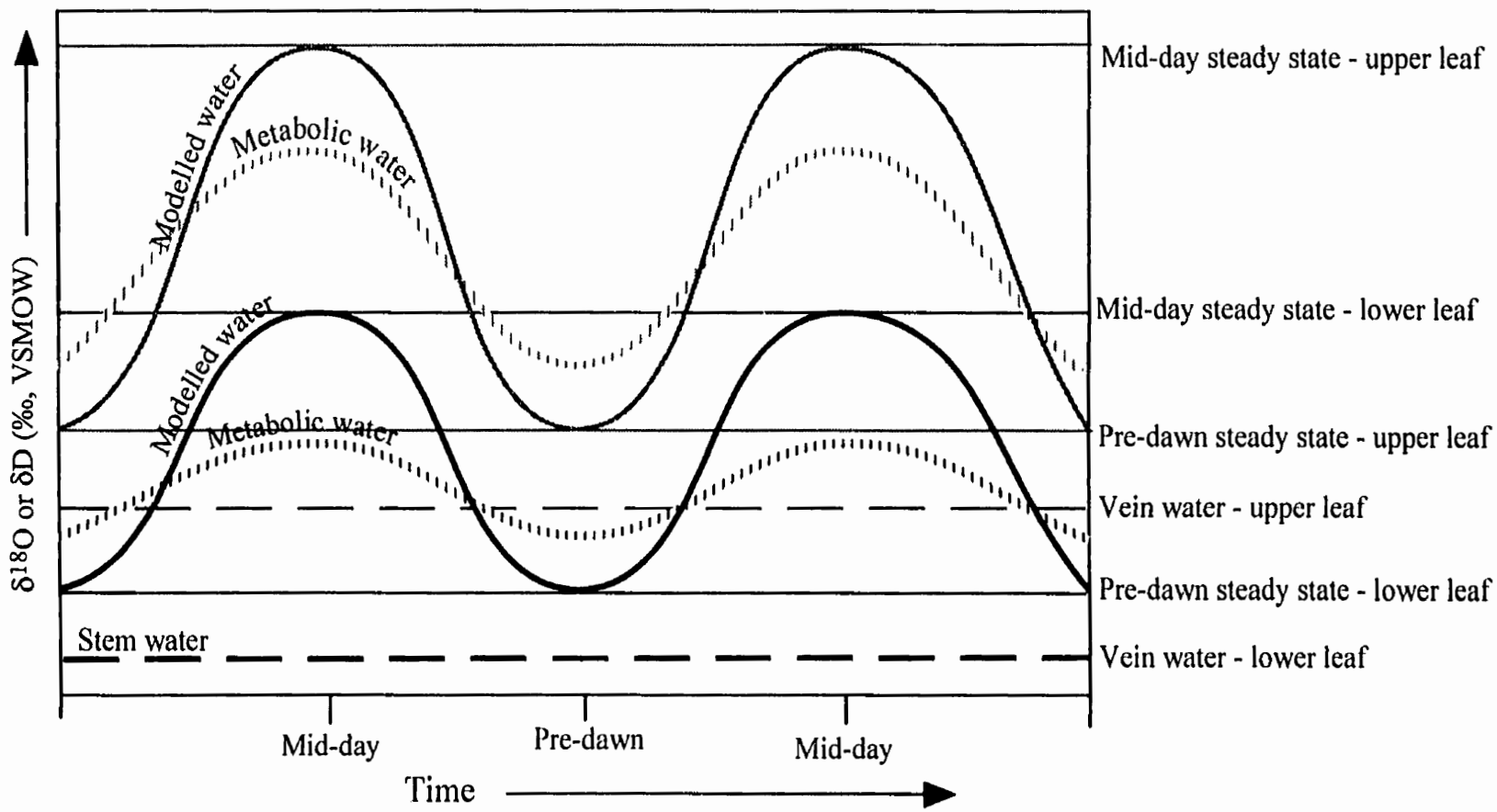

Figure 4-11. Schematic representation of the isotopic variation in distinct leaf-water fractions with time. Modelled water is the water directly involved in transpiration and varies diurnally between the predicted isotopic compositions of leaf water under steady-state conditions (solid lines; see Equation 4-1). Metabolic water experiences restricted mixing with modelled water and vein waters (dashed lines). Upper leaf waters (grey lines) exhibit a greater diurnal variation in $\delta^{18}O$ and δD values than lower leaf water (black lines).

The existence of a secluded fraction of leaf water is also supported by the isotopic composition of guttation collected from the tips of *A. breviligulata* leaves on May 7, 1998 and September 23, 1998 (Table 4-3). Guttation is the loss of liquid water from leaves overnight as it is forced out of the tips of leaf veins by root pressure. The presence of guttation suggests that the rate of root-water uptake has exceeded the evaporative loss of water through the leaves. In May 1998, the isotopic composition of the guttation ($\delta^{18}O$ = -7.0‰, δD = -42‰) is comparable to the range of soil-water isotopic compositions (weighted average soil-water $\delta^{18}O$ = -8.1‰, δD = -57‰; Table 4-1). This indicates that root pressure pushed stem water through the veins of the leaf and flushed out the ^{18}O- and D-enriched leaf water. The isotopic composition of the September guttation ($\delta^{18}O$ = -4.7‰, δD = -45‰) is consistent with a mixture of unfractionated stem water and ^{18}O- and D- enriched leaf waters. In both cases, the guttated water is less enriched than water extracted from the leaf tissues. This indicates that the flux of stem water through the leaf veins does not flush the entire leaf. A component of leaf water is left that retains the isotopic signature of the highly enriched mid-day leaf waters.

As the slope of the transpiration line is dependent on relative humidity, one might expect to see higher slopes during more humid months. Although some variation does occur from month to month, notably higher slopes for *C. longifolia* and *A. breviligulata* in September 1998, these changes do not seem to correspond to relative humidity. The relative humidity under which each plant was sampled varied only slightly from month to month (Table 4-1). For example, mid-day relative humidity recorded in September 1998 (40%) is similar to that in May 1998 (37%) when the transpiration lines have lower slopes (Table 4-5).

The differences in slope may instead be the result of a change in boundary-layer conditions. The boundary-layer conditions at the surface of the leaf may be controlled by the number and activity of stomatal openings, which may change as the leaf matures and

gains more stomatal control or as it loses stomatal control near the end of its life. Differences in slope between species collected over the same time period could also arise from species-dependent boundary-layer conditions. For example, the leaves of *A. breviligulata* have the ability to roll inward during periods or water stress to prevent water loss from the stomatal pores on the upper surface of the leaf. The leaves of *C. longifolia* have abundant stomata on both their upper and lower surfaces and do not display any drought induced modifications such as rolling or folding (Hardy et al., 1995). However, no consistent relationship between the slope of the transpiration line and species was observed in the samples analysed here.

C3 and C4 grasses commonly have comparable slopes for the transpiration line (Table 4-5). Under field conditions, its slope is expected to depend more on the boundary-layer conditions and physiological properties of the leaf than photosynthetic pathway (Flanagan et al., 1991a). For *A. breviligulata* and *C. longifolia*, the two species for which the data are most extensive, there is no clear pattern throughout the growing season of which species has greater leaf-water ^{18}O- and D-enrichment or transpiration lines with greater slopes. Whether this ambiguity is a result of variations introduced by selective sampling, variations in transpiration characteristics between these two species as the plants mature or other factors is unknown at present. Resolution of these questions will require more controlled sampling and a greater knowledge of the physiology of these two species.

4.4.3.5. *δ-values of inflorescence water*

All δ-values for inflorescence water plot on the transpiration lines defined in Figure 4-10 and Table 4-5. However, all of these samples included the portion of the stem from which the flowers radiate. As a result, they are less enriched in ^{18}O and D than the upper leaf water (Fig. 4-8). The inflorescence water represents a mixture among stem water and water that has undergone evaporative ^{18}O- and D-enrichment within the inflorescence and within leaves that supply water and nutrients to the flowering structure.

4.4.4. Implications for silica-phytolith formation

The $\delta^{18}O$ values of silica phytoliths from various tissues of *C. longifolia* and *A. breviligulata* collected at Pinery Provincial Park from 1994 to 1997 are listed in Table 4-6. Also, included are the range of plant-water $\delta^{18}O$ values predicted from the relationship:

$$t\ (°C) = 5.8 - 2.8\ (\delta^{18}O_{silica} - \delta^{18}O_{plant\ water} - 40) \qquad \text{(Eqn. 4-4)}$$

developed by Shahack-Gross et al. (1996) for silica phytoliths. For 1995 phytolith samples from *C. longifolia* and *A. breviligulata* where $\delta^{18}O_{silica}$ and $\delta^{18}O_{water}$ values from corresponding plant parts were known, temperature predictions made using Equation (4-4) were identical to measured temperatures (Webb and Longstaffe, 2000). This confirms its validity for the samples described here.

Temperature ranges that describe two different growing-season intervals were used to calculate the plant-water $\delta^{18}O$ values listed in Table 4-6. Previously, we found a better correlation between measured and calculated $\delta^{18}O_{soil\ water}$ values when the average temperature over the entire growing season (May to August) is used in Equation (4-4) rather than the average temperature for the July to August interval (Chapter 3). However, in most grass species the majority of silica phytoliths are deposited late in the growing season (Johnston et al., 1967; Simkiss and Wilbur, 1989; Shahack-Gross et al., 1996). Hence, it is uncertain whether the climate signals recorded by the oxygen-isotope composition of phytoliths collected from mature plants will reflect the climatic influences over the entire growing season. Shahack-Gross et al. (1996) suggested that late growing-season conditions have the dominant influence on the $\delta^{18}O$ values of phytoliths. In order to consider both possibilities, we have used the average temperature of both growing-season intervals to calculate the possible range of $\delta^{18}O_{plant\ water}$ values involved in silica precipitation.

Because each silica sample comprises phytoliths extracted from approximately a hundred individual plants, the $\delta^{18}O$ values represent an average for opal-A deposited in

Table 4-6. $\delta^{18}O$ values (‰, VSMOW) of silica phytoliths from *C. longifolia* and *A. breviligulata* , Pinery Provincial Park, Ontario.

				$\delta^{18}O$ (‰, VSMOW)										
		Temperature °C		Inflorescence		Leaf		Sheath		Stem		Rhizome		¥Summer
Species	Year	May- Aug	July-Aug	silica	water *	silica	water *	silica	water *	silica	water *	silica	water *	precipitation
A. breviligulata	1994§	17	19	33.0	-3.0 to -2.3	32.0	-4.0 to -3.3	29.6	-6.4 to -5.7	27.9	-8.1 to -7.4	24.7	-11.3 to -10.6	
A. breviligulata	1995	20	22	28.3	-6.6 to -5.9	32.0	-2.9 to -2.2	29.2	-5.7 to -5.0	28.6	-6.3 to -5.6	28.4	-6.5 to -5.8	-5.7
C. longifolia	1994§	17	19	32.9	-3.1 to -2.4	31.7	-4.3 to -3.6	27.8	-8.2 to -7.5	28.2	-7.8 to -7.1	28.0	-8.0 to -7.3	
C. longifolia	1995	20	22	31.8	-3.1 to -2.4	32.4	-2.5 to -1.8	27.7	-7.2 to -6.5	28.1	-6.8 to -6.1	27.5	-7.4 to -6.7	-5.7
C. longifolia	1996[a]	15[a]				28.8	-7.9	27.5	-9.2	27.4	-9.3	28.5	-8.2	-5.8[b]
C. longifolia	1996	18	20	29.8	-5.8 to -5.1	28.7	-6.9 to -6.2	26.7	-8.9 to -8.2	26.4	-9.2 to -8.5	29.1	-6.5 to -5.8	-7.8
C. longifolia	1997	17	19	32.8	-3.2 to -2.5	31.7	-4.3 to -3.6	27.2	-8.8 to -8.1	27.5	-8.5 to -7.8	28.1	-7.9 to -7.2	-8.0

§ 1994 samples were not treated to compensate for oxygen-isotope exchange during analysis (Webb and Longstaffe, in press). [a] grass sample was collected in July, mid-way through the growing season. All other samples were collected at the end of the growing season. * calculated from Equation (4-4) using both the May to August and July to August temperatures. ¥ weighted average summer precipitation collected from April to the end of September each year except for [b] which is the weighted average precipitation from April to the end of July.

the grass over its lifetime. Spatial and temporal heterogeneity of soil-water and precipitation $\delta^{18}O$ values will be smoothed over the course of silica precipitation. We have assumed therefore that the measured differences in $\delta^{18}O_{silica}$ values for *C. longifolia* and *A. breviligulata* at Pinery Provincial Park over the four-year period reflect changes in climatic conditions and/or the $\delta^{18}O$ values of water supplied to the plants from one year to the next.

Over the 1998 growing season (early May to mid-September), the average isotopic compositions of stem water from *A. breviligulata* and *C. longifolia* were $\delta^{18}O$ = -6.0‰, δD = -51‰ and $\delta^{18}O$ = -6.4‰, δD = -56‰, respectively (the average δ-values of rhizome water were substituted for *C. longifolia* stem water for May 1998). These values correspond well to the seasonal average for weighted soil-water δ-values over the same period ($\delta^{18}O$ = -6.5‰, δD = -48‰). On a monthly basis, soil water at Pinery Provincial Park may be either depleted or enriched in ^{18}O and D relative to monthly summer precipitation (Table 4-1). However, the weighted average isotopic composition of precipitation over the entire growing season ($\delta^{18}O$ = -6.8‰ and δD = -46‰) is nearly identical to the δ-values of both average soil water and average stem water from *A. breviligulata* and *C. longifolia* collected in 1998.

Results for the 1997 growing season confirm that the weighted average isotopic composition of soil water and precipitation is recorded reasonably well by the $\delta^{18}O$ values of stem silica. Measured soil-water $\delta^{18}O$ values for Pinery Provincial Park during the 1997 growing season ($\delta^{18}O_{measured\ soil\ water}$ for August = -7.2‰, September = -7.8‰ and October = –6.5‰; Table 4-1; Gage, in progress) are only slightly higher than the 1997 soil-water values that we predicted for this location from stem-silica δ-values ($\delta^{18}O_{stem\ water}$ = -8.5‰ to -7.8‰; Table 4-6). The weighted seasonal (April to September) precipitation for 1997 has a $\delta^{18}O$ value (-8.0‰; Table 4-6) that lies within the range of $\delta^{18}O_{stem\ water}$ values predicted from the stem-silica δ-values. In general, stem water from which phytoliths in the 1997 grass samples precipitated had an isotopic composition

close to the weighted average precipitation or soil water over a depth of one meter from the ground surface.

The δ-values of soil water were not measured prior to 1997. However, based on the above observations we might infer for this location that the weighted average δ-values of precipitation over the growing season could be used to approximate the δ-value of the water from which stem silica precipitated. The $\delta^{18}O$ values of seasonal (April to September) precipitation for the 1995 to 1997 growing seasons are listed in the final column of Table 4-6 and illustrated in Figure 4-12 together with other climatic parameters. A comparison of the data in Table 4-6 shows that the $\delta^{18}O$ values of this seasonal precipitation are higher than the $\delta^{18}O$ values predicted for average stem waters from stem silica δ-values. However, for the most part they differ by less than 1‰ from the predicted composition. The cause of the one large discrepancy between early 1996 summer precipitation (April to July; $\delta^{18}O$ = -5.8‰) and the predicted stem-water composition for the same period ($\delta^{18}O$ = -9.3‰) is unknown. It may be that during the early stages of plant growth and silica precipitation, a large fraction of winter precipitation, which is depleted of ^{18}O and D relative to summer precipitation, remained in the soil-water reservoir and was utilized by the grass. However, the ^{18}O-depleted signature of such early-formed silica is likely obscured later in the season as silica-precipitation rates increase and summer precipitation provides the majority of the water from which stem silica is formed.

For the 1998 growing season the average δ-values of rhizome water ($\delta^{18}O$ = -5.6‰, δD = -52‰ and $\delta^{18}O$ = -5.4‰, δD = -52‰, for *A. breviligulata* and *C. longifolia* respectively), are enriched in ^{18}O relative to average stem waters. However, the $\delta^{18}O$ values of rhizome water predicted from rhizome silica $\delta^{18}O$ values may either be lower (1994 and 1995) or higher (1996 and 1997) than predicted stem-water $\delta^{18}O$ values (Table 4-6). The underground biomass of the grass samples collected for phytolith extraction was sampled only to a depth of approximately 30 centimeters. As shown earlier for the

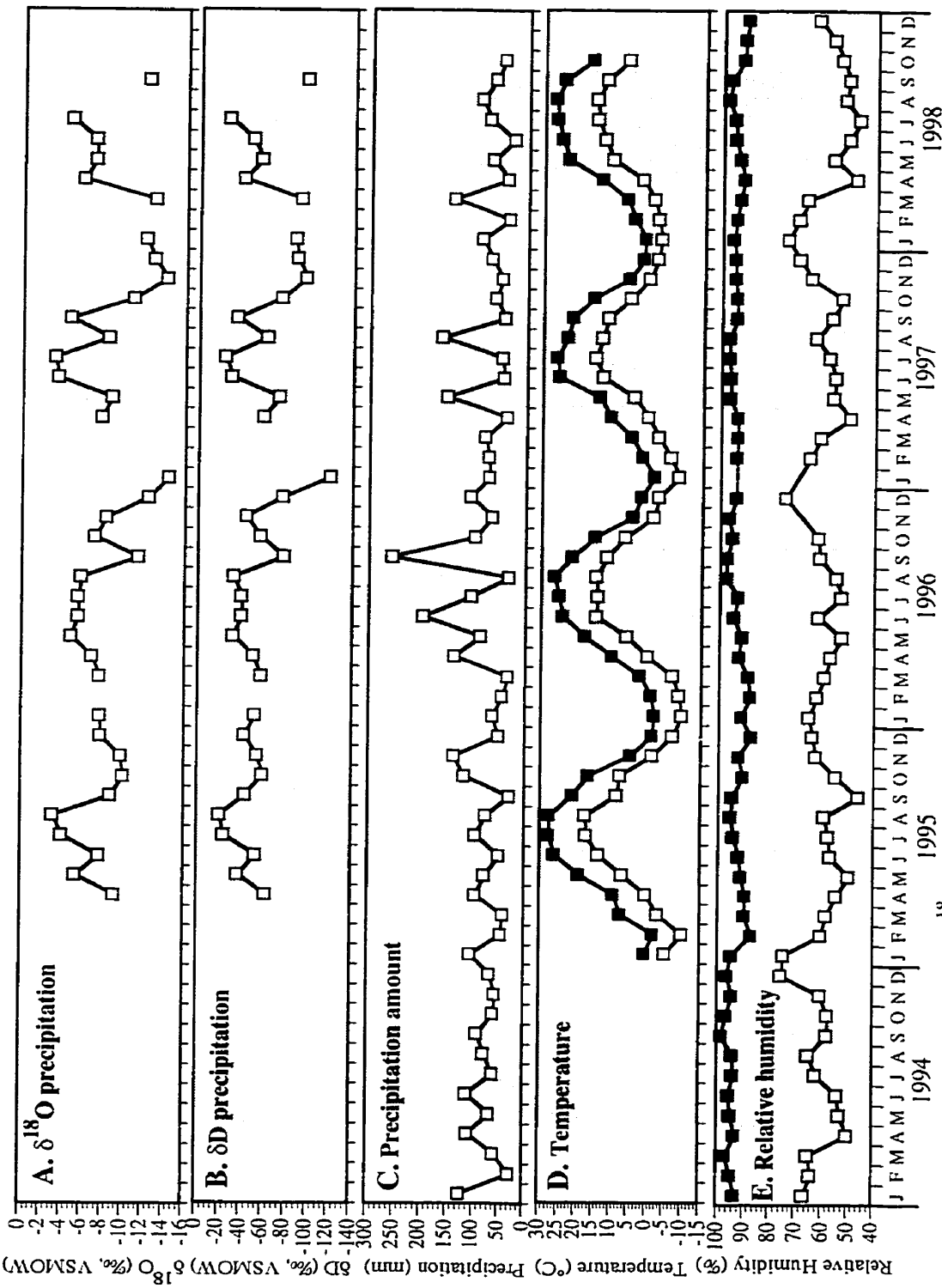

Figure 4-12. Five-year trends of: (A) $\delta^{18}O$ and (B) δD in precipitation, (C) amount of precipitation, (D) maximum (filled symbols) and minimum (open symbols) temperature, and (E) maximum (filled symbols) and minimum (open symbols) relative humidity. The shaded areas highlight the growing-season interval (May to September).

1998 samples (Fig. 4-6), shallow soil water is generally more enriched in ^{18}O than the average for soil water over the entire interval of root-water uptake. This difference is sufficient to explain the discrepancy between the average measured stem-water and rhizome-water δ-values in 1998 and predicted stem-water and rhizome-water δ-values for 1996 and 1997. Rhizome silica that is enriched in ^{18}O relative to stem silica (e.g. 1994 and 1995) may reflect variations in the temperature of silica precipitation in below- versus above-ground tissues or differences in age with the former having the potential to reflect up to five years of growth (see Chapter 3).

The average δ-values of 1998 sheath waters ($\delta^{18}O$ = -4.7‰, δD = -48‰ and $\delta^{18}O$ = -3.9‰, δD = -50‰, for *A. breviligulata* and *C. longifolia* respectively), are enriched in ^{18}O and D relative to average stem waters. However, silica deposited in the sheaths has precipitated from water with very similar $\delta^{18}O$ values to stem water (Table 4-6). Sheath water occurs at the beginning of the string of water pools that leads to ^{18}O- and D-enrichment in leaves (Wang and Yakir, 1995), and therefore can be expected to have $\delta^{18}O$ values quite similar to stem water. During conditions of high transpiration, the greater influx of non-fractionated stem water into the sheath also may counteract back-diffusion of ^{18}O- and D-enriched molecules from sites of evaporative enrichment (Farquhar and Lloyd, 1993). Overall, daily variations in sheath-water δ-values are smoothed by the accumulation of silica over time such that mid-day enrichments are attenuated and sheath silica itself precipitates from water similar in isotopic composition to that of stem water. Hence, we classify the sheath tissues as only weakly transpiring in this regard, and assign them an affinity, in terms of their $^{18}O_{silica}$ behaviour, with the strictly non-transpiring tissues (stems and rhizomes; see Chapter 3).

The calculated $\delta^{18}O$ values of leaf water for the 1994 to 1997 grass samples (Table 4-6) are much lower than the range of leaf-water $\delta^{18}O$ values measured during this study (Fig. 4-8) and during a comparative study of *A. breviligulata* and *C. longifolia* leaf waters grown under monitored greenhouse conditions (Webb and Longstaffe, 2000). The $\delta^{18}O$

values of leaf water from which leaf silica is expected to have precipitated in 1998 can be calculated using the $\delta^{18}O$ values of stem water and the equation (Chapter 3):

$$\Delta^{18}O_{\text{leaf water-stem water}} = \Delta^{18}O_{\text{leaf silica-stem silica}} = (12.5/\text{ relative humidity}) - 13. \quad \text{(Eqn. 4-5)}$$

The values calculated for 1998 leaf water ($\delta^{18}O_{\text{leaf water}}$ = -3.3 to -2.6‰ for *C. longifolia* and $\delta^{18}O_{\text{leaf water}}$ = -2.6 to –2.1‰ for *A. breviligulata*) are also much lower than the measured leaf-water $\delta^{18}O$ values (Fig. 4-8). The reasons for this discrepancy are not entirely clear, but likely are related to the presence of different water reservoirs in the leaf. Biogenic silica can be deposited in intercellular spaces and as intracellular deposits within epidermal cells of the leaf tissues (Kaufman et al., 1981). If the water associated with intracellular deposits is "metabolic", and experiences only limited mixing with the leaf water that is directly affected by transpiration, then leaf silica may be precipitated in equilibrium with leaf waters that are depleted of ^{18}O relative to the bulk water extracted from upper or lower leaf portions.

Based on the isotopic analysis of water from the leaves of sunflower (*Helianthus annuus*) and ivy plants (*Hedera helix*), Yakir et al. (1989) estimated that "metabolic" water constitutes approximately 69% of total leaf water. Vein water, which is unfractionated from stem-water $\delta^{18}O$ values, constitutes 2% of the bulk leaf water. The remaining 29% is composed of water directly involved in transpiration (δ_{modelled} in Equation 4-1). If we can extrapolate such results to our samples, the measured δ-values for bulk upper-leaf and bulk lower-leaf water primarily represent the restricted "metabolic" water pool. The isotopic composition of the "metabolic" water fraction can be calculated using a mass balance equation (Yakir et al., 1990):

$$\delta_{\text{metabolic water}} = (\delta_{\text{measured leaf water}} - 0.29\delta_{\text{modelled}} - 0.02\delta_{\text{stem water}})/0.69 \quad \text{(Eqn. 4-6).}$$

This calculation for our samples shows that the isotopic composition of "metabolic" water in lower-leaf samples falls above, within and below the range of leaf-water values predicted from Equation (4-5) for 1998 (hatched area, Fig. 4-13). Nevertheless, the calculated δ-values of "metabolic" water provide a better match than measured δ-values of

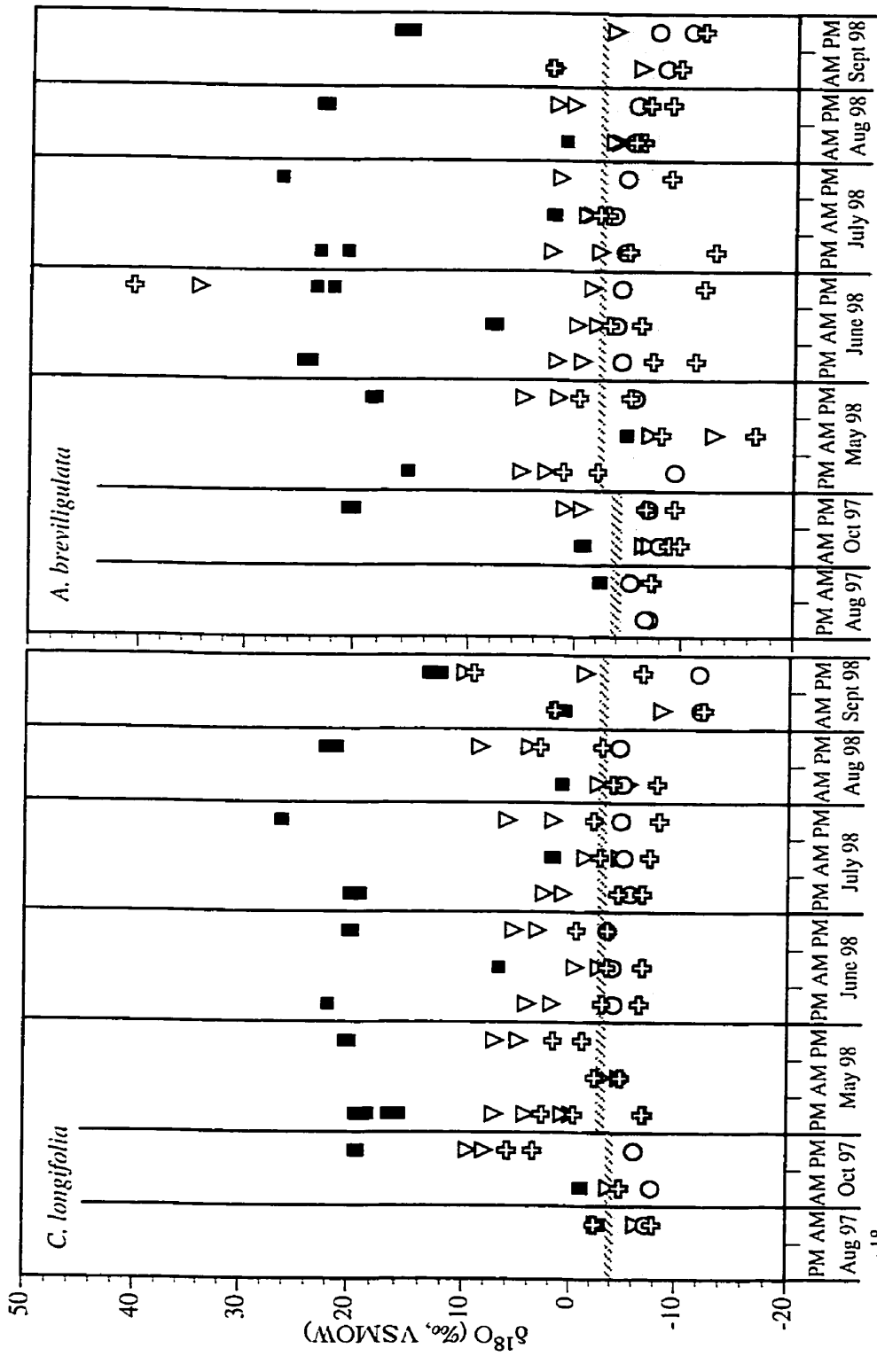

Figure 4-13. $\delta^{18}O$ values of (▽) lower leaf water, (■) modelled leaf water, (○) stem water and (✠) metabolic water (calculated from Equation 4-6) of *C. longifolia* and *A. breviligulata* at Pinery Provincial Park. The shaded area represents the range of $\delta^{18}O$ values for leaf water from which leaf silica was precipitated during 1994-1997 at Pinery Provincial Park calculated from Equation (4-4). The hatched areas are the $\delta^{18}O$ values predicted leaf water, based on measured leaf-silica $\delta^{18}O$ values and temperatures for 1997, and on average stem-water $\delta^{18}O$ values and Equation (4-5) for 1998.

bulk lower-leaf water to the $\delta^{18}O$ values predicted for leaf water over the previous four years at Pinery Provincial Park (shaded area, Fig. 4-13).

Similar calculations using upper leaf water are not possible as the $\delta^{18}O_{modelled}$ values likely do not reflect steady state conditions that occur in the upper leaf. Adjustments would also be necessary for the isotopic composition of vein waters, which become more enriched in ^{18}O towards the tip of the leaf. If a correction for these parameters could be devised, we predict that the integrated $\delta^{18}O_{metabolic\ water}$ value over the length of the leaf, weighted for relative volumetric water content, will have an isotopic composition similar to the predicted leaf-water $\delta^{18}O$ values in Table 4-6.

4.5. CONCLUSIONS

The $\delta^{18}O$ values of stem water represent a mixture of evaporated soil water near the ground's surface and winter and summer precipitation stored in the soil-water reservoir at greater depths. Because stems contain water that is drawn up by roots over the entire rooting zone, their $\delta^{18}O$ and δD values provide a good proxy for the stable isotopic composition of water available for root uptake in the unsaturated zone.

Variations in the $\delta^{18}O$ and δD values of water within a single plant and between corresponding tissues of plants of the same species were as large as those observed between different species. Although point sampling shows large variations in the $\delta^{18}O$ and δD values of stem water, seasonal averages approximate the isotopic values for average soil water and weighted average precipitation over the 1998 growing season. In addition, the average $\delta^{18}O_{precipitation}$ values lie close to the range of $\delta^{18}O_{stem\ water}$ values predicted from measured stem-silica oxygen-isotope compositions over a four-year period at Pinery Provincial Park.

The majority of rhizome and root waters are more enriched in ^{18}O and D than the corresponding stem water from the same plant. This reflects the selective sampling of root and rhizomes tissues in the upper portions of the soil profile. The greater spatial

and temporal variation in the $\delta^{18}O$ and δD values of waters in the top 5 centimeters of the soil, as well as the greater sensitivity of waters at this depth to evaporative ^{18}O and D enrichment, are reflected in the generally higher δ-values of water extracted from these underground tissues. In turn, these variations may be transferred to the $\delta^{18}O$ values of rhizome phytoliths, which may be more enriched in ^{18}O relative to stem silica. The difference in $\delta^{18}O$ values between shallow rhizome and stem silica may provide a useful way to infer the extent of evaporative enrichment of water in the upper layers of the soil relative to the average soil-water $\delta^{18}O$ values over the depth of the active rooting zone. However, $\delta^{18}O$ values of rhizome silica may also be lower than corresponding stem silica because of differences in age and average temperature of silica precipitation between these two tissues.

Interpretation of the $\delta^{18}O$ and δD values of leaf water is complicated by their spatial variation within individual leaves, and by the diurnal and seasonal variations in isotopic composition that also occur. Leaf silica is not precipitated from waters that experienced the extreme ^{18}O- and D- enrichments that typify water from the upper leaf at mid-day. The ^{18}O- and D-enrichment of leaf water does not occur according to the model of a single evaporating pool; multiple water pools are involved, which are affected differently during transpiration. Water in the sheath and lower and upper leaf tissues behaves as a string of evaporating pools that become increasing more enriched in ^{18}O and D as the water moves toward the tip of the leaf. Sheath water, which is located at the beginning of this string of pools, has isotopic compositions quite similar to stem-water $\delta^{18}O$ and δD values. There may be a secluded fraction of water within the leaf (so-called "metabolic" water) that undergoes much smaller diurnal fluctuations in isotopic composition than those measured for bulk-leaf water. It may be that leaf silica is preferentially deposited from such "metabolic" water.

4.6. REFERENCES

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Chapter 5. Closing Remarks

5.1. Major Conclusions

1. The $\delta^{18}O$ values of phytoliths are determined directly by the temperature of silica crystallization and the corresponding plant-water oxygen-isotope composition. The temperature-dependent, oxygen-isotope equilibrium fractionation between silica and plant water for *C. longifolia* and *A. breviligulata* can be accurately predicted using the geothermometer of Shahack-Gross et al. (1996). However, accurate determination of the temperature of phytolith formation is only possible when plant-water $\delta^{18}O$ values are known.

2. Phytoliths formed in non- or weakly transpiring tissues (rhizomes, stems and sheaths) are precipitated in equilibrium with plant water that is unfractionated in oxygen-isotope composition from that of soil water. Under natural conditions, the variation of $\delta^{18}O_{silica}$ values for *C. longifolia* is highly dependent on soil-water $\delta^{18}O$ values. Stem-water $\delta^{18}O$ values represent the cumulative average of the soil water drawn up over the entire depth of the active rooting zone. Hence, the $\delta^{18}O$ values of stem silica provide a good proxy for the $\delta^{18}O$ values of soil water available for root uptake in the unsaturated zone. The magnitude of any difference between the average $\delta^{18}O$ values of plant water in non-transpiring tissues and local meteoric water reflects the extent of evaporation experienced by the soil-moisture reservoir that feeds the grass. This difference increases as the environment becomes more arid.

3. Average relative humidity has a role in determining the degree of ^{18}O enrichment between silica from transpiring and non- or weakly transpiring tissues. The magnitude of the enrichment increases as relative humidity decreases. Interpretation of leaf-water δD and $\delta^{18}O$ values is complicated by the spatial heterogeneity in isotopic composition of water within individual leaves and the diurnal and seasonal variations in leaf water δ-

values. Water within the sheath and lower- and upper-leaf tissues behaves as a string of evaporating pools. The water in these tissues experiences continual evaporation and becomes progressively more enriched in ^{18}O as it moves towards the tip of the leaf. This often results in the enrichment of upper-leaf water above the $\delta^{18}O$ values predicted for steady-state conditions. The sheath tissues are at the beginning of the string of pools and therefore contain water and phytoliths that are most similar in their $\delta^{18}O$ and δD values to stem water and stem phytoliths, respectively. Leaf silica, however, is precipitated from plant water that has not acquired the extreme ^{18}O-enrichment predicted using steady-state models, or measured for leaf waters sampled under mid-day conditions. Silica within the leaves may be precipitated from an isotopically secluded fraction of water, which experiences smaller diurnal variations in oxygen isotopic composition than the water present at sites of evaporation within the leaf.

4. Soil-phytolith assemblages are composed of silica from both transpiring and non-transpiring plant parts. Temperature and soil-water $\delta^{18}O$ signals carried by phytoliths from the stems, sheaths and rhizomes may be masked by the $\delta^{18}O$ values of phytoliths from the leaves and inflorescence. These are variably further enriched in ^{18}O, depending on relative humidity. Accurate reconstruction of temperature, $\delta^{18}O_{soil\ water}$ values, and relative humidity for ancient phytolith assemblages requires, as a first prerequisite, the recognition and physical separation of phytoliths produced in transpiring and non-transpiring plant parts.

5.2. Future Work

The scope of this study was insufficient to test for species-related effects associated with silica polymerization, which may influence the isotopic composition of silica phytoliths in different grasses. Although the $\delta^{18}O$ values of silica from a C3 and C4 grass were similar at Pinery Provincial Park, differential adaptation to water stress in

more arid regions may emphasize differences in water-uptake patterns, water-holding capacity and transpiration rates between these two species. Such effects have the potential to alter the $\delta^{18}O$ values of plant water and ultimately the oxygen isotopic compositions of silica phytoliths between different species. A more detailed investigation of the variation in oxygen isotopic composition of silica phytoliths from a wide selection of C3 and C4 grasses grown at the same location is required to test for species effects. In addition, measurements of a selection of C3 and C4 grasses growing under a variety of climatic conditions are required to investigate the potential magnification of species-related effects in arid versus humid conditions.

Establishing the $\delta^{18}O$ values of the water from which phytoliths precipitate is an important direction for future work. It is conceivable that soil-water $\delta^{18}O$ values could be determined independently, if phytolith studies were combined with the isotopic study of an additional soil component formed in the unsaturated zone, such as soil carbonates. Once soil-water $\delta^{18}O$ values are determined, stem-water $\delta^{18}O$ values can be inferred and the temperature of phytolith formation can be calculated. Investigations into the hydrogen isotopic composition of the hydrous components of opal-A phytoliths may also provide information concerning the original plant-water $\delta^{18}O$ values. In particular, differential thermal analysis and nuclear magnetic resonance studies may provide detailed knowledge regarding the varying nature of phytolith hydration. This information is required to interpret properly hydrogen-isotope data obtained from various water fractions that can be extracted from phytolith silica. If water is bound in phytoliths in a similar manner as diatom silica, it should be possible to extract original plant waters trapped in the porous silica structure. These may have retained the hydrogen isotopic signature of the plant water from which the silica precipitated.

It may also be possible to calculate the temperature of plant growth without prior knowledge of plant-water $\delta^{18}O$ values by combining the oxygen-isotope analysis of

phytoliths with that of another biogenic mineral phase that co-precipitates with the silica. Biogenic calcium oxalate, which can also be precipitated in the leaves of grasses, is one such possibility. The following questions should be addressed: Does calcium oxalate form in isotopic equilibrium with plant water? Does calcium oxalate co-precipitate with biogenic silica within the plant? Does the $\delta^{18}O$ value of calcium oxalate preserve climatic information? Does the temperature-dependent fractionation between calcium oxalate and water differ sufficiently from that of silica to provide a useful comparison?

To use soil-phytolith assemblages successfully as a paleoclimatic indicator, further investigation into the stability and perseverance of phytoliths in the soil is also needed. The extent of phytolith dissolution over time and the effects of diagenesis on the isotopic composition of phytoliths in the soil-record, all must be assessed. Preferential dissolution of biogenic silica may occur if phytoliths have different morphologies or originate from different plant species. In addition, partial dissolution of individual phytoliths may remove some of the more delicate surface features, by which they can be identified. The oxygen-isotope composition of the soil-phytolith assemblages could also be altered during diagenesis if ^{16}O is removed preferentially over ^{18}O from the silica structure during dissolution. Even if there is no oxygen-isotope fractionation associated with dissolution processes, the isotopic composition of the overall soil phytolith assemblage may become biased if specific phytolith morphologies typical of transpiring versus non-transpiring tissues dissolve completely. Finally the effect of fire on the oxygen-isotope composition of silica phytoliths requires full assessment. High temperatures associated with grassland fires have the potential to alter the oxygen-isotope composition of the residual silica phytoliths. Once these questions are addressed, full efforts can then be placed into using this paleoclimate proxy in the study of Holocene climate change in a variety of terrestrial settings.

5.3. REFERENCES

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Appendix A

GLOSSARY

arabinoxylan – A nucleotide sugar used in the synthesis of most polysaccharides.

bulliform cells – Large, thin walled cells in the leaf epidermis situated on either side of the main central vein. Under periods of water stress the bulliform cells may partially collapse, causing the leaf blade to fold or roll reducing transpiration.

casparian strip – A band of waterproof, corky material that is found on the walls of endodermal root cells, preventing water from entering the vascular tissue except through the cytoplasm of the endodermal cells.

cellulose – A complex, insoluble carbohydrate that constitutes the principal component of most plant-cell walls.

Chloridoid – A morphological classification of phytoliths that includes saddle-shaped silica bodies, which are generally found in grasses belonging to the sub-family Eragrostoideae (Chloridoideae).

cortex – The tissue between the epidermis and the vascular cylinder of stems and roots.

cortical cells – Cells that occur in the cortex.

culm – A jointed stem of a grass or sedge that may be hollow, filled with pith or solid.

cuticle – A waxy or fatty protective layer of varying thickness on the outer wall of the epidermal cells of the stems and leaves of plants, which protects the internal leaf tissue from excessive water loss.

dicotyledon – A class of angiosperms (flowering plants) whose seeds commonly have two cotyledons (embryo leaves). They have a net-like system of veins in the leaves.

endodermis – The innermost, single-layer of cells of the cortex, which surrounds the vascular tissue in roots and some stems. Many endodermal cells have casparian strips.

epidermis – The outer most layer of cells of leaves, stems and roots, which is usually one cell thick.

fatty acids – A lipid.

Festucoid – A morphological classification of phytoliths that includes circular, elliptical, rectangular or oblong shaped silica bodies, which are generally found in grasses belonging to the sub-family Festuceae.

forb – A herbaceous species other than a grass sedge or rush (e.g. legumes).

geotropism – The upward-growth response to the stimulus of gravity.

gibberellic acid – One of a group of plant hormones that can stimulate the growth of leaves and promote the elongation of stems.

glaucous – Covered with a waxy coating that gives a sea-green or bluish green colour.

glucan – A polysaccharide composed of glucose units (e.g. cellulose and starch).

Gramineae – The grass family.

guttation – The exudation of water in liquid form from leaves resulting from root pressure.

herb – A plant that does not develop a persistent above-ground stem.

hydric - Wet soil conditions.

inflorescence – A flowering structure that consists of more than a single flower.

intercalary meristem – A region within the stem and sheaths in which undifferentiated cells are capable of dividing indefinitely.

intercellular – Between cells.

internode – The part of the stem between successive nodes or joints.

intracellular – Within cells.

involute – Having edges that roll inwards or under.

leaf – A thin expanded organ generated at a node on the stem of a plant. Leaves are usually green and the main site of photosynthesis.

lignin – a complex, cross-linked polymer that is found in many plant cell walls. Its function is to cement together and anchor cellulose fibers and to stiffen the cell wall.

lipids – A general term for fats, fatty substances and oils. Lipids may serve as energy storage compounds, hormones, vitamins and as structural components of cells (e.g. membranes). Lipids include any variety of compounds insoluble in water but soluble in ether and alcohol.

long cells – Elongate, epidermal leaf cells with the long axis parallel to the axis of the leaf.

lumen – The space or cavity within a tube, sac or cell.

macro-hair – A large hair.

mesophyll – Parenchyma (chlorenchyma) tissue between the upper and lower epidermis of a leaf. Its main functions are photosynthesis and the storage of starch.

mesic - Moist soil conditions.

monocotyledons – A class of angiosperms (flowering plants) whose seeds have one cotyledon (embryo leaf). They have a parallel vein system in the leaves.

node – The regions of a stem where one or more leaves or buds are attached.

panicle – Any complex, branched inflorescence.

Panicoid – A morphological classification of phytoliths that includes dumb-bell and cross-shaped silica bodies, which are generally found in grasses belonging to the sub-family Panicoideae.

perennial – A plant that normally produces above-ground parts from the same root system for at least three growing seasons and produces flowers annually.

phenols – An aromatic compound that bears one or more hydroxyl groups

phloem – A tissue that transports dissolved organic and inorganic material in vascular plants.

polysaccharide –Any carbohydrate that is a linear or branched polymer of 10 or more simple sugars bonded together in long chains (e.g. starches).

protein – An organic compound containing carbon, hydrogen, oxygen, nitrogen and frequently sulfur, in complexes molecules composed of numerous amino acids linked by peptide bonds. Structurally they are divided in to two groups: globular and fibrous. They function as enzymes, structural elements, hormones, respiratory pigments, contractile elements, antibodies and genes.

pulvinus – The swelling at the base of the sheath, part of the node.

rhizome – A horizontally creeping underground stem, which has definite nodes and internodes, bears roots and new shoots, and usually persists from season to season.

roots – The lower part of a plant, usually underground, through which water and mineral nutrients enter the plant. The roots also serve to anchor plants to the soil.

sheath – The lower, tubular base of a leaf blade that encloses the stem.

sieve cell – A long slender tapering conductive cell of the phloem that has cytoplasm but lacks a nucleus. Each one has a porous sieve plate on the bottom.

sieve tube – A series of sieve cells that lie end to end forming a tube in the phloem.

silica cells – A short cell of the leaf epidermis that has been completely filled with silica.

spike – An unbranched inflorescence in which the flowers lack a stalk.

stem – The part of a vascular plant that bears buds, leaves and flowers, forms central axis of the plant and provides mechanical strength.

stoma – A small opening found in epidermal layers of plants that allows access for carbon dioxide and an escape for water. The stoma is surrounded by guard cells that control the pore size.

stomata – The plural of stoma.

suberin – A hydrophobic material that prevents water movement in suberized cell walls. Suberin may be found in localized areas of the root and stem epidermis and bundle sheath cells and cork cells.

trichome – Any hair-like outgrowth on the epidermis of a plant.

vascular cylinder/bundle – A discrete longitudinal strand that consists primarily of vascular tissue. Groups of vascular bundles form a continuous conductive system throughout the plant along which water and soluble nutrients pass. The vascular cylinder also contributes to the structural support of the plant.

vascular tissue – Tissues through which water and nutrients move through a plant (xylem and phloem).

xylem – A plant tissue that consists of various types of cells which transport water and dissolved substances towards the leaves. The xylem may be distinguished from the phloem by the presence of a vertical system of dead cells with thick lignified walls.

xeric – A soil deficient in available moisture, such as in deserts.

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Appendix B. Meteoric water $\delta^{18}O$ values (‰, VSMOW).

Location	Source of water	Source of Data	$\delta^{18}O$ surface water	$\delta^{18}O$ ground water	$\delta^{18}O$ precipitation annual	winter	summer
1. Pinery, Ontario, CAN							
Pinery, Ontario	Lake Huron	this study	-7.1				
Pinery, Ontario	tap water (Lake Huron)	this study	-7.2*				
Pinery, Ontario	Ausable River	this study	-8.0				
Parkhill, Ontario	Parkhill reservoir	this study	-6.4				
Ontario	ground water	Fritz et al. (1987)		-10*			
Simcoe, Ontario	IAEA precipitation	Rozanski et al. (1993)			-9.3		
Grand Bend, Ontario	1995 precipitation	Huddart (1998)			-7.4		-6.0*
Grand Bend, Ontario	May-July precipitation 1996	Huddart (1998)			-7.7		-5.8*
Grand Bend, Ontario	1996 precipitation	Huddart (1998)					-6.8*
Grand Bend, Ontario	1997 precipitation	Huddart (1998)			-7.8		-6.1*
2. Colorado Springs, CO, USA							
Colorado Springs, CO	tap water	this study		-13.5*			
Colorado Springs, CO	Monument Creek	this study	-5.1*				
Colorado Springs, CO	drain pipe	this study					
Nothern New Mexico	Canadian River	this study	-6.5*				
SE Colorado	Dakota Aquifer	Clark et al. (1998)		-11.4 to -8.5			
3. Havanna, Illinois, USA							
Monon, Indianna	tap water	this study		-6.4			
Monon, Indianna	Wabash river	this study	-6.6*				
Havanna, Illinois	drainage creek	this study	-6.8*				
Havanna, Illinois	Illinois river	this study	-5.6*				
Quincy, Illinois	Mississippi River	this study	-5.7*				
Quincy, Illinois	tap water	this study		-6.4*			
Chicago, Illinois	IAEA precipitation	IAEA (1992)			-6.0		-4.1*
Illinois	ground water	Siegal (1989)		-7			
Illinois	precipitation	Siegal (1989)			-6		
4. Kellogg, Minnesota, USA							

Kellogg, Minnesota	pond	this study	-1.6				
Minnesota	ground water	Siegel (1989)		-9 to -8*	-7		
Southern Wisconsin	meteoric water	Yapp (1979)	-7.5*				
Southern Wisconsin	meteoric water	Yapp (1979)	-8.5*				
Southern Wisconsin	precipitation	Hunt et al. (1997)					-6*
Minnesota	precipitation	Cerling and Quade (1993)			-11.4		
5. Aroya, Colorado, USA							
Aroya, Colorado	South Rush Creek	this study	-7.2*				
Rush, Colorado	tap water	this study		-10.0*			
Eastern Colorado	Dakota Aquifer	Clark et al. (1998)		-11.4 to -8.5			
6. Quinter, Kansas, USA							
Quinter, Kansas	tap water	this study		-10.5*			
Quinter, Kansas	Turkey Creek	this study	-4.7*				
Aroya, Colorado	South Rush Creek	this study	-7.2*				
Western Kansas	High Plains Aquifer	Clark et al. (1998)		-8.9*			
Western Kansas	ground water	Clark et al. (1998)		-9.9*			
Western Kansas	shallow ground water	Clark et al. (1998)		-9.4*			
Kansas	precipitation	Cerling and Quade (1993)			-5.3		
7. Thedford, Nebraska, USA							
Thedford, Nebraska	pond	this study	1.4				
Thedford, Nebraska	Middle Loup River	this study	-10.5*				
Nebraska/ Iowa	Missouri River	this study	-11.3				
Nebraska	IAEA precipitation	Bryant et al. (1994)			-9.8		
DesMoines, Iowa	precipitation	Fricke et al. (1998)				-9.4	-5.8*
Alliance, Nebraska	tap water	this study		-12.1*			
Nebraska	precipitation	Cerling and Quade (1993)			-9.0		
Nebraska	precipitation	Cerling and Quade (1993)			-6.2		
8. Bonner, Nebraska, USA							
Alliance, Nebraska	tap water	this study		-12.1*			
Alliance, Nebraska	pond water	this study	5.2				
Alliance, Nebraska	pond water	this study	0.8				

Nebraska	IAEA precipitation	Bryant et al. (1994)			-9.8		
Nebraska	precipitation	Luz et al. (1990)			-12.0		-9.6*
Nebraska	precipitation	Cerling and Quade (1993)			-9.0		
Nebraska	precipitation	Cerling and Quade (1993)			-6.2		
9. Fertile, Minnesota, USA							
Fertile, Minnesota	Sand Hill River	this study	-9.0*				
Fertile, Minnesota	well water	this study		-12.7*			
middle Minnesota	lake	LaBaugh et al. (1997)	-2.1				
middle Minnesota	ground water	LaBaugh et al. (1997)		-8.9			
middle Minnesota	precipitation	LaBaugh et al. (1997)			-11.0		-12 to -5*
Minnesota	precipitation	Siegel (1989)			-8		
Minnesota	precipitation	Cerling and Quade (1993)			-11.4		
10. Cheyenne, Wyoming, USA							
Meridian, Wyoming	tap water	this study	-15.1				
Torrington, Wyoming	tap water	this study	-14.4*				
Torrington, Wyoming	Tristae Canal	this study	-14.3*				
Eastern Wyoming	ground water	Back et al. (1983)		-17*			
11. Onefour, Alberta, CAN							
Onefour, Alberta	pond	this study	-3.9				
Northern Montana	Milk River	this study	-14.6*				
Southern Alberta	precipitation	Hendry and Schwartz (1988)				-23 to -21	-15 to -12.5
Southern Alberta	Milk River aquifer	Hendry and Schwartz (1988)		-18*			
Southern Alberta	ground water	Fritz et al. (1987)		-18*			
Lethbridge, Alberta	average stem water	Flannagan et al. (1991)	-14.5*				
Southern Alberta	precipitation	Luz et al. (1990)			-17.4		-14.2*
12. Dundurn, Saskatchewan, CAN							
Regina, Saskatchewan	shallow creek	this study	-11.1*				
Dundurn, Saskatchewan	deep puddle	this study	-5.3				
Dundurn, Saskatchewan	controlled creek	this study	-12.0*				
Saskatchewan	ground-water contours	Fritz et al. (1987)		-19*			
Wynard, Saskatchewan	IAEA precipitation	IAEA (1992)			-15.6		-13.6*

Southern Saskatchewan	ground water & precip	Dowuona et al. (1993)		-15 to -11		-27 to -22	-15 to -12
Saskatchewan	precipitation	Cerling and Quade (1993)			-15.2		
Saskatchewan	precipitation	Cerling and Quade (1993)			-14.5		
13. Sprucewoods, Manitoba, CAN							
Sprucewoods, Manitoba	pump water	this study		-14.5*			
Sprucewoods, Manitoba	Assiniboine River	this study	-11.0*				
Manitoba	ground water	Fritz et al. (1987)		-15			
Gimli, Manitoba	IAEA precipitation	Rozanski et al. (1993)			-14.2		
14. Rawlins, Wyoming, USA							
Rawlins, Wyoming	Seminoe river	this study	-16.5				
Rawlins, Wyoming	small creek	this study	-17.9				
Rawlins, Wyoming	Seminoe reservoir	this study	-16.0*				
Rawlins, Wyoming	North Platte River	this study	-15.2*				
Southern Wyoming	precipitation	Luz et al. (1990)			-16.6		-14.9
15. Broadus, Montana, USA							
Southern Montana	Tongue River	this study	-16.9*				
Southern Montana	Powder River	this study	-11.9*				
Southern Montana	Little Powder River	this study	-8.1				
Southern Montana	Powder River aquifer	USGS (1984)		-20.7 to -17.5*	-14.9		-12.2*
Northern Wyoming	precipitation	Luz et al. (1990)					
16. Kinsella, Alberta, CAN							
Kinsella, Alberta	N. Saskatchewan River	this study	-18.3*				
Kinsella, Alberta	well water	this study		-18.9*			
Kinsella, Alberta	marsh	this study	-7.8				
Drumheller, Alberta	Red Deer River	this study	-17.8				
Drumheller, Alberta	Red Deer River tap water	this study	-17.2				
Alberta	ground water contours	Fritz et al. (1987)		-20			
North of Saskatoon	recharged ground waters	Fortin et al. (1991)		-16.3 to -21.9			
North of Saskatoon	average ground waters	Fortin et al. (1991)		-18.9*			
Edmonton, Alberta	precipitation	Maulé et al. (1994)			-18.1	-25.6	-15.6*
Edmonton, Alberta	soil water	Maulé et al. (1994)	-18.7*				

near Kinsella, Alberta	precipitation	Luz et al. (1990)		-17.4	-14.5
17. **Kortes Dam, Wyoming, USA**					
Rawlins, Wyoming	Seminoe river	this study	-16.5*		
Rawlins, Wyoming	small creek	this study	-17.9		
Rawlins, Wyoming	Seminoe reservoir	this study	-16.0		
Rawlins, Wyoming	North Platte River	this study	-15.2		
Southern Wyoming	precipitation	Luz et al. (1990)		-16.6	-14.9

* values used in Chapter 3, Table 3-1. For references see Chapter 3.

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