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Cold Season Physiology of Arctic Plants

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COLD SEASON PHYSIOLOGY OF ARCTIC PLANTS

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Jonathan George Moser

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To: Dean Kenneth G. Furton
College of Arts and Sciences

This thesis, written by Jonathan George Moser, and entitled Cold Season Physiology of Arctic Plants, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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DEDICATION

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ABSTRACT OF THE THESIS

COLD SEASON PHYSIOLOGY OF ARCTIC PLANTS

by

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The cold season in the Arctic extends over eight to nine months during which ecosystem gas exchange and water balance of arctic plants have been largely unexplored. The overall objective of this thesis was to examine two critical gaps in our knowledge about tundra cold season processes – ecosystem respiration at very low temperatures and water uptake during the winter-spring transition. I determined the temperature response of ecosystem respiration of tundra monoliths down to temperatures as low as can be expected under snow-covered conditions (-15 °C). Temperature responses fit the Arrhenius function well with Q_{10} values over the range of -15 to 15 °C varying from 6.1 to 4.8. I used deuterium-enriched water (^2H_2O) as a tracer to evaluate water uptake of evergreen plants at snowmelt when soils are largely frozen. The results revealed that evergreen plants take up water under snow cover, possibly via roots but undoubtedly by foliar uptake.

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CHAPTER I. INTRODUCTION

Arctic tundra ecosystems have the potential to release enormous stores of carbon into the global biogeochemical environment that could affect numerous processes and feedbacks. Post *et al*. (1982) estimated that over 13 % of the global soil carbon pool resides in arctic tundra, though they lacked detailed data concerning permafrost (soils remaining below 0 °C for 2 consecutive years). Present-day permafrost research now places 50 % of the total global soil carbon stock in belowground organic matter of the Arctic (Tarnocai *et al*. 2009). Arctic tundra ecosystems have historically been a carbon sink (Billings 1987), though the recent onset of warming in high latitude regions during the last few decades (Lachenbruch and Marshall 1986; Jones and Wigley 1990) has led to an increase in permafrost degradation in arctic regions (Jorgenson *et al*. 2006). Arctic tundra ecosystems have begun to reduce their capacity as carbon sinks as a result of increases in microbial and plant respiration (Oechel *et al*. 1993). The subsequent increase in ecosystem carbon uptake via plant growth resulting from warming would not come close to offsetting the release of previously stabile carbon (0.8 - 1.1 Pg C yr^{-1}) in tundra soils and permafrost (Schuur *et al*. 2009).

Total carbon sequestration and release depends both on photosynthesis during the growing season as well as summer and winter respiratory losses. Which of these processes will become more significant in the future is uncertain, although most high latitude warming is occurring during the winter (Intergovernmental Panel on Climate Change 2007). Unfortunately, very little is known about tundra ecosystem processes during winter compared to those in the growing season. The Intergovernmental Panel on Climate Change (2007) predicts that arctic regions will have earlier and stronger impacts

resulting from global climate change than in other regions of the globe, which highlights the susceptibility of large carbon stores currently residing in arctic soils (McGuire *et al*. 2006; Grosse *et al*. 2011).

The cold season in the Arctic extends over eight to nine months during which air temperatures often reach as low as -40 °C. However, these extreme temperatures are rarely reached at the level of the soil surface and plants, as a result of the insulating layer created by snow cover. Under the snow, temperatures seldom fall below -15 °C (Toolik Environmental Data Center Team 2009-2011). Arctic ecosystem respiration (ER) continues during the winter at low rates, but over the extended winter ER can be a substantial fraction of the annual carbon budget. Photosynthesis by arctic evergreens also occurs under the snow in springtime (Starr and Oberbauer 2003; Starr *et al*., unpublished). Recent research shows that one evergreen is photosynthetically competent over the entire winter (Lundell *et al*. 2010). Cold season photosynthesis by evergreens may help offset winter respiratory carbon losses. How these evergreens maintain their water balance under the snow while the soils are still frozen is uncertain. Physiological processes of tundra at very low temperatures during the arctic winter are largely unknown and unstudied. Vegetation clipping experiments suggest a large fraction of winter respiration is from aboveground plant biomass (Grogan *et al*. 2001).

Deciduous shrubs are now expanding throughout the Arctic (Sturm *et al*. 2001; Hollister *et al*. 2005; Sturm *et al*. 2005b; Wahren *et al*. 2005; Tape *et al*. 2006). The expansion of shrubs is apparently a summer phenomenon; the International Tundra Experiment (ITEX) has found evidence that summer time temperatures could be responsible for driving changes in vegetation (Elmendorf *et al*. 2012). Sturm *et al*.

(2005b) proposes that these changes are driven by changes in soil temperature resulting from snow deposition or increased winter temperature, allowing faster nutrient turnover. Simulated increases in soil temperature have also been found to increase aboveground biomass as well as root tissues in shrub growth forms, further increasing winter plant respiration (Brooker and Van der Wal 2003; Hudson *et al*. 2011). Projected changes in the timing of snowmelt and growing season will favor abundant, taller plants, which increases overall vegetative biomass, while at the same time decreasing the occurrence of uncommon species (Walker *et al*. 2006; Rixen and Mulder 2009; Rammig *et al*. 2010). Increases in abundance of larger shrub species will adversely affect snow albedo, having global implications (Sturm *et al*. 2005a; Loranty *et al*. 2011). Deciduous shrubs are expanding, possibly at the expense of nonvascular plants and evergreen shrubs, which will very likely affect the ecosystem carbon balance by increasing ecosystem carbon losses during winter and reducing carbon uptake through photosynthesis under the snow. The arctic winter season is long, so even small changes in winter rates may translate into large effects on the overall carbon balance. Reliable temperature response information is critical to understanding carbon balance and making accurate predictions about arctic tundra ecosystem respiration in the face of future climate change.

BACKGROUND

Characteristics of Tundra Ecosystems

Tundra vegetation is characterized by the absence of trees and is made up of communities of low stature vegetation including shrubs, grasses, forbs, mosses and lichens (Callaghan 2001). Viereck *et al*. (1992) provides a detailed classification system of Alaskan vegetation. Tussock-forming tundra, dominated by the graminoid,

Eriophorum vaginatum, is very common throughout Alaskan, Canadian, and Russian arctic landscapes (Bliss and Matveyeva 1992; Walker *et al*. 1994). Arctic tundra plants face many challenges including freezing temperatures during winter and growing seasons, low soil temperatures and shallow soil thaw depths, short growing seasons, drought and inundation of water, strong winds, and infertile soils (Pielou 1994). Tundra soils become frozen several months into the winter season and remain frozen until the onset of spring, severely constraining physiological access to water.

Low Temperature Ecosystem-Level Respiration

Although the cold season is three times longer than the growing season, little research on arctic plants and tundra has been conducted during the cold season (winter-spring), when the largest changes are expected to occur with climate warming (Chapman and Walsh 2007; Christensen *et al*. 2007). The primary emphasis of cold season research has been on rates of winter ecosystem respiration to develop estimates of annual carbon balance. The majority of all CO_2 flux measurements during the winter or at winter temperatures indicate that some amount of respiration is occurring, and while low, may contribute substantially to the annual carbon balance because of the length of the cold season. In arctic locations, accumulation of respiration during the cold season has the potential to shift the carbon balance to a source (Zimov *et al*. 1996).

Methodologies of CO_2 measurement during/in the harsh winter environment range from infrared gas analyzers with static chambers, steel probes for determination of subnivean CO_2 concentrations for application in diffusion models, alkali CO_2 absorption, syringe extractions with gas chromatographs, and subnivean CO_2 sensors. Most of these methods have serious limitations. As a result, estimates of winter ecosystem respiration

rates are highly variable. One issue is that ecosystem CO_2 production may be temporally disconnected to CO_2 release to the atmosphere by resistance created by snow cover (Jones *et al*. 1999) or impermeable barriers produced by soil surface ice layers (Björkman *et al*. 2010b). Another important issue is the performance of electronics at very low temperatures for measurement of very low rates. Several cold-temperature CO_2 methods have employed syringe extraction techniques (Zimov *et al*. 1993; Zimov *et al*. 1996; Panikov and Dedysh 2000), thereby eliminating the need to expose electronics to the severe cold. Of existing wintertime CO_2 studies, few are directly comparable. Studies differ in site location, vegetation community, snow depth, treatment, and soil temperature (Table 1). In the few *in situ* ecosystem studies of winter CO_2 efflux measurements that report both soil temperatures and the depths at which they were measured, minimum soil temperatures vary from -2 to -25 °C and associated soil depths range from 0 to 10 cm (Table 2). Björkman *et al*. (2010b) summarized research on winter CO_2 rates, methodologies, habitats, and locations and felt that variation in CO_2 efflux rates resulted primarily from different techniques. However, several of the studies lacked CO_2 efflux rates at subfreezing temperatures, did not report the depth at which the associated soil temperatures were recorded, or presented data that had low replication or even data recorded on a single sensor. Few presented responses with temperatures that would be amenable to application in ecosystem models.

Low Temperature Soil Respiration

The assumption that soil microbial activity discontinues when soils freeze has been refuted by low temperature research on soil respiration rates (Clein and Schimel 1995). At the onset of winter, the active, unfrozen layer of arctic soils has reached its

greatest thaw, however, soils resist below-freezing temperatures over several months (Schimel and Mikan 2005). During the height of the winter season, soils can fall to temperatures below -25 °C (Schimel *et al*. 2004). Respiration rates from soil cores 0-25 cm have been measured by Mikan *et al*. (2002) at temperatures of -10 °C, and Elberling and Brandt (2003) were able to observe CO_2 increases in soil at -18 °C. Tundra soils incubated for over a year at below-freezing temperatures had positive and measurable CO_2 efflux rates at temperatures as low as -39 °C (Panikov *et al*. 2006).

Low Temperature Photosynthesis

Only a few studies have looked at photosynthetic and respiration of bryophytes and vascular plants at cold temperatures, and some key studies have been conducted in the Antarctic. Both the Arctic and Antarctic experience similar harsh, cold temperatures during the winter season. Antarctica plant life consists of mosses, lichens, and only two vascular plant species, *Colobanthus quitensis* (Antarctic pearlwort) and *Deschampsia antarctica* (Antarctic hairgrass, (Lewis Smith 2003). Two of the lowest temperatures at which photosynthesis and respiration measurements were conducted on Antarctic mosses were -5 and -6 °C by Pannewitz *et al*. (2005) and Kappen *et al*. (1989), respectively. One of the lowest temperature studies on Antarctic vascular plants was by Xiong *et al*. (1999), who performed photosynthesis and respiration field studies on both *C. quitensis* and *D. antarctica* at temperatures as low as -1.5 °C.

Arctic tundra vegetation has been shown to be photosynthetically ready during the winter-spring transition (Oberbauer *et al*. 1996), and evidence published a decade ago provided evidence of photosynthetic activity under the snow (Oberbauer and Starr 2002). Starr and Oberbauer (2003) measured positive photosynthetic rates of arctic Evergreens

(*Eriophorum vaginatum*, *Ledum palustre*, *Vaccinium vitis-idaea*, and *Cassiope tetragona*) under snow. Subsequent research on *V. vitis-idaea* has found that in addition to being photosynthetically capable prior to snowmelt, this species is photosynthetically active throughout the winter (Lundell *et al*. 2008; Lundell *et al*. 2010). It has yet to be determined how these evergreens manage their water balance over the winter and acquire the water required to sustain photosynthesis during snowmelt.

Water Balance During the Winter-Spring Transition

Arctic tundra plant water balance during winter-spring transition is a function of a number of interacting factors that are not well understood. When plant stem water freezes, embolisms may form in the xylem vessels, blocking further water uptake in that vessel. Water is more viscous when cold and uptake rates are lower both from greater stem resistance and loss of conductivity of roots (Brodribb and Hill 2000). Tundra soils remain frozen during most of the winter and partially so after snow melts in the spring. Plants may be able to uptake unfrozen water bound to soil particles when most soil is frozen (Watanabe and Mizoguchi 2002). As snow begins to melt in the spring, a process that may take a week or more, water percolates from the upper snow layers down to the snow-soil interface where it might be available to plant roots. During cold season photosynthesis, such as that described by Starr and Oberbauer (2003) and Lundell *et al*. (2008, 2010), plants may be transpiring, implying that the water losses are replaced or the plants undergo water deficits.

Addressing Winter Water Balance and Availability

One approach to evaluating plant water sources is by use of stable isotopes as a tracer or label. Elements typically have at least two stable isotopes in different

abundances that have allowed scientists to study biological, ecological, and environmental processes (Ehleringer and Rundel 1989). Hydrogen has two stable isotopes, ^{1}H and ^{2}H (also referred to as deuterium, D) with terrestrial abundances of 99.985 and 0.015 %, respectively (Dawson *et al*. 2002). Variation in stable isotope contents arise from kinetic (chemical), equilibrium (physical), or diffusive fractionation processes relating to the properties of the isotope in question (Ehleringer and Cerling 2002). Water does not generally experience kinetic fractionation as it is taken up by terrestrial plants, allowing plant water to have the same isotopic signal as the water source (Wershaw *et al*. 1966; Dawson and Ehleringer 1993). However, equilibrium fractionation occurs during leaf transpiration, which tends to enrich leaf water with ^{2}H (Marshall 2007). Terrestrial plants have naturally occurring $\delta^{2}H$ values typically ranging from +35 to -350 ‰ depending on latitude, elevation, continental position, season, and frequency of precipitation (Dawson and Siegwolf 2007).

Deuterium has been used to study many aspects of plant physiology, including but not limited to photosynthetic pathways (Ziegler *et al*. 1976; Sternberg 1986; Flanagan *et al*. 1991), evapotranspiration (Walker and Brunel 1990), ecosystem water vapor (Lai and Ehleringer 2011), nighttime transpiration (Dawson *et al*. 2007), ramet water movement (De Kroon *et al*. 1996; Matlaga and Sternberg 2009), and water uptake (Ehleringer and Dawson 1992; Corbin *et al*. 2005). Multiple studies have been conducted looking into the source of water uptake in plants, whether comparing isotopic signals of precipitation with soil moisture (Lin *et al*. 1996; Sugimoto *et al*. 2002; Gat *et al*. 2007; West *et al*. 2007; Xu *et al*. 2011) or within the vertical or horizontal profile of soils (Thorburn and Ehleringer 1995; Moreira *et al*. 2000; Sternberg *et al*. 2002; Schwinning *et al*. 2005; Ewe

et al. 2007). Several deuterium studies on redwood species have found evidence that foliar uptake is one strategy plants use to obtain water (Dawson 1998; Burgess and Dawson 2004; Limm *et al*. 2009).

THESIS OVERVIEW

The overall objective of my thesis is to examine two critical gaps in knowledge about tundra cold season processes – low temperature respiration and water uptake during winter-spring transition. My project addresses these needs by: 1) testing the limitations and rates of respiration of arctic tundra monoliths at low temperatures (Chapter 2); and 2) examining how arctic tundra plants manage water balance when snow covered and soils are frozen during the arctic winter-spring transition (Chapter 3). I hypothesize that:

1) Respiration rates of arctic tundra ecosystems continue at significant rates to soil temperatures as low as -15 °C,

2) Tundra plants are able to take up water during the winter-spring transition while under snow and soils are frozen.

Chapters 2 and 3 are written in manuscript format intended to be submitted for publication in peer-reviewed journals. The research presented here fills important gaps in our understanding of arctic tundra vegetation during cold season conditions. Chapter 2 presents the temperature response of ecosystem respiration down to temperatures as low as can be expected under snow-covered conditions (-15 °C), providing both above- and below-freezing Q_{10} responses, as well as developing parameters for the Arrhenius function. Chapter 3 demonstrates that plants are able to take up water under snow, possibly via roots but undoubtedly by foliar uptake. By furthering our knowledge of the temperature responses of ecosystem respiration at low temperatures, researchers will be

able to obtain improved model estimates of rates of CO_2 efflux during the cold season, thus improving annual carbon balance estimates. Increased biomass, carbon sources, and changes in species composition are all components that may significantly change as winter temperatures increase. By understanding the physiology of dominant species at low temperatures, researchers should be better able to predict how tundra communities may respond to changes in temperature and snow cover.

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Table 1. Summary of *in situ* ecosystem winter CO_2 efflux comparisons from literature.

Paper	CO_2 Efflux Comparison(s)	Location
Björkman *et al*. 2010a	date of measurement, site location, snow depth, soil temperature (5 and 10 cm, via graph comparison), vegetation type, winter average	Svalbard and Sweden
Björkman *et al*. 2010b	CO_2 measurement methodology, snow depth, date of measurement, winter average	Svalbard and Sweden
Elberling 2007	soil temperature (5 cm depth), date of measurement, winter average	Svalbard
Fahnestock *et al*. 1998	site location, snow depth, date of measurement, vegetation community, winter average	Alaska
Fahnestock *et al*. 1999	snow depth, tundra community type, winter average	Alaska
Grogan and Chapin 1999	site location, date of measurement, temperature (of what unclear), vegetation type	Alaska
Grogan and Jonasson 2005	experimental treatment, soil temperature (3-5 cm), date of measurement, winter average, month average	Sweden
Grogan and Jonasson 2006	site location, month of measurement, time since snow clearance, vegetation type	Sweden
Grogan *et al*. 2001	experimental treatment, soil temperature (3 cm), time since snow removal, winter average	Sweden

Larsen *et al*. 2007	month average, temperature (of what unclear)	Sweden
Morgner *et al*. 2010	date of measurement, experimental treatment, snow depth, soil temperature (2 cm, via graph comparison), surface soil temperature, time since ice layer break-through, vegetation type, winter average	Svalbard
Nobrega and Grogan 2007	winter average, experimental treatment	Canada
Oechel *et al*. 1997	date of measurement, experimental treatment, month average, season average, site location, soil surface temperature, time since initial measurement	Alaska
Oechel *et al*. 2000	date of measurement, winter average	Alaska
Panikov and Dedysh 2000	snow depth, winter sample incubation time	Siberia
Schimel *et al*. 2004	date of measurement, snow cover	Alaska
Sjögersten *et al*. 2008	experimental treatment, winter average	Norway
Sullivan *et al*. 2008	date of measurement, soil temperature (10 cm)	Alaska
Sullivan *et al*. 2010	date of measurement, soil temperature (10 cm)	Alaska
Walker *et al*. 1999	experimental treatment, vegetation type, winter average	Alaska

Welker *et al*. 2000	vegetation type, winter average	Alaska
Welker *et al*. 2004	experimental treatment, vegetation type, winter average	Canada
Zimov *et al*. 1993	air temperature (via graph comparison), date of measurement	Siberia
Zimov *et al*. 1996	air temperature (via graph comparison), date of measurement	Russia

Table 2. Summary of minimum soil temperature, soil depth, and CO_2 efflux of *in situ* ecosystem winter CO_2 efflux measurements from literature. All soil temperatures and CO_2 effluxes were estimated from figures.

Paper	Minimum Soil Temperature (°C)	Soil Depth (cm)	CO_2 efflux ($\mu mol\ m^{-2}\ s^{-1}$)
Björkman *et al*. 2010a	-21	5	0.00568*
Elberling 2007	-13	5	0.0750
Grogan and Jonasson 2005	-9	3-5	0.121*
Grogan *et al*. 2001	-8.6	3	0.0772*
Larsen *et al*. 2007	-7	unclear	0.125
Morgner *et al*. 2010	-23	2	0.0625
Oechel *et al*. 1997	-25	surface	0.121*
Sullivan *et al*. 2008	-15	10	0.0603*
Sullivan *et al*. 2010	-2	10	0.125

*Recalculated from original publication into units of $\mu mol\ m^{-2}\ s^{-1}$

CHAPTER II. ECOSYSTEM RESPIRATION RATES OF ARCTIC TUNDRA AT LOW TEMPERATURES

INTRODUCTION

Although the cold season in the Arctic is three times longer than the growing season, little research on arctic plants and tundra has been conducted during the cold season (winter-spring), when the largest changes are expected to occur with climate warming (Chapman and Walsh 2007; Christensen *et al*. 2007). The primary emphasis of cold season research has been on rates of winter ecosystem respiration (ER) and to develop estimates of annual carbon balance. The majority of all winter CO_2 flux measurements during the winter or at winter temperatures indicate that some amount of respiration is occurring, and while low, may contribute substantially to the annual carbon balance because of the length of the cold season. In arctic locations, accumulation of respiration during the cold season has the potential to shift the carbon balance to a source (Zimov *et al*. 1996).

The cold season in the Arctic extends over eight to nine months during which air temperatures often reach as low as -40 °C. However, as a result of the insulating layer created by snow cover, these extreme temperatures are rarely reached at the soil surface and plant level. Under the snow, temperatures seldom fall below -15 °C (Toolik Environmental Data Center Team 2009-2011). Physiological processes of tundra plants under the snow during the arctic winter remain largely unknown. Vegetation clipping experiments suggest a large fraction of winter respiration is from aboveground plant biomass (Grogan *et al*. 2001).

Methodologies of CO_2 measurement during/in the harsh winter environment range from infrared gas analyzers with static chambers, determination of subnivean CO_2 concentrations for application in diffusion models, alkali CO_2 absorption, syringe extractions with gas chromatographs, and subnivean CO_2 sensors. Most of these methods have serious limitations. As a result, estimates of winter ecosystem respiration rates are highly variable. One issue is that ecosystem CO_2 production may be temporally disconnected to CO_2 release to the atmosphere by resistance created by snow cover (Jones *et al*. 1999) or impermeable barriers produced by soil surface ice layers (Björkman *et al*. 2010). Another important issue is the performance of electronics at very lower temperatures for measurements of very low rates. Several cold-temperature CO_2 methods have employed syringe extraction techniques (Zimov *et al*. 1993; Zimov *et al*. 1996; Panikov and Dedysh 2000) thereby eliminating the need to expose electronics to the severe cold. Of existing wintertime CO_2 studies, few are directly comparable. Studies differ in site location, vegetation community, snow depth, experimental treatment, and soil temperature. In the few *in situ* ecosystem studies of winter CO_2 efflux measurements that report both soil temperatures and soil depths, minimum soil temperatures vary from -2 to -25 °C and associated soil depths range from 0 to 10 cm. Björkman *et al*. (2010) summarized research on winter CO_2 rates, methodologies, habitats, and locations and felt that variation in CO_2 efflux rates resulted primarily from different techniques. Several of the studies lacked CO_2 efflux rates at subfreezing temperatures, did not report the depth at which the associated soil temperatures were recorded, or present data that have low replication or even data obtained from a single sensor. Few present responses with temperatures that would be amenable to application within ecosystem models.

The objective of my study was to determine the response of arctic tundra ER to a range of temperatures from moderate temperatures above-freezing (15 °C) to as low as can be expected under snow-covered conditions (-15 °C).

By furthering our knowledge of the temperature responses of ecosystem respiration at low temperatures, researchers will able to obtain improved model estimates of rates of CO_2 efflux during the cold season, thus improving annual carbon balance estimates.

METHODS AND MATERIALS

Sample Collection and Culture

Tundra monoliths (blocks of intact soil and vegetation) used for this study were collected near Toolik Field Station from moist acidic tundra (Viereck *et al*. 1992; Walker *et al*. 1994; Hobbie *et al*. 2005). The vegetation of the monoliths was cotton grass (*Eriophorum vaginatum)* tussock tundra with (*Carex bigelowii*), evergreen shrubs (mainly *Andromeda polifolia*, *Cassiope tetragona*, *Ledum palustre*, *and Vaccinium vitis-idaea* with *Empetrum nigrum* and *Pyrola grandiflora*), deciduous shrubs (mainly *Betula nana* and *Salix pulchra* with *Vaccinium uliginosum*), forbs (*Saxifraga cernua* and *Stellaria laeta*), and mosses (primarily *Sphagnum* spp., *Hylocomium splendens*, *Aulacomnium turgidum*, and *Dicranum* spp.). The climate at the site is continental arctic, with mean monthly temperatures ranging from -22.5 (January) to 11.2 ºC (July), (Toolik Environmental Data Center Team 1997-2000). Three hour moss surface and 5 cm soil depth temperatures from the Arctic LTER (posted on the Toolik Field Station Weather Station Data Query website) during 2009 through 2011 was averaged every 24 hour time step and graphed (Figure 1, Toolik Environmental Data Center Team 2009-2011). The

soils at the site of collection are Pergelic Cryaquepts (Genet *et al*. 2012). The six tundra monoliths used in this study were collected in July of 2009, 2010, or 2011 during peak permafrost thaw. Monoliths (average height and area of 12 cm and 915 cm^2, respectively), were collected to approximately 15 cm depth below the top of the moss layer down to mineral soil. Samples were either express shipped or brought directly back to Florida International University in luggage. Monoliths were immediately placed in opaque rectangular tubs in growth chambers (Environmental Growth Chambers GC-2H Plant Chamber) set at 15 °C with 100 % lighting and 24 hr photoperiod. Samples were watered twice weekly with deionized water so that a small amount of standing water was present at the bottom of the tubs (1-2 cm). The day prior to CO_2 measurements, plants were well-watered and soil moisture (0-5 cm depth) was monitored with an EC-5 sensor (Decagon Devices, Pullman WA). At least 2 months before measurements were begun, growth chamber settings were changed to 15/10 °C day/night temperatures with 12 hr daylight at 50 % full lighting at 15 °C and darkness at 10 °C to cold harden the monoliths. Chambers were set to ramp between temperature set points. Light levels in the chambers, which were measured with a quantum sensor LI-190SA and read with a LI-1400 datalogger (LI-COR, Lincoln, NE), were 310 and 135 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) at the canopy surface for 100 and 50 % lighting, respectively.

Carbon Dioxide Efflux

Measurements of CO_2 efflux were made using static chamber methods whereby the rate of change in the concentration of CO_2 within a chamber enclosing a tundra monolith was used to determine the CO_2 efflux rate. Concentrations of CO_2 were

measured from samples taken by syringe and injected into an infrared gas analyzer (IRGA, Ehleringer and Cook 1980). By using syringe samples rather than a closed-loop transient or differential IRGA system, we avoided exposing the gas analyzer to very low temperatures or warming the air in the chamber by cycling through an IRGA.

The measurement process consisted of monolith temperature incubation and stabilization, IRGA calibration, chamber gas extraction, and gas injection into the IRGA gas line. Prior to the first measurement at a target temperature, the monolith was placed in the chamber (previously kept at the same target temperature). When the internal monolith temperature had stabilized, the Luer-Lock extraction point was sealed and the chamber covered with a black cloth to ensure darkness during extractions. The monolith temperature was again monitored until the temperature had stabilized again.

Incubation Chamber

A five-sided polycarbonate chamber (36 x 40 x 36 cm, total volume 66,240 cm^3) was designed to enclose the monoliths and the plastic tub in which they were established. The chamber contained two fans in opposite corners to ensure internal chamber air mixing, a coil of Excelon Bev-A-Line® tubing (Thermoplastic Processes, Stirling, NJ) vented to the outside to equalize pressure resulting from syringe extractions, and an extraction point with Luer-Lok fitting inserted into the chamber for Luer-Lok cap seal and Luer-Lok syringe connectivity. For measurements, the tub containing a monolith was placed on aluminum braces within a 51 x 43 x 5 cm polycarbonate tray filled to 1 cm depth with 100 % antifreeze. The five-sided polycarbonate chamber was then placed over the monolith with a seal created by the antifreeze in the bottom of the polycarbonate tray.

Infrared Gas Analyzer Gas Line

An infrared gas analyzer (LI-6262 CO_2/H_2O, LI-COR, Inc, Lincoln, NE) was set up in absolute mode for CO_2 concentration measurement (Ehleringer and Cook 1980). A 6262-04 auxiliary pump installed with a Gelman Acro® 50 Filter and soda lime (CO_2) and Drierite (H_2O) scrub cycled zeroed gas through the IRGA reference line. The gas flowing through the sample line was Ultra High Purity N_2 that had passed through a soda lime and Drierite scrub and a Gelman Acro® 50 Filter. Flow rates were measured with a rotameter (Dwyer Instruments, Michigan City, IN), and a three-way Luer-Lok stopcock was used as the injection site. Excelon Bev-A-Line® was used for all tubing.

Carbon Dioxide Measurements

Measurements on tundra monoliths previously collected took place from May 2012 through July 2012 and were conducted at seven target temperatures: 15 to -15 °C at 5 °C intervals. The IRGA was calibrated each day of measurements, prior to the first chamber CO_2 measurement, with 450 ppm $\pm$ 1 % CO_2 calibration gas. A Becton-Dickinson 3 cc Luer-Lok Precisionglide sterile syringe with marked 0.1 cc increments was used for calibrations as well as extractions and injections of chamber air for concentration measurements. Volumes from 0.5 to 3.0 ml at 0.5 ml intervals were extracted from the calibration gas at 0.5 l min^{-1} and injected into the IRGA gas line. The IRGA gas line flow was maintained at 0.75 l min^{-1}.

The integrated areas under the peaks resulting from the different volumes of CO_2 were calculated using the peak integration function of the IRGA with a 0.5 ppm activation threshold. The IRGA outputs were captured by computer using the RS-232 port with data output set to 1 s, and data were collected via HyperTerminal (Hilgraeve

Inc., Monroe, MI). Peak integration and volumes were used to calculate the calibration equation relating peak height to concentration of carbon dioxide.

For measurement of change in chamber CO_2 concentrations, five gas samples were collected over time for each of the seven target temperatures. Periods between samplings ranged from 30 to 180 min, with longer periods used for lower temperatures (Table 1); see Figure 2 for an example of one monolith temperature series. Multiple extractions of 1.5 ml were taken from the chamber at each sample interval and injected one at a time into the IRGA gas line after temperature stabilization of the tundra monolith. Peak integration values for chamber extraction ranged 38.957 to 120.083 units. The IRGA sometimes logged two peak integration data points for the same injection instead of single peak, with the first data point lasting ~1 s and ranging from 0.5 to 6.0 units and the other data point being several units less than the expected range for integrations. In these cases, the two points were combined to give a total injection peak integrated area. All peak values were filtered to exclude values exceeding one standard deviation to better elucidate linear trends in the data.

Temperature Measurements

Tundra monoliths were placed in an EGC-2H growth chamber for target temperatures 15, 10, and 5 °C, while target temperatures 0, -5, -10, and -15 °C were conducted in a chest freezer (Kenmore Model 1654) regulated by a temperature controller (Ranco Model ETC-111000) with a 3 °C temperature differential. Monoliths were incubated at their target temperature until the monolith temperature, 3 cm beneath the tundra surface, was stable. Monolith temperature was measured as the mean of three thermoplastic-insulated copper-constantan thermocouples placed throughout the monolith

at 3 cm depth. A separate thermocouple measured chamber air temperature approximately 5 cm above the monolith surface. All thermocouples were connected to a CR23X datalogger (Campbell Scientific, Inc, Logan, UT) scanning at 5 s intervals and logging temperatures as 1 min averages. Measurements of ER were made from high to low temperatures. Upon placement in a new, lower temperature regime (prior to incubation chamber placement and measurements), monolith soil temperature began to immediately decline for all target temperatures except for -5 °C, which took between 30 to 50 hr before a decrease in soil temperature began (Figure 3). For the growth chamber measurements, air temperatures varied only slightly ($\pm$ 0.25 to $\pm$ 0.50 °C) around the set point. However, for the freezer-based measurements with the 3 °C differential set by the controller, air temperatures varied slightly more ($\pm$ 0.63 to $\pm$ 0.75 °C). The soil temperature variation at 3 cm depth was about half that of air temperature, with variations of $\pm$ 0.13 to $\pm$ 0.19 °C and $\pm$ 0.13 to $\pm$ 0.44 °C for growth chamber and freezer-based measurements, respectively.

Normalizing Monoliths for Comparisons

Monolith effluxes are presented on a per unit surface area basis. To test if variation in CO_2 fluxes among the monoliths was related to differences in soil depth or vegetation, curves were derived from monolith volume, normalized difference vegetation index (NDVI), and sum percent plant cover. Normalized difference vegetation index, a reflectance metric for live green vegetation, was measured on all monoliths using a Unispec-SC spectroradiometer (PP Systems, Haverhill MA). Sum percent plant cover was determined for each monolith using a 5 cm x 5 cm cell quadrat (25 cm × 30 cm; 0.075 m^2) to estimate percent plant cover of vascular species and growth forms

(graminoids, evergreen shrubs, deciduous shrubs, forbs, and moss) as modified from Laidler *et al*. 2008.

Data Analysis

A unique calibration equation calculated for each individual target temperature measurement was determined using a linear trendline in Microsoft Excel (Part of Microsoft Office Professional Edition), Release Version 14.0 (© Microsoft, Inc., 2011, Redmond, WA). The calibration equation was then used to estimate the CO_2 concentration at each of the five samplings for each temperature. The average change in CO_2 concentration was used to estimate the monolith ecosystem respiration efflux rate by Equation (1).

$$ER = \frac{10VP_0}{RS(T_0 + 273.15)}\left(\frac{dC'}{dt}\right) \quad (1)$$

where ER is the soil CO_2 efflux rate (μmol m^{-2} min^{-1}), V is volume (cm^3), P_0 is the initial pressure (kPa), R is the gas constant (8.314 Pa m^3 K^{-1} mol^{-1}), S is soil surface area (cm^2), T_0 is initial air temperature (°C), and dC'/dt is the initial rate of change in CO_2 mole fraction (μmol mol^{-1} min^{-1}) (LI-COR Biosciences 2010).

A temperature response curve was graphed for ER as calculated from Equation 1. Additional curves were fit based on ecosystem respiration CO_2 efflux and monolith volume, NDVI, and sum percent plant cover. Linear regressions using PASW Statistics 18, Release Version 18.0.0 (© SPSS, Inc., 2009, Chicago, IL) were used to compare all raw CO_2 rates with monolith volume, NDVI, and sum percent plant cover for each target temperature. The vegetation of one of the monoliths died after the -15 °C measurement but prior to NDVI measurement, and was excluded from NDVI comparisons.

Carbon dioxide efflux at temperatures 15 to -15 °C at 5 °C intervals were fit to the Arrhenius equation (Equation 2) in Microsoft Excel, Release Version 14.0 using the linear relationship between the natural logarithm of CO_2 efflux and the inverse temperature to estimate activation energy and the pre-exponential factor. The Arrhenius equation is derived as:

$$ER = Ae^{\frac{-E_a}{RT}} \quad (2)$$

where A is the pre-exponential factor, E_a is the activation energy, R is the universal gas constant (8.314×10^{-3} J $mol^{-1}K^{-1}$), and T is the temperature (K) (Fang and Moncrieff 2001).

Ecosystem respiration effluxes derived from the Arrhenius equation at temperatures 15 to -15 °C at 5 °C intervals were used to calculate Q_{10} values between every 10 °C change in temperature for the range of temperatures measured in this study (15 to -15 °C at 5 °C intervals). The Q_{10} equation is calculated as:

$$Q_{10} = \frac{K_{t+10}}{K_t} \quad (3)$$

where Q_{10} is the increase in reaction rate per 10 ° increase in temperature, K_t is the rate of reaction at temperature t, and K_{t+10} is the rate of reaction at temperature 10 ° greater than temperature t (Winkler *et al*. 1996).

RESULTS

Carbon dioxide efflux rates were collected from six tundra monoliths at soil equilibrium temperatures from +15 to -15 °C at 5 °C intervals. Calibrations for all tests were linear, and r^2 values averaged >0.99 (SE = $3.21 \cdot 10^{-4}$). Raw rates of CO_2 increase in

the chamber ranged from 0.038 to 4.037 ppm min^{-1} with standard errors of 0.010 to 0.224, respectively (Table 2). The average r^2 for the increase in CO_2 concentration with time for the equilibrium temperatures ranged from 0.98 (± SE 0.004) at +15 °C to 0.47 (± SE 0.089) at -15 °C (Table 2). Low r^2 values at low temperatures are the result of very low slopes rather than variability of linear fits (Figure 2).

Carbon dioxide efflux calculated on an area basis using monolith area and chamber volume (Equation 1) showed an exponential response and closely fit a second order polynomial, $r^2 = 0.97$ (Figure 4). The linear relationship between the natural logarithm of corrected CO_2 efflux and inverse temperature as defined by the Arrhenius equation (Figure 5) was used to estimate the pre-exponential factor and activation energy constants, $1.44737 \cdot 10^{19}$ and 103.2 kJ, respectively. A plot of the natural logarithm of respiration efflux vs. inverse temperature shows a break in the relationship above-freezing (Figure 6).

The Q_{10} values of tundra monoliths at 10 °C intervals over the range of soil equilibrium temperatures ranged from 4.77 to 6.10 (Table 3). The Q_{10} values decreased with increasing temperature, with the lowest 10 °C interval having the highest value.

Temperature response curves derived from monolith volume, NDVI, and sum percent plant cover can be viewed in Figures 7, 8, and 9. The fit to the response expressed on a volume basis (polynomial fit $r^2 = 0.97$) was slightly less than for area. The fit for NDVI-derived fluxes (polynomial fit $r^2 = 0.95$) was marginally lower than those expressed on an area and volume basis. The fit of the response to sum percent plant cover (polynomial fit $r^2 = 0.98$) barely exceeded that of area.

Linear regressions comparing all the raw rate of change of CO_2 concentration with monolith volume, NDVI, and sum percent plant cover for each target temperature Tables 4, 5, and 6, respectively, were only significant at 15 °C for sum percent plant cover (Figure 10).

A summary of percent plant cover for vascular species and growth forms of each monolith is provided in Table 7. Graminoids and mosses comprised approximately 36 and 35 %, respectively, of the cover for the six monoliths used in this study, followed by evergreen shrubs (24 %), with few deciduous shrubs (3 %) and forbs (2 %).

DISCUSSION

The temperature response of area-derived ER of tundra monoliths (Figure 4) showed a clear exponential increase as temperature increased, with a very high second order polynomial r^2 (0.97). The response also fit the Arrhenius function very well (Figure 5).

Values of Q_{10} estimated from the Arrhenius fit of the data (Table 3) were considerably higher (4.77 to 6.1) than the standard value of 2 usually associated with biological reactions. Raich and Schlesinger (1992) reviewed Q_{10} values for soil respiration, a major component of ER, and found that most fell within the range of 1.3-3.3, which is the same range that many plant processes fall in as well (Ryan 1991). Previous research on arctic tundra soils have calculated Q_{10} values between 1.8 and 3.6 for temperatures above 0 °C (Elberling 2003; Elberling and Brandt 2003; Elberling 2007; Morgner *et al*. 2010), corroborating Raich and Schlesinger's (1992) range. However, Mikan *et al*. (2002) reported significantly higher Q_{10} values of 4.6 to 9.4. Values of Q_{10} reported for below-freezing temperatures are highly variable, with values as low as 3

(Morgner *et al*. 2010), increasing to 7.1 (Elberling 2007), 8.5 (Panikov *et al*. 2006), 18.2 (Sullivan *et al*. 2008), 50.8 (Elberling and Brandt 2003), and as high as 237 (Mikan *et al*. 2002). The high Q_{10} values of soil respiration at low temperatures indicate that small increases in temperature during the cold season will have greater proportional effects than increases during the growing season (Schleser 1982 cited in Raich and Schlesinger 1992). The Q_{10} of 6.0 in my research translates to a ~20 % increase in ER for a 1 °C increase in temperature. Temperatures in the North Slope of Alaska have already increased 2.9 °C since 1976 (Wendler *et al*. 2010). A Q_{10} of 6.0, assuming no acclimation of respiration rates to temperature, implies that respiration rates currently are 70 % higher than 35 years ago. However, because most of the temperature increase in the last 35 years has been for winter months, when respiration rates are very low, the absolute increase in respiration is likely relatively small, so far.

Most studies providing Q_{10} values for tundra soil respiration have used some form or derivation of the Arrhenius equation. The use of the Q_{10} and Arrhenius functions in this paper follows the accepted application characteristic of ecosystem and soil respiration research. Lloyd and Taylor (1994) and Fang and Moncrieff (2001) point out estimation bias and underestimate limitations of the current Arrhenius equations applied to soil respiration, but consistent and widely used alternatives are still lacking. Menzinger and Woldgang (1969) cautioned that the Arrhenius equation overestimates the activation energy constant; however, alternatives are overcomplicated and inconsistent. There are both short- and long-term temporal concerns for the use of Q_{10} values to describe temperature responses of respiration, including the short-term deviation of the respiration rate from a simple exponential function and possible long-term temperature

response shifts resulting from temperature acclimation (Tjoelker *et al*. 2001; Atkin and Tjoelker 2003). The equilibrium of air and soil temperatures and the relatively short sampling times (ranging 2 to 12 hours) in my research help to minimize short- and long-term concerns that could arise from temperature response data.

The high Q_{10} values from other low temperature studies and this project suggest the presence of a threshold for ecosystem and soil respiration at the freezing point. Mikan *et al*. (2002) found that the exponential parameter and activation energy constants for his data were significantly different at above- vs. below-freezing temperatures and calculated different constants for temperatures 0.5 to 14.0 °C and -0.5 to -10 °C. While the data reported here closely fit the linearized Arrhenius function (Figure 5), a plot of the natural logarithm of respiration vs. inverse temperature show a break in the relationship above-freezing (Figure 6), similar to that found in Mikan *et al*. (2002). Consistent with this difference, the Q_{10} values here were also higher at temperatures below-freezing than above-freezing (Table 3).

Multiple hypotheses have been suggested to explain low respiration rates and high Q_{10} values for soils at and below-freezing temperatures. Elberling and Brandt (2003) suggested that the onset of ice reduced CO_2 release by trapping CO_2 in frozen soil. Mikan *et al*. (2002) argued the diffusion processes of substrates, nutrients, and waste products were all adversely indirectly affected by temperature via the unfrozen water content of soils. The more widely accepted explanation for decreased respiration from soils at temperatures below 0 °C is the effect of soil water availability and moisture limitations on microbial activity (Lloyd and Taylor 1994, Mikan *et al*. 2002; Davidson *et al*. 2006; Panikov *et al*. 2006). Freezing is known to affect numerous soil microbial

properties including community composition (Lloyd and Taylor 1994), growth of microbial populations and root tissues (Davidson *et al*. 2006), kinetic energy of reactants (Panikov *et al*. 2006), enzymatic respiratory activity (Davidson *et al*. 2006), diffusion of O_2 and soluble carbon substrates across cellular membranes (Davidson *et al*. 2006), extracellular barriers to diffusion (Mikan *et al*. 2002), and intracellular desiccation (Mikan *et al*. 2002).

While this research provides precise ecosystem CO_2 efflux rates at equilibrium for temperatures as low as -15 °C, laboratory experiments have limitations compared to *in situ* measurements. Unlike natural temperature conditions for arctic tundra, both air and soil temperatures in these experiments were in equilibrium across the full temperature range. Under natural conditions, especially in the absence of snow cover, soil and canopy temperatures are often not the same, with soil temperatures often colder than surface temperatures in the fall before the development of snow cover and warmer in the spring than surface temperatures after snowmelt. The data from the current experiment are representative of the field conditions at low temperatures during winter with deep snow cover when canopy and soil temperatures are most similar. These conditions were the highest priority of this study, as at winter temperatures field measurements are often problematic as a result of low temperature operation limitations of the analytical devices used to measure CO_2. Furthermore, these results were not complicated by possible CO_2 storage below snow, ice, or frozen soils layers.

During the onset of winter, the liquid to solid phase change releases energy in the form of latent heat that results in a warming of soil (Luo *et al*. 2002; Zhang and Sun 2011). The postponement to decrease temperature during the -5 °C equilibrium for all

monoliths (ranging 30 to 50 hr, Figure 3), clearly displays this same release of latent heat as a resistance to temperature change at subzero temperatures. For Toolik tundra, this period may last or exceed a month (see September of 2009, 2010, and 2011 in Figure 1). Under these conditions, the relationship between air and soil temperature is temporarily disrupted, which may strongly affect the contribution of above- and below-ground components to ecosystem respiration.

While the response of the monoliths presented very similar responses to temperature, some variation in ER of the monoliths was present, possibly as a result of differences in the amount of soil volume or green plant biomass. When rates were normalized to monolith volume (Figure 7) to account for soil depth, the correlation was only slightly below area-based normalization (Figure 4), indicating that depth of tundra soil may contribute to ecosystem respiration. Rates normalized to NDVI (Figure 8) were marginally below volume correlations, and sum percent plant cover rates (Figure 9) faintly exceeded area, suggesting above-ground vegetation components may also affect tundra ecosystem respiration. Area, volume, NDVI, and sum percent plant cover relationships indicate that both above- and below-ground factors are important drivers of variation in ER across the full temperature range. These findings somewhat support Grogan *et al*. (2001) above-ground vegetation removal experiments that indicated a large fraction of winter respiration is from aboveground plant biomass. My results suggest that above- and below-ground components are equally significant concerning cold season ecosystem respiration.

CONCLUSION

The research of tundra ecosystem CO_2 efflux in this study provides some of the first precise temperature response data for Arctic tundra at low temperatures. The temperature response was exponential and best fit by a second order polynomial. The Q_{10} values were generally higher than typically found in soil and plant respiration, and were inversely related to temperature. These results translate into a ~20 % increase in ER for a 1 °C increase in temperature. Temperatures in the North Slope of Alaska have already increased 2.9 °C since 1976 (Wendler *et al*. 2010), which implies that ecosystem respiration rates could be 70 % higher now than before temperature warming. Carbon dioxide efflux was best explained by monolith surface area and sum percent plant cover; however, monolith volume and NDVI were only marginally different than area and sum percent plant cover. The equal significance of both above- and below-ground components suggests that further research is required to determine which components will contribute most to cold season ecosystem respiration.

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Table 1. Time intervals between the five extractions at target temperatures.

Target Temperature (°C)	Extraction Interval (min)
15	30
10	30
5	30
0	60
-5	120
-10	120
-15	180

Table 2. Mean slope and slope r^2 of raw rate of CO_2 increase vs. time for each temperature.

Target Temperature (°C)	n	Mean Slope	SE	Mean r^2	SE
15	6	4.037	0.224	0.98	0.004
10	6	2.729	0.181	0.96	0.017
5	6	1.695	0.092	0.92	0.020
0	6	0.921	0.093	0.90	0.024
-5	6	0.235	0.045	0.71	0.141
-10	6	0.131	0.012	0.51	0.145
-15	6	0.038	0.010	0.47	0.089

Table 3. The Q_{10} values as calculated from data fit to the Arrhenius equation.

Q_{10} Temperature (°C)	Q_{10}
15, 5	4.77
10, 0	5.04
5, (-5)	5.35
0, (-10)	5.70
(-5), (-15)	6.10

Table 4. Linear regressions for raw rate of CO_2 increase vs. monolith volume for each target temperature.

Treatment	n	r^2	Slope	*p*	*t*	df
15	6	0.45	$4 \cdot 10^{-6}$	0.145	1.80	4
10	6	0.33	$3 \cdot 10^{-6}$	0.232	1.41	4
5	6	0.33	$1 \cdot 10^{-6}$	0.234	1.40	4
0	6	0.13	$8 \cdot 10^{-7}$	0.484	0.77	4
-5	6	0.21	$5 \cdot 10^{-7}$	0.358	1.04	4
-10	6	0.18	$-1 \cdot 10^{-7}$	0.402	-0.94	4
-15	6	0.0035	$2 \cdot 10^{-8}$	0.912	0.12	4

Table 5. Linear regressions for raw rate of CO_2 increase vs. monolith NDVI for each target temperature.

Treatment	n	r^2	Slope	*p*	*t*	df
15	5	0.23	0.0534	0.414	0.95	3
10	5	0.18	-0.0553	0.473	-0.82	3
5	5	0.036	-0.0122	0.760	-0.34	3
0	5	0.46	-0.0365	0.210	-1.59	3
-5	5	0.48	0.0195	0.195	1.66	3
-10	5	0.47	0.00442	0.199	1.64	3
-15	5	0.59	0.00504	0.129	2.08	3

Table 6. Linear regressions for raw rate of CO_2 increase vs. monolith sum percent plant cover for each target temperature.

Treatment	n	r^2	Slope	*p*	*t*	df
15	6	0.69	$9 \cdot 10^{-4}$	0.0416	2.96	4
10	6	0.075	$2 \cdot 10^{-4}$	0.599	0.57	4
5	6	0.13	$1 \cdot 10^{-4}$	0.474	0.79	4
0	6	$7.5 \cdot 10^{-5}$	$-3 \cdot 10^{-6}$	0.987	-0.02	4
-5	6	0.49	$1 \cdot 10^{-4}$	0.124	1.94	4
-10	6	0.16	$1 \cdot 10^{-5}$	0.425	0.89	4
-15	6	0.0066	$2 \cdot 10^{-4}$	0.637	0.48	4

Table 7. Summary of percent plant cover for vascular species and growth forms of each monolith.

Growth Form	Species	Monolith					
		1	2	3	4	5	6
		Percent Plant Cover					
Graminoid		56	56	48	71	40	32
	Carex bigelowii	0	0	0	14	12	27
	Eriophorum vaginatum	56	56	48	57	28	6
Evergreen Shrub		20	44	40	27	33	45
	Andromeda polifolia	0	4	10	5	11	21
	Cassiope tetragona	0	11	14	13	14	19
	Empetrum nigrum	0	12	0	0	0	0
	Ledum palustre	13	3	3	3	1	0
	Pyrola grandiflora	0	0	0	0	1	1
	Vaccinium vitis-idaea	6	15	13	6	7	4
Deciduous Shrub		0	0	1	4	3	17
	Betula nana	0	0	1	4	0	5
	Salix pulchra	0	0	0	0	3	12
	Vaccinium uliginosum	0	0	0	0	0.3	0
Forb		0	0	6	1	9	0
	Saxifraga cernua	0	0	0	1	0	0
	Stellaria laeta	0	0	6	0	9	0
Moss		58	34	61	37	62	51

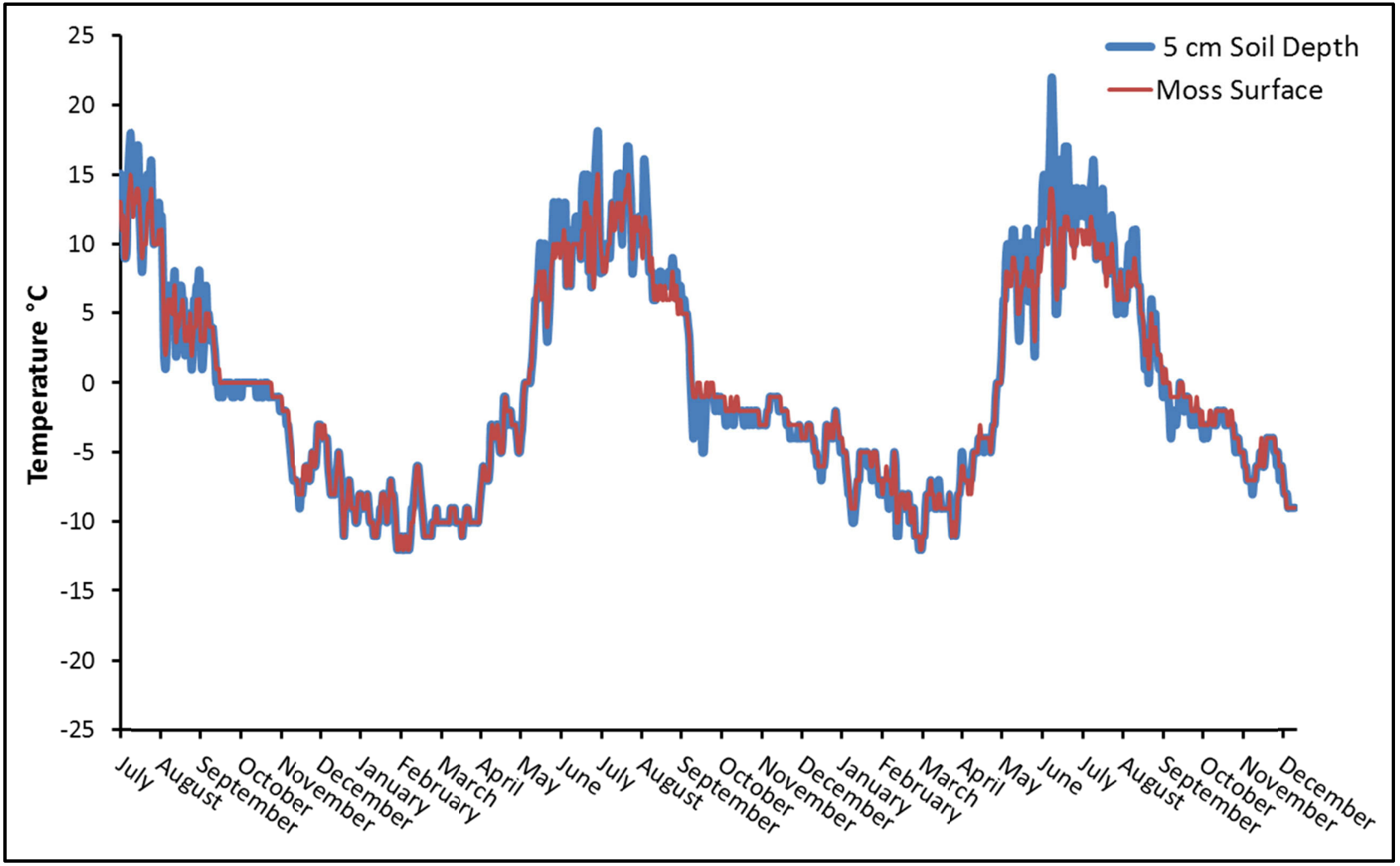

Figure 1. Moss surface and 5 cm soil depth temperatures at Toolik Lake from July 2009 through December 2011.

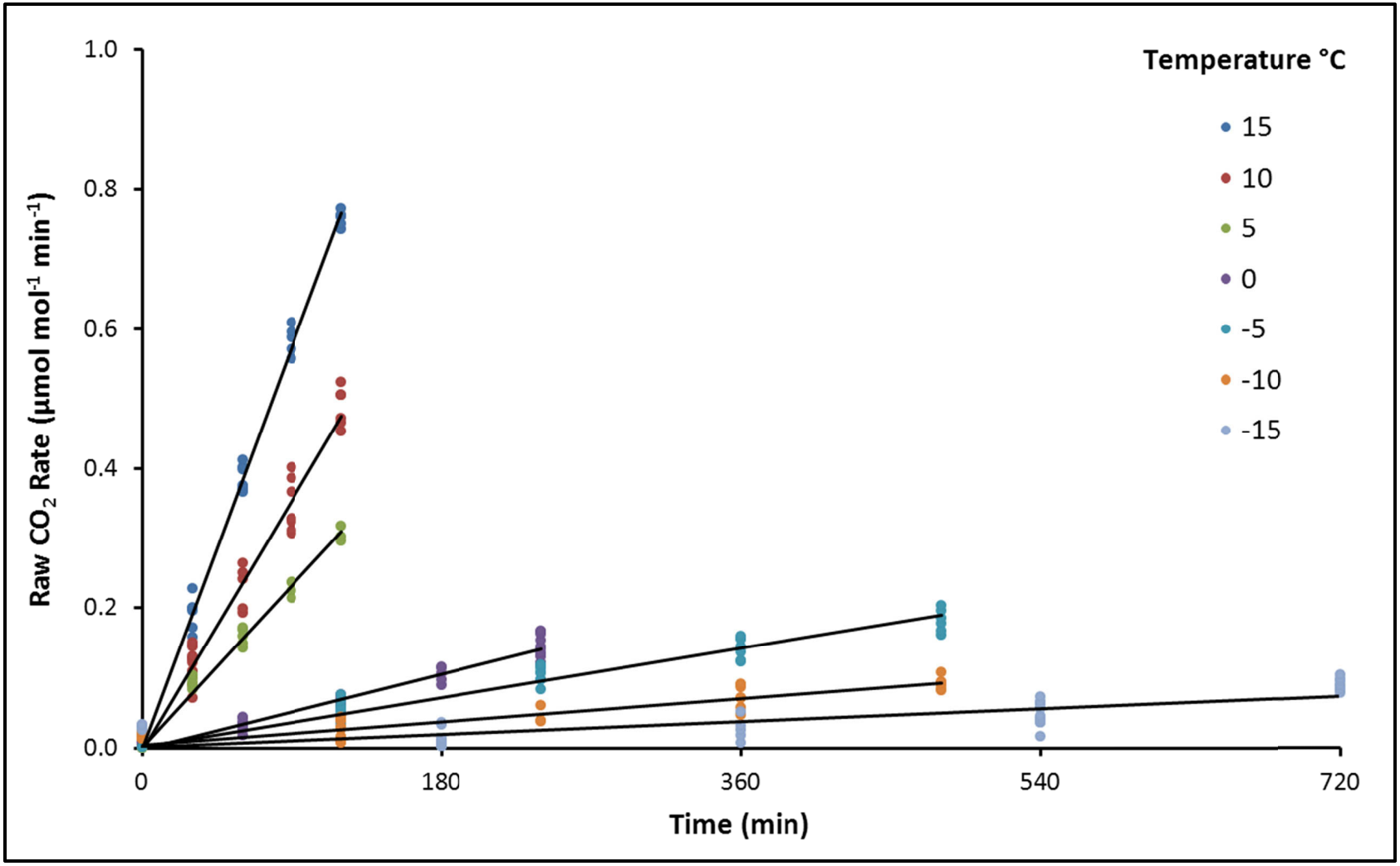

Figure 2. Carbon dioxide concentration accumulation lines for the seven target temperatures for one monolith. Lines are normalized to have a zero y-intercept.

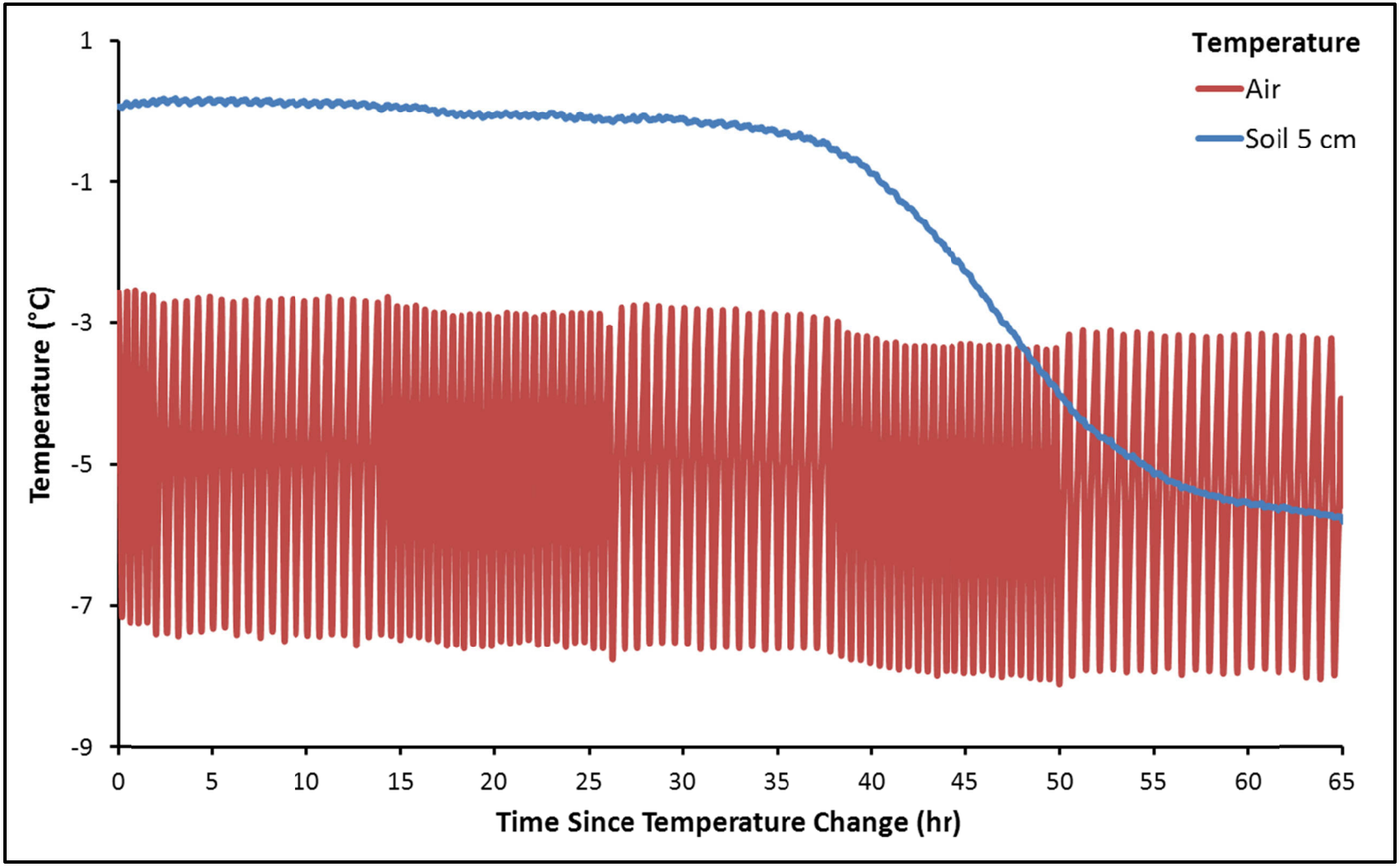

Figure 3. Example of the time series of soil and air temperatures moving toward equilibrium in response to the change from 0 to -5 °C for one monolith.

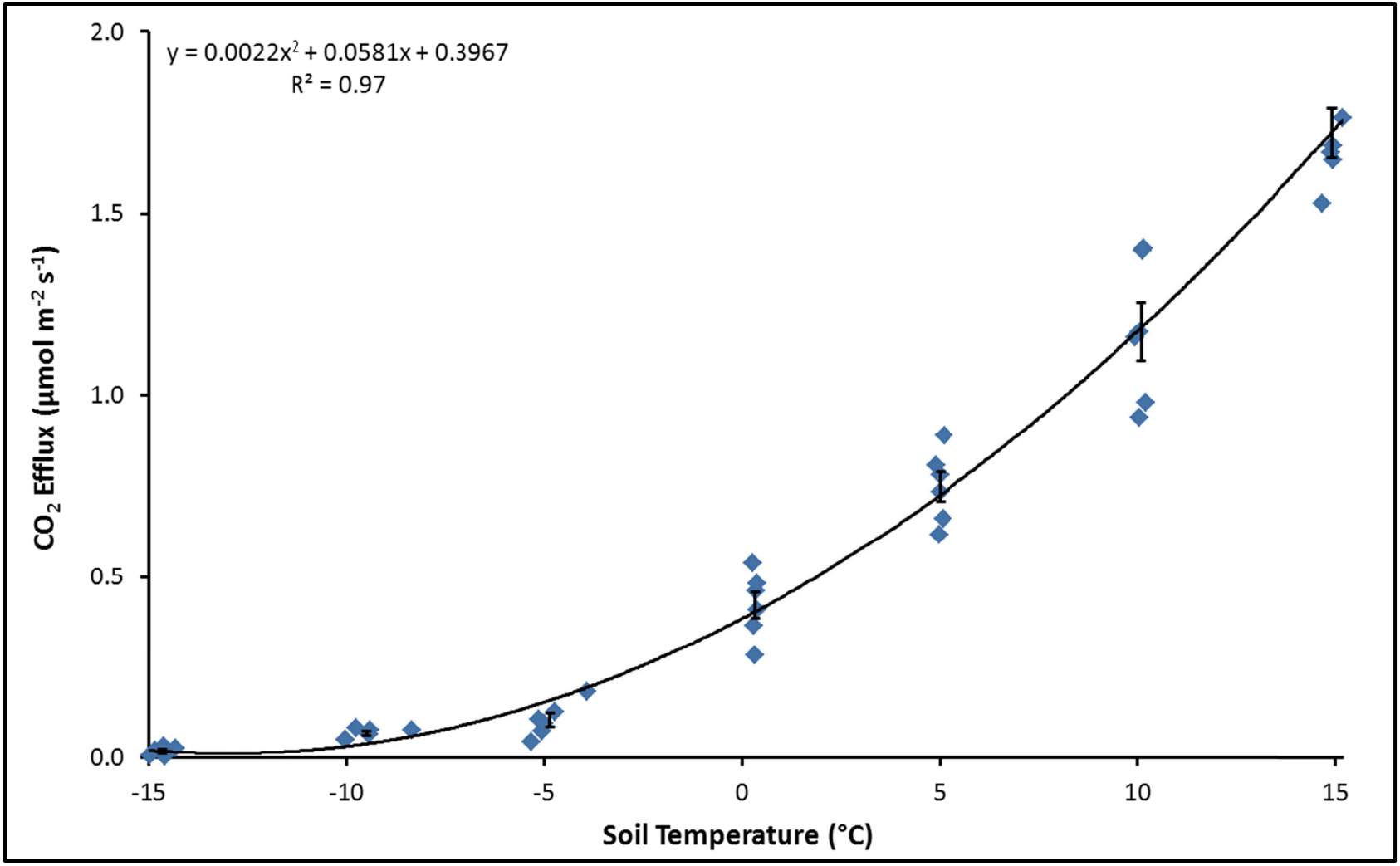

Figure 4. Polynomial fit of CO_2 efflux per unit surface area. Error bars ± 1 SE.

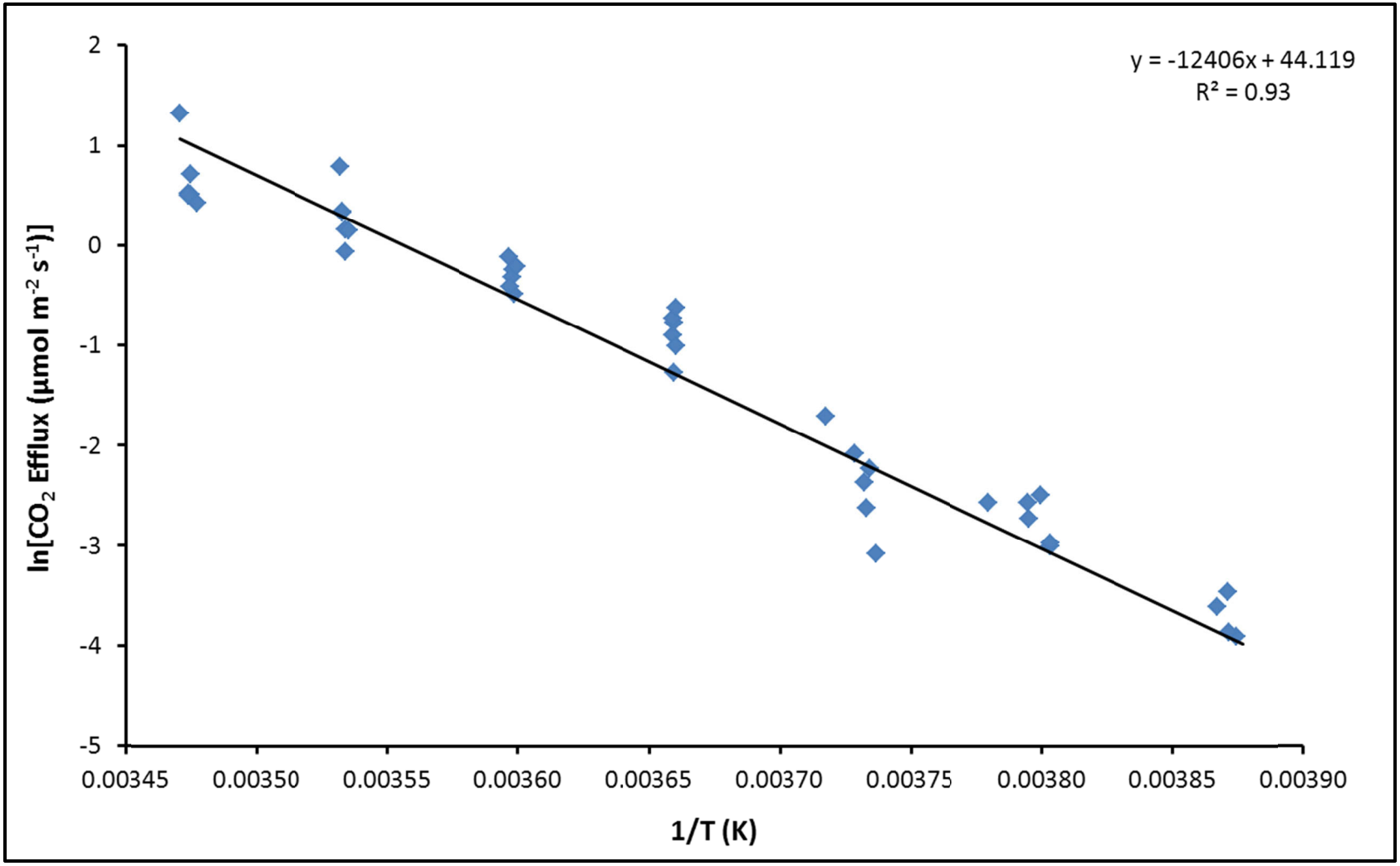

Figure 5. Linearized Arrhenius plot using all data (natural logarithm of CO_2 efflux data per unit area vs. inverse temperature).

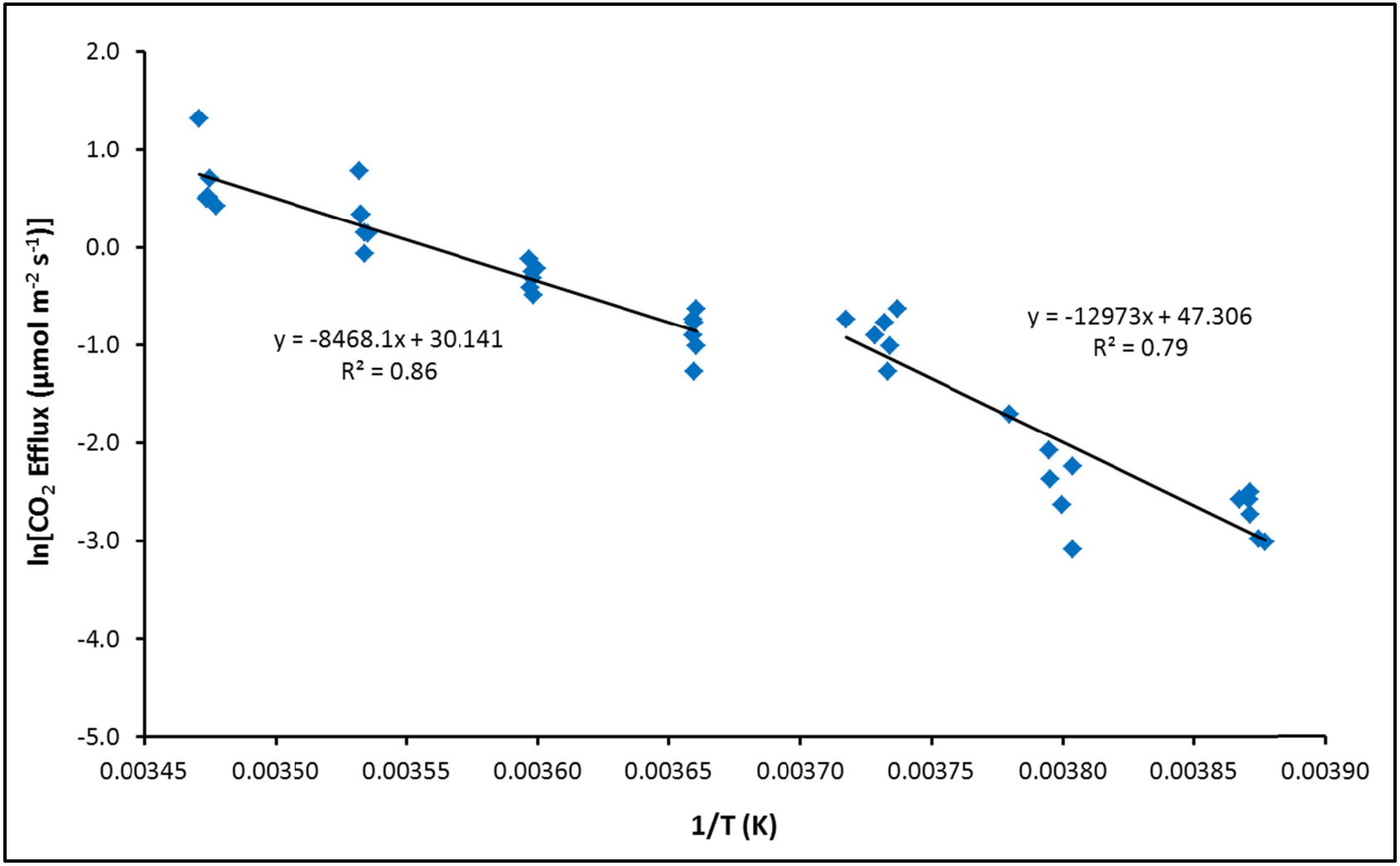

Figure 6. Plots of natural logarithm of CO_2 efflux and inverse temperature for above- and below-freezing temperatures.

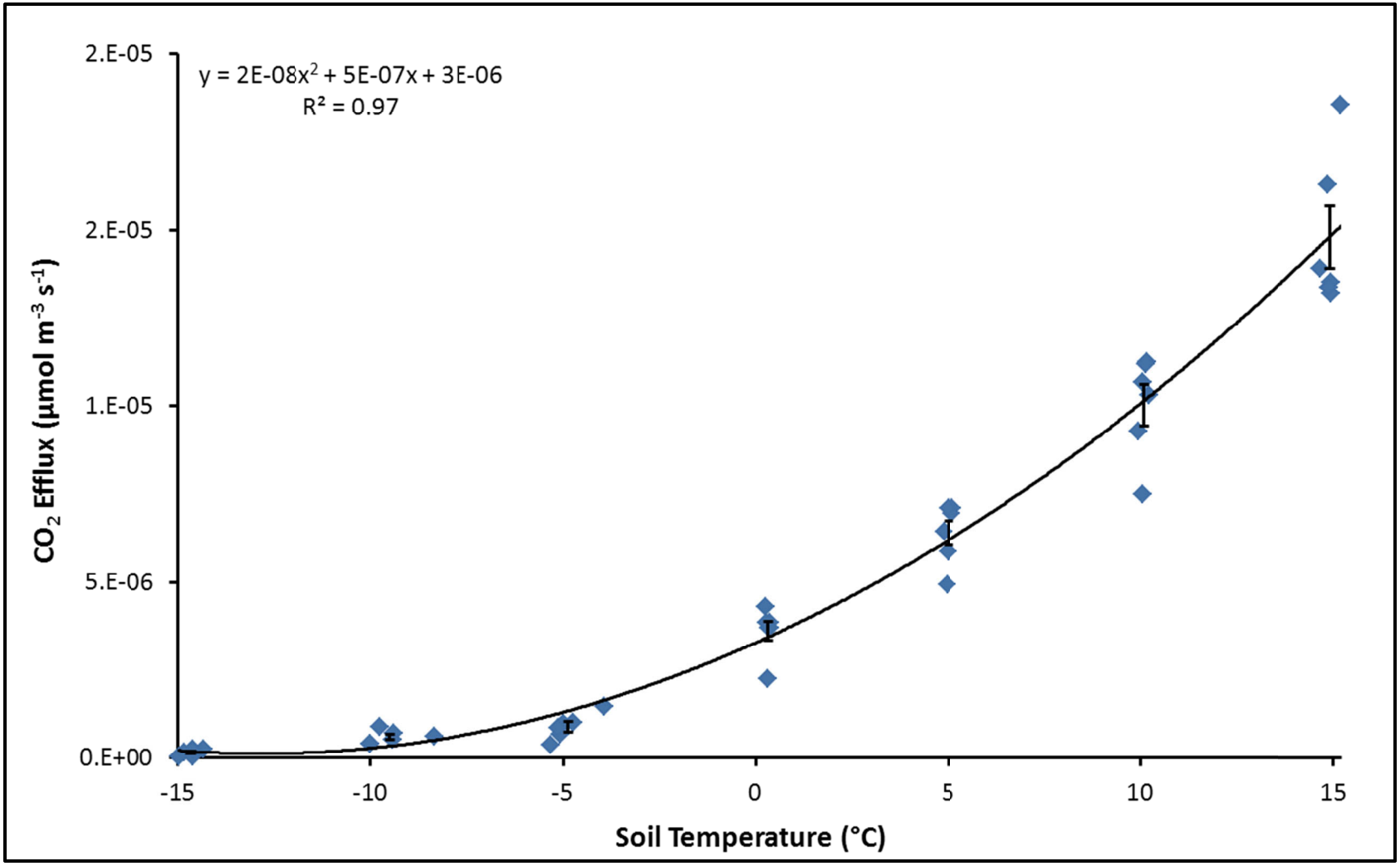

Figure 7. Polynomial fit of CO_2 efflux divided by monolith volume. Error bar 1 ± SE.

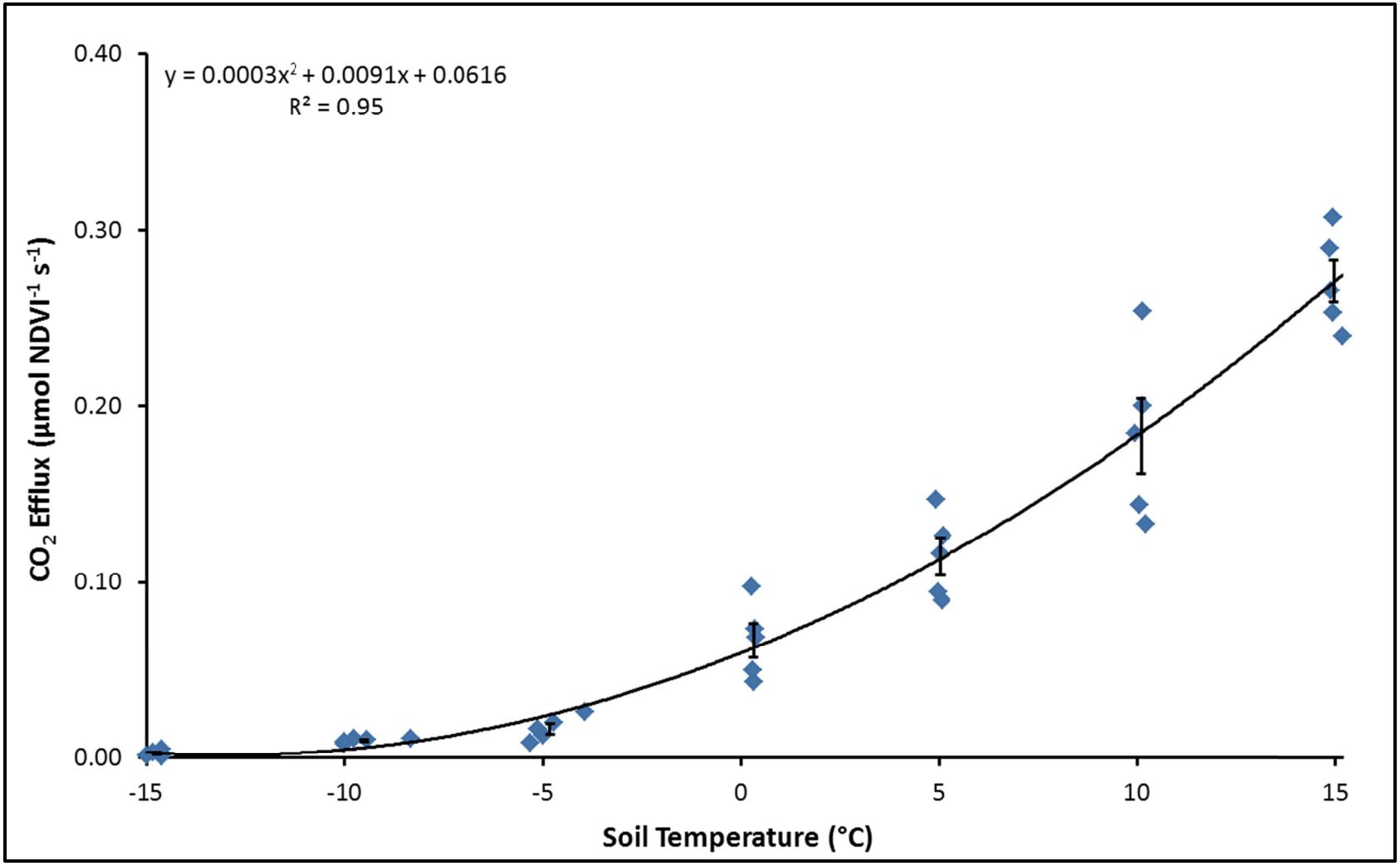

Figure 8. Polynomial fit of CO_2 efflux divided by monolith NDVI. Error bars ± 1 SE.

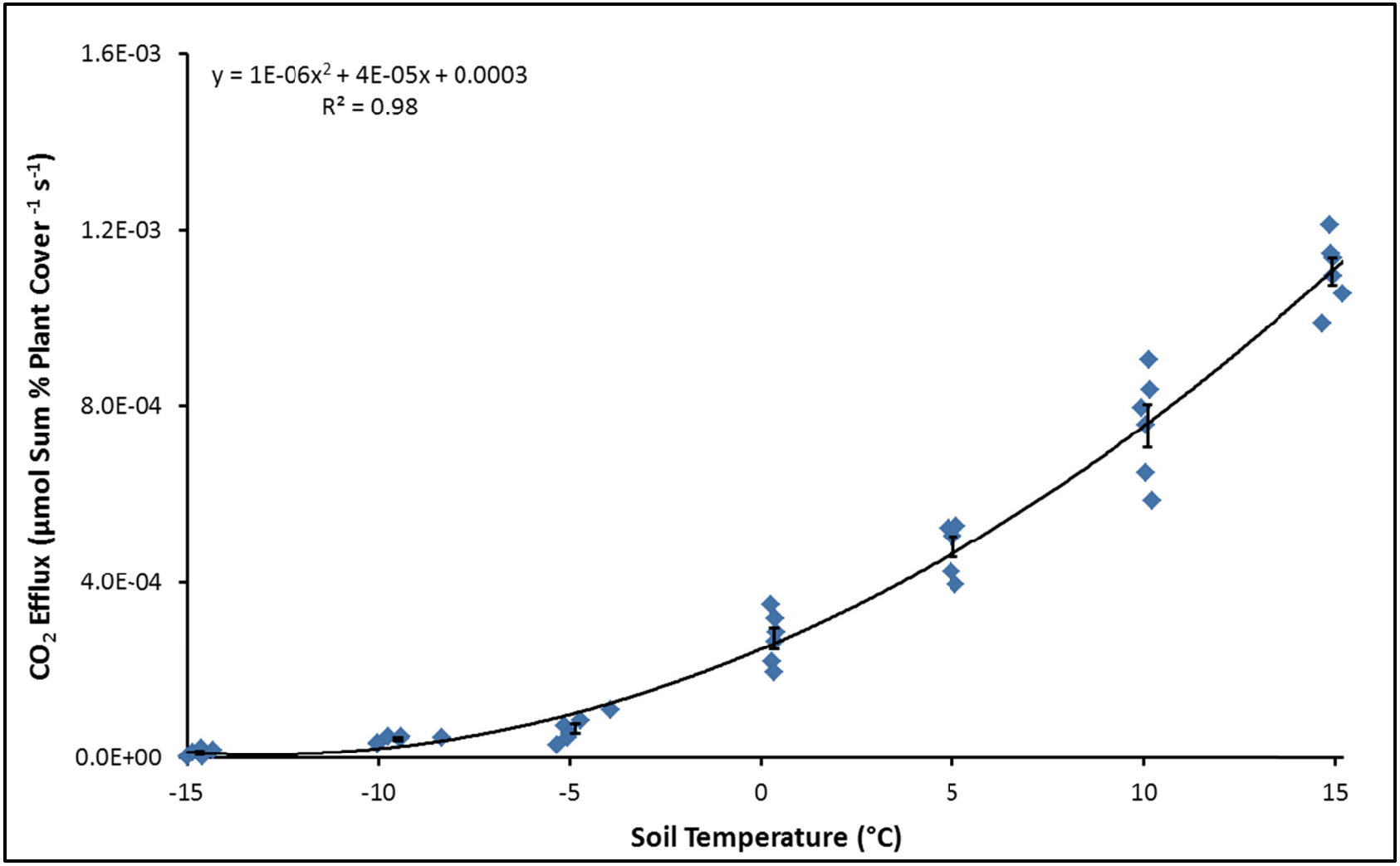

Figure 9. Polynomial fit of CO_2 efflux divided by monolith sum percent plant cover. Error bar 1 ± SE.

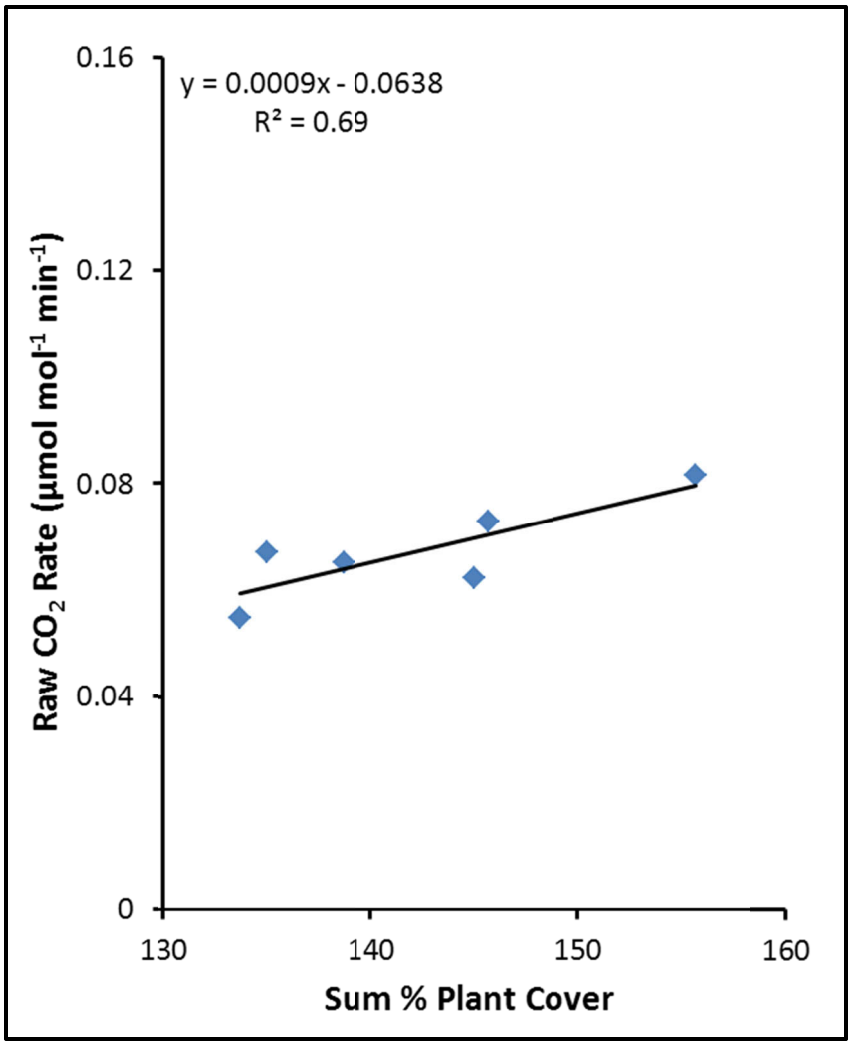

Figure 10. Linear regression for raw rate of CO_2 increase vs. monolith sum percent plant cover at 15 °C.

CHAPTER III. WATER UPTAKE OF ARCTIC TUNDRA EVERGREENS DURING THE ALASKAN WINTER-SPRING TRANSITION

INTRODUCTION

One advantage of the evergreen habit is that photosynthesis can continue over a greater proportion of the year than in deciduous species. An extreme example occurs in arctic evergreens that photosynthesize under the snow in the spring (Oberbauer and Starr 2002; Starr and Oberbauer 2003; Starr *et al*., unpublished). Starr and Oberbauer (2003) measured positive photosynthetic rates of four common arctic evergreen species (*Eriophorum vaginatum*, *Ledum palustre*, *Vaccinium vitis-idaea*, and *Cassiope tetragona*) under snow. Subsequent research on *V. vitis-idaea* has found that in addition to being photosynthetically capable prior to snowmelt, this species is photosynthetically active throughout the winter (Lundell *et al*. 2008; Lundell *et al*. 2010). Cold season photosynthesis by evergreens may help offset winter respiratory carbon losses. However, photosynthetic CO_2 uptake means that stomata are open, which in turn implies that plants are transpiring. How these evergreens replace water losses under the snow and during the winter-spring transition while the soils are still frozen is unknown. Indeed, relatively little is known about the physiological processes of tundra plants during the cold season.

The water balance of arctic tundra plants during the winter-spring transition is a function of a number of interacting factors, some of which are not well understood. When plant stem water freezes, embolisms may form in the xylem vessels, blocking further water uptake in that vessel. However, in small plants, such as tundra plants, root pressure may refill embolized vessels if liquid water is available (Hacke and Sauter 1996; Cochard *et al*. 2001). Water is more viscous when cold, and uptake rates are lower in

cold weather compared to the growing season because of increased stem resistance and reduced conductivity of roots (Brodribb and Hill 2000). Tundra soils remain frozen during most of the winter and partially so after snow melts in the spring. However, some water bound to soil particles remains unfrozen even in very cold soils, and plants may be able to access that water (Seyfried and Murdock 1997). As snow begins to melt in the spring, a process that may take a week to several weeks, water percolates from the upper snow layers down to the snow-soil interface, where it might be available to plant roots. The environment under snow is warmer than above-snow air temperature, it is also likely considerably more humid, especially with percolating meltwater. Under these conditions, plants may not lose any water and may even take up water. Foliar uptake has been shown to be an important source of water in some evergreens, such as redwoods (Burgess and Dawson 2004).

One approach to evaluating plant water uptake and its sources is by use of stable isotopes as a tracer or label. Elements typically have at least two stable isotopes, with one being in greater abundance than the other(s); these stable isotopes have allowed scientists to study biological, ecological, and environmental processes (Ehleringer and Rundel 1989). Hydrogen has two stable isotopes, ^{1}H and ^{2}H (also referred to as deuterium, D) with terrestrial abundances of 99.985 and 0.015 %, respectively (Dawson *et al*. 2002). Variation in stable isotope contents arise from kinetic (chemical), equilibrium (physical), or diffusive fractionation processes relating to the properties of the isotope in question (Ehleringer and Cerling 2002). Water does not generally experience kinetic fractionation as it is taken up by plants, allowing plant water to have the same isotopic signal as the water source (Wershaw *et al*. 1966; Dawson and

Ehleringer 1993). However, equilibrium fractionation occurs during leaf transpiration, tending to enrich leaf water with deuterium (Marshall 2007). Terrestrial plants tend to have naturally occurring δ^2H values ranging from +35 to -350 ‰, depending on latitude, elevation, continental position, season, and frequency of precipitation (Dawson and Siegwolf 2007).

Deuterium has been used to study many aspects of plant physiology, including but not limited to photosynthetic pathways (Ziegler *et al*. 1976; Sternberg 1986; Flanagan *et al*. 1991), evapotranspiration (Walker and Brunel 1990), ecosystem water vapor (Lai and Ehleringer 2011), nighttime transpiration (Dawson *et al*. 2007), ramet water movement (De Kroon *et al*. 1996; Matlaga and Sternberg 2009), and water uptake (Ehleringer and Dawson 1992). Multiple studies have been conducted looking into the source of water uptake in plants, whether comparing precipitation with soil moisture (Lin *et al*. 1996; Sugimoto *et al*. 2002; Gat *et al*. 2007; West *et al*. 2007; Xu *et al*. 2011) or within the vertical or horizontal soil profile (Thorburn and Ehleringer 1995; Moreira *et al*. 2000; Sternberg *et al*. 2002; Schwinning *et al*. 2005; Ewe *et al*. 2007). Isotopes have been used to determine that foliar uptake is one strategy plants use to obtain water (Burgess and Dawson 2004; Corbin *et al*. 2005; Limm *et al*. 2009).

The objective of the present study was to use deuterium as a tracer to determine if evergreen tundra plants are actively taking up water during the winter-spring transition when soils are mostly frozen. A δ^2H isotopic solution was made available to experimental plants so that any water uptake would be confirmed with enriched δ^2H values. I hypothesized that tundra plants under snow in frozen soils are rehydrating either by root or foliar uptake of water.

My research provides evidence that evergreen plants are indeed able to take up water under snow, possibly via roots but undoubtedly by foliar uptake. By understanding the physiology of dominant species at low temperatures, researchers should be better able to predict how tundra communities may respond to changes in temperature and snow cover with climate change.

METHODS AND MATERIALS

Study Site

This study was conducted near Toolik Field Station (68º 38'N, 149º34'W, 760 m above sea level) in the northern foothills of the Brooks Range, Alaska. Samples were taken from moist acidic and dry heath tundra (Viereck *et al*. 1992; Walker *et al*. 1994; Hobbie *et al*. 2005). The moist acidic vegetation was dominated by graminoids (*Carex bigelowii*), deciduous shrubs (*Betula nana* with *Vaccinium uliginosum* and *Salix pulchra*), evergreen shrubs (mainly *Ledum palustre, Vaccinium vitis-idaea, Cassiope tetragona, Empetrum nigrum, and Andromeda polifolia*), and mosses (primarily *Sphagnum* spp., *Hylocomium splendens*, *Aulacomnium turgidum*, and *Dicranum* spp.). The dry heath vegetation was dominated by *Dryas octopetala, Kalmia procumbens, Diapensia lapponica, Empetrum nigrum, Ledum palustre, Vaccinium vitis-idaea, and Arctous alpina*. The climate at the site is continental arctic, with mean monthly temperatures ranging from -22.5 (January) to 11.2 ºC (July), (Toolik Environmental Data Center Team 1997-2000).

Samples for the current study were collected in mid-May of 2010 and 2011 during the final stages of snowmelt. Soil (5 cm depth) temperature during field experiments averaged 0.063 and -3.77 °C for 2010 and 2011, respectively (Toolik Environmental Data

Center Team 2010, 2011). Air (3 m height) temperatures during field experiments averaged -0.38 and -2.26 °C for 2010 and 2011, respectively (Toolik Environmental Data Center Team 2010, 2011). Three different deuterium isotopic labeling experiments (*in situ* community, *in situ* species, and *ex situ* foliar uptake) were conducted.

In Situ Community Labeling Experiment

In situ community labeling experiments were designed to investigate root water uptake by photosynthetically capable evergreen vascular plants during the winter-spring transition, while the ground was frozen. Ten 100 x 100 cm snow-covered plots were set up in an area of moist acidic tundra in mid-May 2010. Prior to isotopic treatment, baseline leaf and soil samples were collected for deuterium analysis from five nearby snow-covered 50 x 50 cm plot (Table 1). Three snow samples were also collected as snow deuterium references from the snow surface among plots and, using methods described in the Deuterium Analysis section of Materials and Methods, averaged -173.1 ‰ δ^2H (Table 1). Two different treatments were used during initial application of the deuterium isotopic solution of 1:1000 99.8 % deuterium oxide and deionized water (δ^2H of 6319.4 ‰), with five plots designated for each treatment. A metal rod was used to make a grid of 50 vertical tunnels in the snow to the surface of the ground for the no-tap treatment. For the tap treatment, the same grid of tunnels were made, however, the metal rod was tapped with a hammer for each tunnel to break the surface of the frozen soil. Each plot was treated twice with 500 ml of the chilled deuterium isotopic solution, equally distributed over the grid of 50 vertical tunnels of each plot using a 30 ml syringe and hollow rod, similar to Koeniger *et al*. (2010). These two dosings were made at 22:00 - 01:00, the night before the first sampling, and at 7:00 - 9:30, the morning of the first

sampling. Thus, the solutions were applied during the colder part of the day to ensure the introduced solutions minimized changes to the natural thermal environment of the plots. Each of the ten plots were divided into four 50 x 50 cm subplots, with a different subplot sampled during each of the four consecutive sampling days. A sampling event consisted of excavating snow from the subplot of interest, collecting all evergreen leaves and stems, and a single soil core of 3-5 cm in depth and one inch diameter. To avoid contamination, hands were covered with latex gloves and all specimens were collected with tweezers and immediately placed in 15 ml BD Falcon™ polypropylene conical centrifuge tubes, sealed with parafilm, and placed in a freezer at -18 °C prior to express shipping frozen in an insulated box to Miami, FL.

***In Situ* Species Labeling Experiment**

In situ species labeling experiments were conducted to examine root water uptake while the ground was frozen by photosynthetically capable evergreen species during the winter-spring transition. Individual large, spreading plants of two evergreen shrubs, *Empetrum nigrum* and *Ledum palustre,* growing in dry heath tundra at Toolik Lake Field Station were flagged while snow-free in summer 2010. Species were selected from dry heath because of the large spreading form and large numbers of individuals present in that vegetation type. Specimen size was critical because subsamples from each individual plant were harvested on three separate occasions and leaves contain relatively low amounts of water. Prior to isotopic treatment, six snow samples each were also collected within the study area from the snow surface and the bottom layer of the snow, and, using methods described in the Deuterium Analysis section of Materials and Methods, averaged -181.2 and -146.3 ‰ δ^2H, respectively (Table 1). For each species,

24 plants were divided into four groups of six individuals each, with two groups used as a control and the other two groups as experimental. In mid-May 2011 the soil at the base of each experimental plant was dosed twice with 90 ml of a chilled deuterium isotopic solution of 1:1000 99.8 % deuterium oxide and deionized water (δ^2H of 6316.8 ‰), equally distributed among three separate pre-selected points arranged around the individual plant to avoid contact with aboveground tissue of the plant. These dosings were conducted at 16:00 - 16:45 and 20:25 - 21:00 the day before the first sampling. As a result of lower snow cover and faster snowmelt than expected, some plants were freed from snow prior to the last day of sampling. A different area of each individual plant was sampled on each of the three consecutive sampling days following dosings. During sampling, snow was excavated from the area of interest and leaves were collected, after which snow was replaced. All specimens were collected using latex gloves and tweezers and immediately placed in 15 ml BD Falcon™ polypropylene conical centrifuge tubes, sealed with parafilm, and placed in a freezer at -18 °C prior to express shipment frozen in an insulated box to Miami, FL.

Ex Situ Foliar Uptake Labeling Experiments

Ex situ foliar uptake labeling experiments were designed to explore whether evergreen shrubs are capable of direct foliar water uptake of simulated snow water during the winter-spring transition. Branches from large individuals of the evergreen shrubs *Cassiope tetragona*, *Empetrum nigrum*, *Ledum palustre*, and *Vaccinium vitas-idea* were collected from plants in moist acidic tundra that had just been released from snow cover. Experiments were performed on *C. tetragona* and *E. nigrum* during mid-May 2010, with all four evergreen shrub species tested in mid-May 2011. Each of the ten samples

collected for each species were placed outdoors in shade on paper for 1 hr to air dry the leaf surfaces and to lower water potential. Five samples from each species were then submerged for 1 hr in a deuterium isotopic solution of 1:1000 99.8 % deuterium oxide and deionized water (δ^2H of 6319.4 ‰ in 2010 and 6316.8 ‰ in 2011) without submerging cut stems or roots. After 1 hour of submersion, the samples were shaken off, rinsed three times in two different fresh batches of deionized water, and allowed to air dry until leaves were dry. The remaining five samples of each species were submerged in the same deuterium isotopic solution without submerging cut stems or roots, and then immediately shaken off and rinsed three times in two different fresh batches of deionized water, and allowed to air dry until leaf surfaces (and dead leaves in the case of *Cassiope tetragona*) were no longer wet. These samples were used as a "control" to determine potential isotopic contamination of the leaf surfaces that might be interpreted as uptake. All specimens were collected using latex gloves and tweezers and immediately placed in 50 ml (2010) or 15 ml (2011) BD Falcon™ polypropylene conical centrifuge tubes, sealed with parafilm, and placed in a freezer at -18 °C prior to express shipment frozen in an insulated box to Miami, FL.

Deuterium Analysis

All leaf, stem, and soil specimens were processed at the Stable Isotope Laboratory, Department of Biology, University of Miami (Coral Gables, FL). Glass tubes were custom-handmade, loaded with leaf and stem specimens and vacuum sealed before water was extracted from specimens through a distillation process described in Vendramini and Sternberg (2007). Three water samples were lost (*in situ* community: stem day 1 no-tap treatment and *in situ* species: *L. palustre* day 3 control and *L. palustre*

day 2 labeled) during distillation as a result of complications during the vacuum sealing process. Soil samples were compressed to express an adequate amount of water for analysis. All water samples were analyzed in a multiflow system connected to an Isoprime mass spectrometer (GV, Manchester, UK). Hydrogen gas was equilibrated with water vapor by using 5 mg of platinum black powder (Sigma-Aldrich, St. Louis, MO, USA) and waiting for 24 hr before hydrogen isotope ratio analysis as described in Vendramini and Sternberg (2007). The deuterium hydrogen isotopic ratio expressed as δ^2H and in per mil units (‰) was calculated as:

$$\delta^2H\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000 \qquad (1)$$

where R_{sample} is the ratio of deuterium to hydrogen in the sample and $R_{standard}$ is the ratio of deuterium to hydrogen in the international standard Vienna-Standard Mean Ocean Water.

Data Analysis

All statistical analyses were conducted with PASW Statistics 18, Release Version 18.0.0 (© SPSS, Inc., 2009, Chicago, IL). Treatment and sampling day of δ^2H data from the *in situ* community labeling experiments were analyzed by a two-way analysis of variance. Baseline leaf and soil data were compared with sampling day for both treatments using independent-samples t-tests. Levene's test for equality of variances, which tests the null hypothesis that the error variance of the dependent variable is equal across groups, was used for all independent-samples t-tests and the appropriate t-tests results were reported. Linear regressions were additionally conducted comparing all pairs of soil, leaf, and stem data within each treatment. Sampling day and control δ^2H

data from the *in situ* species labeling experiment were analyzed by a two-way analysis of variance. Two δ^2H values for *L. palustre* (one short and one long submersion) were the inverse of what would be expected on the basis of what the group data showed. Upon review of tube labels and processing methodology, it is highly likely that these tubes were mislabeled. If these interchanged values were removed from data analysis, the results were the same as if they were exchanged and included. The δ^2H of each species pair of short and long submersions in the e*x situ* foliar uptake labeling experiments were compared using independent-samples t-test. Levene's test for equality of variances was used for all independent-samples t-tests and the appropriate t-tests results were reported.

RESULTS

In Situ Community Labeling

Plots in which deuterium-labeled water was added to frozen soil where the surface had been broken became increasingly enriched for leaf δ^2H over the four sample days (Figure 1a). The mean leaf δ^2H of the no-tap treatment plots declined slightly until day 4 when δ^2H increased (Figure 1a). Mean stem δ^2H of tap treatment plots were enriched on two of three days of sampling (Figure 1b). In contrast, the no-tap treatment showed no enrichment (Figure 1b). Variation in soil δ^2H was largely the result of two samples with highly enriched δ^2H values (380.7 and 467.0 ‰ on the first and second day for the no-tap and tap treatments, respectively) likely the result of sampling directly on a label delivery site (Figure 2). Mean soil δ^2H of tap treatment plots was also enriched on the fourth day of sampling (Figure 2). Comparison of δ^2H for effects of collection day, treatment (no-tap or tap), and day*treatment interaction using an ANOVA were not significant for leaf or soil water, although stem water was significant for treatment (Tables 2-4).

Independent-samples t- tests comparing the baseline leaf and soil δ^2H values with community labeling data showed no significance for either treatment for soil δ^2H, except for leaf δ^2H on the second collection day for both treatments (Tables 5-6).

Linear regressions comparing all pairs of soil, leaf, and stem data within each treatment (Table 7) were significant only for soil and leaf δ^2H of the no-tap treatment, and stem and leaf δ^2H for the tap treatment (Figure 3). Significance of the linear regression comparing soil and leaf δ^2H of the no-tap treatment (Figure 3a) is the result of a single highly enriched soil value, likely resulting from soil sampling at a dosed location.

***In Situ* Species Labeling**

Mean δ^2H of control plants decreased slightly for *E. nigrum* and evenly over the three days of sampling for *L. palustre* (Figure 4). Mean δ^2H of labeled plants was enriched slightly on all three days of sampling for both species (Figure 4). Comparison of δ^2H for collection day, treatment (control or labeled), and day*treatment interaction using an ANOVA showed no significant effects for *E. nigrum* (Table 8). Mean δ^2H of *Ledum palustre* was significantly different between control and labeled treatments and among days in the control (Table 9).

***Ex Situ* Foliar Uptake Labeling**

Mean δ^2H of shoots of evergreen species submerged for 60 min in deuterium-labeled water increased substantially compared to shoots submerged only briefly (Figure 5). Independent-samples t-tests for all species in both years were highly significant (Table 10), demonstrating significant foliar uptake potential for plants during the winter-spring transition.

DISCUSSION

I tested two possible mechanisms for water uptake by evergreen plants while the ground may be partially or completely frozen during the winter-spring transition, root uptake and foliar uptake. I found evidence for both.

My e*x situ* foliar uptake labeling results suggest that leaf water uptake from melting snow may be another mechanism by which arctic evergreen species obtain water while ground water remains frozen. *Ex situ* foliar uptake labeling shoots from multiple evergreen shrubs submerged for 1 hour took up significantly more deuterium than controls (shoots briefly submerged only), indicating that their leaves and or stems are capable of direct water uptake. The only species showing enriched δ^2H values after being briefly submerged was *Cassiope tetragona* (Figure 5), which retains many years of dead leaves appressed on the stems that trap and hold water. It is quite likely that these dead leaves either were not completely dry when samples were collected or that they effectively retained water that could be taken up by the stems and or green leaves while the samples were air drying. Foliar uptake has been previously demonstrated as a source of water for redwoods (Dawson 1998; Burgess and Dawson 2004; Limm *et al*. 2009; Simonin *et al*. 2009), pines (Stone *et al*. 1956; Boucher *et al*. 1995), elms (Meidner 1953), junipers (Breshears *et al*. 2008), tomatoes (Breazeale *et al*. 1950), lavender (Mumme-Bosch *et al*. 1999), rosemary (Mumme-Bosch *et al*. 1999), Crassulas (Martin and Willert 2000), chasmophytes (Gouvra and Grammatikopoulos 2003), and ferns (Limm and Dawson 2010). Oliveira *et al*. (2005) found evidence in a desiccation-tolerant monocot of direct water absorption of shoots. To our knowledge, this is the first

demonstration that foliar uptake may be important for the water balance of snow-covered tundra plants.

In situ δ^2H labeling indicates arctic vascular evergreens may be taking up some water in frozen soil via roots. Leaf δ^2H from living vascular tissue collected from the *in situ* community experiments were more enriched, though not significantly so on every day, than the baseline δ^2H values. The differences between baseline and label treatment leaf δ^2H were largest on day 4, and for the tap treatment that broke the soil surface, δ^2H steadily increased with sample date. Differences in baseline leaf water were only significant on day 2 for both treatments, because of the small variation in δ^2H on that day for both treatments (Figure 1a). This variation arose in part because the plots were of necessity selected while under cover of snow and variation in plant community composition and topography could not be controlled. Nevertheless, the presence of highly enriched leaf tissue indicates at least some individuals took up the labeled water.

I did not collect baseline samples for plant stems prior to the community labeling experiment. However, the δ^2H values found for stems for the no-tap treatment that showed no sign of enrichment were very close to the δ^2H of the leaf baseline sample, suggesting that leaf and stem baselines were likely very similar to each other. The δ^2H of stems from the tap treatment showed strong increases in δ^2H compared to the baseline leaf value. Stem δ^2H was significantly higher for the tap treatment than the no-tap treatment suggesting that penetrating the soil surface by tapping with the steel rod somehow enhanced water availability for root uptake. That the elevated δ^2H values of the tap treatment were a result of root uptake is supported by the significant positive correlation between δ^2H of leaves and stems for the tap treatment (Figure 3b). These

elevated values are likely a result of root uptake because water was delivered at the soil level below any leaves.

The significant difference in leaf δ^2H between control and labeled individuals of *L. palustre* in dry heath tundra indicate that the roots of this species were actively taking up δ^2H-labeled water that had been added directly to the soil surface. A similar difference between treatments was found for *E. nigrum*, although the difference was not statistically significant.

During the onset of winter freezing, water from within the soil profile flows upwards and accumulates at the soil surface (Zhang and Sun 2011). As liquid water phase changes into solid ice, significant amounts of latent heat are released (Zhang and Sun 2011). Some amount of water remains unfrozen after the bulk of soil water has frozen (Seyfried and Murdock 1997). The absorption force and curvature of particle surfaces results in a thin layer of unfrozen water among particles in porous soil, and the melting point depression of water determined by the concentration of solutes results in potentially available unfrozen water in soils (Watanabe and Mizoguchi 2002).

During spring as snow meltwater percolates into the snowpack, it refreezes, releasing latent heat (Illangasekare *et al*. 1990; Williams *et al*. 1999). A large percentage of water flows in unique preferential horizontal and vertical paths, referred to as flow fingers, which expedites the distribution of meltwater into the rest of the snowpack and onto the soil surface (Marsh 1988; Schneebeli 1995; Williams *et al*. 1999).

The permeability of meltwater into the soil is determined by both soil temperature and soil pores being blocked by ice (Colbeck and Davidson 1973; Seyfried and Murdock 1997; Carey and Pomeroy 2009). Percolating meltwater seeps into the soil, releasing

heat as it freezes, allowing for more water to infiltrate into the soil (Alexeev *et al*. 1973). The degree of ice formation in pores depends on temperature, and will either allow infiltration to continue or cease altogether (Alexeev *et al*. 1973). Stadler *et al*. (2000) lists the prevailing factors influencing infiltration of freezing soils as ice content, soil-water moisture content, soil structure, the soil surface temperature, and the amount of freezing and thawing cycles. The melt out of snow is one of the most important annual events for arctic soils because it is allows for renewal of moisture in soils (Gray *et al*. 2001).

Bliss *et al*. (1981) discussed root water uptake of tundra plants while the ground remains frozen at and below-freezing temperatures, noting that following the onset of permafrost thaw, plant roots begin to grow quickly, citing literature confirming root growth at low temperatures. Two arctic tundra species, *Kalmia* and *Diapensia,* are able to absorb surface meltwater via adventitious roots while soils remain frozen (Courtin 1968; Larcher 1963 as cited in Bliss 1981). Several root water uptake strategies were suggested by Bliss *et al*. (1981) including root growth in freezing soils, cushion plants growing roots above the soil surface, and absorption of meltwater from soil via superficial adventitious roots.

Adult conifers in boreal and alpine habitats have been shown to maintain adequate water balance to replace winter transpiration losses (Havas and Hyvärinen 1990; Boyce *et al*. 1991; Sowell *et al*. 1996). Sevanto *et al*. (2006) found that subalpine and boreal evergreens use stored stem water during the winter. Both Boyce and Lucero (1999) and Sevanto *et al*. (2006) indicate that plants were able to uptake soil water via roots at low winter surface soil temperatures (near freezing).

The low δ^2H enrichments of labeled *in situ* leaves compared with *ex situ* leaves dipped in an isotopic solution imply that water uptake rates are relatively low during the winter-spring transition and/or that our delivery methods did not make the labeled water easily available. Complications in the movement of added water could have arisen from evaporation or uneven dispersion of water over the plots or into the frozen soil under the snow. The large δ^2H variability of *in situ* soil samples likely resulted when the sampling location (a 3 cm diameter subsample taken from each 50 x 50 cm subplot) was either directly on a dosing location, or if the labeled mixture had pooled at the sampling location. The movement of labeled water in snow covered, frozen soils is likely complicated by local topography, soil surface ice layers, and diurnal refreezing, all of which may act to limit or expedite the movement of snowmelt water within the local topography.

Additional evidence for water uptake during the winter-spring transition comes from the controls of the *in situ* species experiment. Snow δ^2H from the surface of the snowpack in dry heath tundra was significantly depleted compared to snow collected from the bottom of the snow profile, as observed by Moser and Stichler (1974), resulting from isotopic fractionation occurring in the melt-freeze mass exchange within the melting snow (Zhou *et al*. 2008b). Variations in isotopic content of snow layers are the result of individual precipitation events (Gat 1996; Unnikrishna *et al*. 2002). Snow surface layers are more enriched as a result of snow evaporation, condensation of air humidity into the snow cover, snow melt, sublimation, and crystallization metamorphism, with deeper layers of the snow profile being unaffected by these processes (Moser and Stichler 1974).

The mass-dependent isotope fractionation effect explains why meltwater is relatively more enriched than the snowpack (Gat 1996; Zhou *et al*. 2008a).

Mean δ^2H of surface snow from the dry heath site in 2011 were significantly depleted compared to *E. nigrum* and *L. palustre* leaf water, especially at the first day of sampling. The leaf water δ^2H of *L. palustre* and *E. nigrum* controls became naturally depleted over the short, 3-day sampling period, likely the result of plants taking up comparatively-depleted snow surface water that melted and drained down to the soil. It should be noted that the stony mineral soils at the dry heath site thawed quickly in the spring, once snow cover melted, meaning that free water for plant uptake may be available relatively soon after snowmelt. Furthermore, as mentioned in the Methods and Materials, the low snow cover in 2011 at the dry heath site led to early melt out around control plants, which may have led to uptake of meltwater during the 3-day sampling window.

CONCLUSION

Tundra evergreens face a unique situation during the winter-spring transition where water may be lost during subnivean photosynthesis. How much water is lost is uncertain, but recent data (Oberbauer *et al*. unpublished) suggests that some evergreen plants are water stressed under the snow. My research suggested plants may be replacing some of this water during the winter-spring transition, either through foliar or root water uptake during this time. The capacity of liquid water to infiltrate into the frozen soil is directly related to the ability of roots to take up water. The present study is to my knowledge the first study to have found evidence of foliar uptake of water in arctic

evergreens shrubs. The approach used here increases our understanding of how arctic tundra evergreens cope with water loss during winter-spring transition conditions.

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Table 1. Baseline sample data for *in situ* labeling experiments.

Substrate	*In situ* Labeling	n	Mean δ^2H (‰)	SE
Leaf	Community	5	-165.6	2.4
Soil	Community	5	-155.6	2.5
Snow Top	Community	3	-173.1	6.3
Snow Top	Species	6	-181.2	7.5
Snow Bottom	Species	6	-146.3	10.6

Table 2. Two-way Analysis of Variance (ANOVA) for leaf *in situ* community labeling.

Subject Effect	n	p	F	df
Treatment	5	0.660	0.20	1,36
Day	5	0.562	0.69	3,36
Day*Treatment	5	0.847	0.27	3,36
No-Tap Days	5	0.784	0.36	3,16
Tap Days	5	0.702	0.48	3,16

Table 3. Two-way Analysis of Variance (ANOVA) for soil *in situ* community labeling.

Subject Effect	n	p	F	df
Treatment	5	0.897	0.02	1,36
Day	5	0.317	1.22	3,36
Day*Treatment	5	0.0887	2.35	3,36
No-Tap Days	5	0.335	1.22	3,16
Tap Days	5	0.140	2.11	3,16

Table 4. Two-way Analysis of Variance (ANOVA) for stem *in situ* community labeling.

Subject Effect	n	p	F	df
Treatment	5	0.00318	10.84	1,23
Day	5	0.529	0.65	2,23
Day*Treatment	5	0.252	1.46	2,23
No-Tap Days	5	0.724	0.33	2,11
Tap Days	5	0.245	1.58	2,12

Table 5. Independent-samples t-tests between baseline sampling and leaf *in situ* community labeling data.

Day	Treatment	n	*p*	*t*	df
1	No-Tap	5	0.232	1.29	8
	Tap	5	0.320[1]	1.11	4.55
2	No-Tap	5	0.02	2.92	8
	Tap	5	0.03	2.66	8
3	No-Tap	5	0.175[1]	1.57	5.26
	Tap	5	0.0734[1]	2.31	4.60
4	No-Tap	5	0.131	1.68	8
	Tap	5	0.225[1]	1.43	4.11

[1]Equal variances not assumed; based on Levene's Test for Equality of Variances

Table 6. Independent-samples t-tests between baseline sampling and soil *in situ* community labeling data.

Day	Treatment	n	*p*	*t*	df
1	No-Tap	5	0.283[1]	1.24	4.00
	Tap	5	0.153[1]	1.70	4.71
2	No-Tap	5	0.338[1]	1.08	4.19
	Tap	5	0.176[1]	1.64	4.01
3	No-Tap	5	0.233	1.29	8
	Tap	5	0.133	-1.67	8
4	No-Tap	5	0.209[1]	1.41	5.96
	Tap	5	0.232[1]	1.40	4.05

[1]Equal variances not assumed; based on Levene's Test for Equality of Variances

Table 7. Linear regressions for *in situ* community labeling data.

Substrate	Treatment	n	r^2	Slope	*p*	*t*	df
Soil*Stem	No-tap	14	0.0015	-0.0194	0.895	-0.13	12
	Tap	15	0.073	0.0344	0.330	1.01	13
Stem*Leaf	No-tap	14	$9.1 \cdot 10^{-4}$	0.0432	0.919	0.10	12
	Tap	15	0.50	1.083	0.00299	3.64	13
Soil*Leaf	No-tap	20	0.35	0.0896	0.00584	3.13	18
	Tap	20	0.011	-0.0214	0.666	-0.44	18

Table 8. Two-way Analysis of Variance (ANOVA) for *E. nigrum in situ* species labeling.

Subject Effect	n	*p*	*F*	df
Treatment	12	0.089	2.98	1,66
Day	12	0.770	0.26	2,66
Day*Treatment	12	0.654	0.43	2,66
Control Days	12	0.908	0.097	2,33
Labeled Days	12	0.651	0.44	2,33

Table 9. Two-way Analysis of Variance (ANOVA) for *L. palustre in situ* species labeling.

Subject Effect	n	*p*	*F*	df
Treatment	12	<0.001	12.31	1,64
Day	12	0.772	0.26	2,64
Day*Treatment	12	0.0567	3.00	2,64
Control Days	12	0.03	3.83	2,32
Labeled Days	12	0.399	0.95	2,32

Table 10. Independent-samples t-tests for *ex situ* foliar uptake labeling.

Species	Year	n	*p*	*t*	df
C. tetragona	2010	5	<0.001	-8.22	8
E. nigrum	2010	5	<0.001	-6.90	8
C. tetragona	2011	5	0.001[1]	-6.37	5.01
E. nigrum	2011	5	0.004[1]	-5.90	4.02
L. palustre	2011	5	<0.001	-6.25	8
V. vitis-idaea	2011	5	0.01	-4.63	4.02

[1]Equal variances not assumed; determined by Levene's Test for Equality of Variances

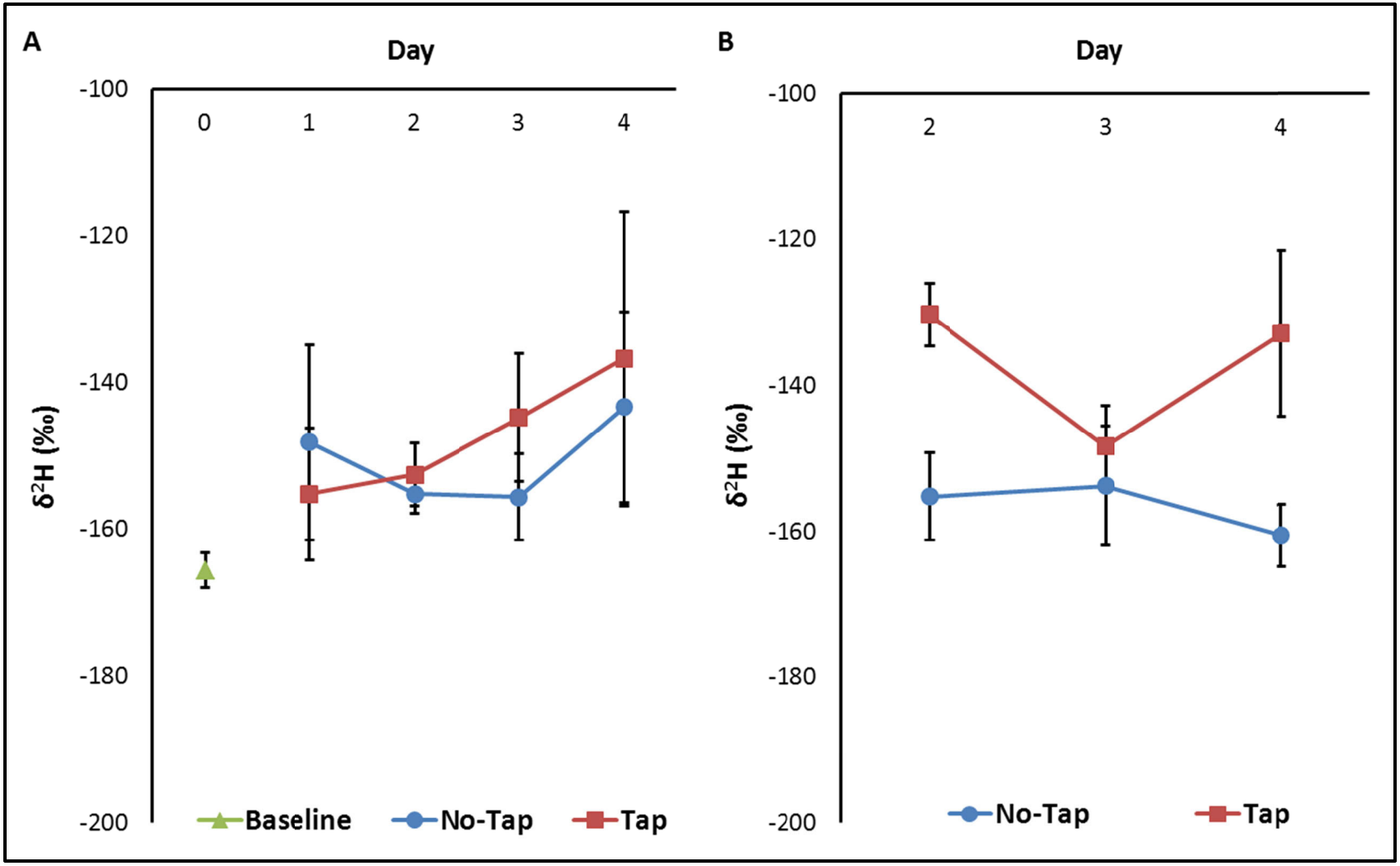

Figure 1. *In situ* community labeling leaf (A) and stem (B) data. Error bars ± 1 SE.

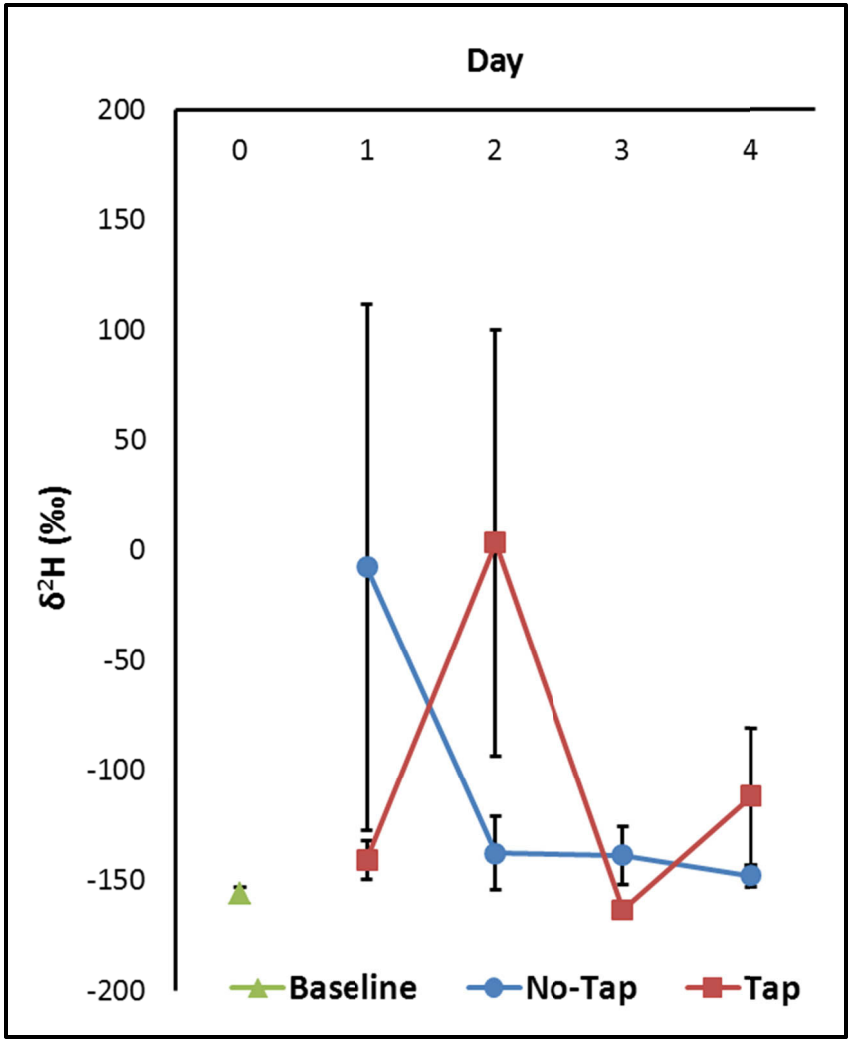

Figure 2. *In situ* community labeling soil data. Error bars ± 1 SE.

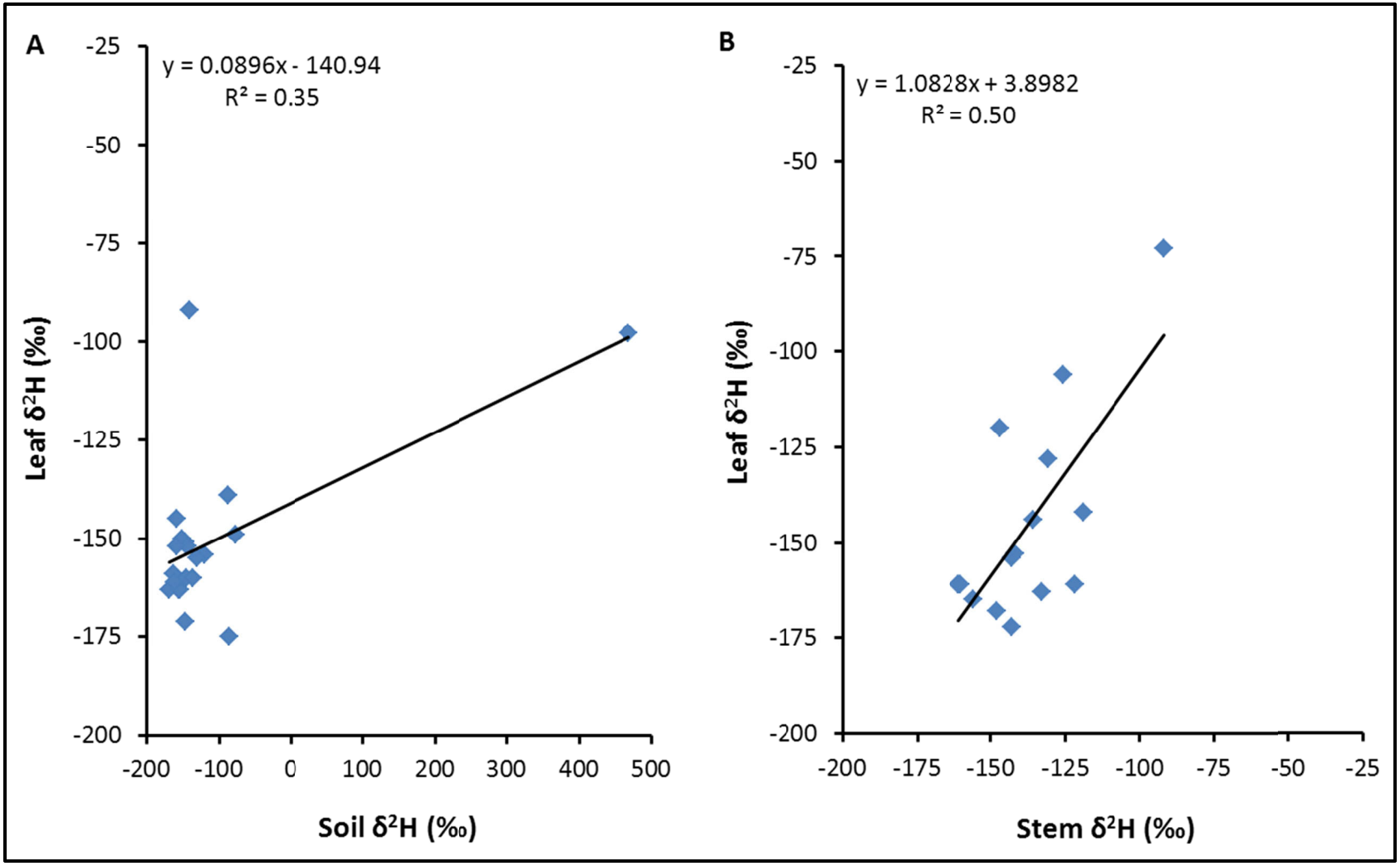

Figure 3. Linear regressions for *in situ* community labeling soil*leaf no-tap treatment (A) and stem*leaf tap treatment (B).

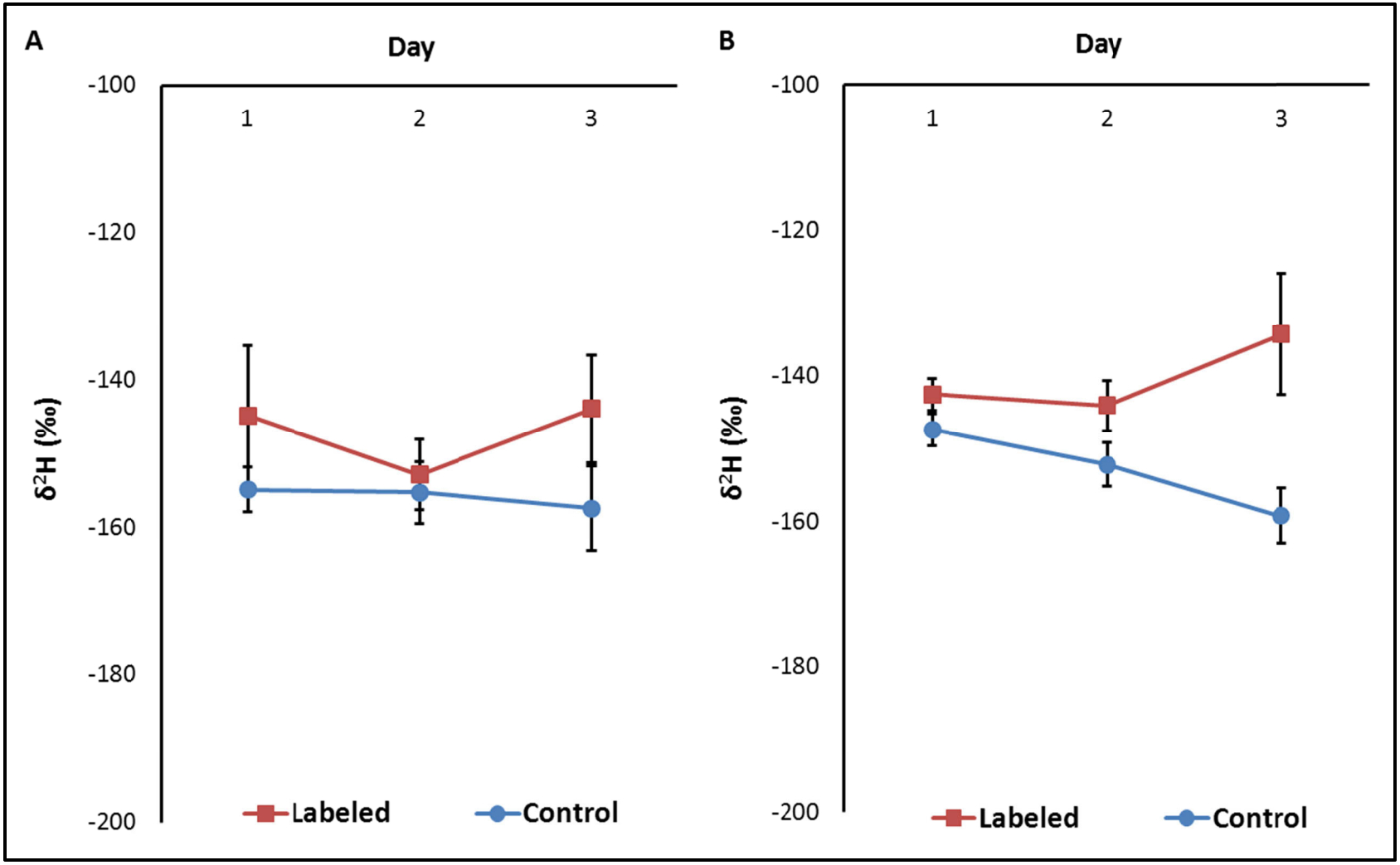

Figure 4. *In situ* species labeling for *E. nigrum* (A) and *L. palustre* (B). Error bars ± 1 SE.

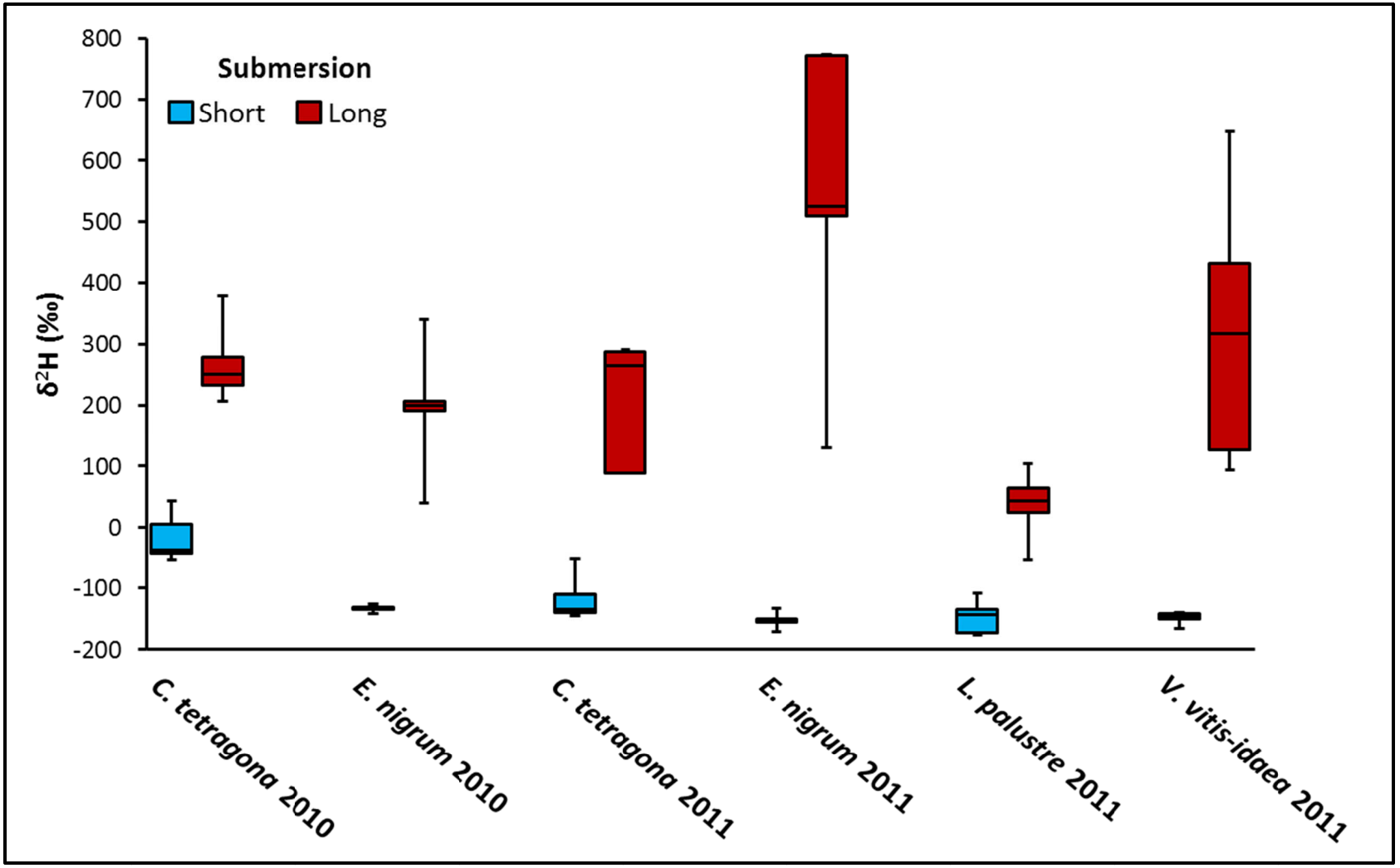

Figure 5. *Ex situ* foliar uptake labeling data box-and-whisker distribution for all test species over two years.

CHAPTER IV. CONCLUSION

Despite the fact that the cold season forms 75 % of the arctic annual cycle, and the largest effects of climate warming in the Arctic will be in winter, we know far more about the growing season physiology of arctic plants than we do about their winter physiology. Granted, obtaining reliable measurements during winter is very difficult, both on equipment and investigators. In the deep cold of winter, just obtaining measureable rates of ecosystem respiration is an enormous challenge. As a result, estimates of CO_2 balance for Arctic sites are frequently based on growing season values only or use guesstimates for what winter values might be.

In Chapter 2, I addressed the uncertainty about ecosystem respiration at low temperatures by determining the temperature response of ER of intact blocks (monoliths) of Alaskan tundra. The resulting responses from six blocks were extremely consistent, showing Q_{10} values near 6 at temperatures below-freezing and near 5 at temperatures between 0 and 15 °C, validating some previous determinations of the responses of ER to low temperatures. These results translate to a ~20 % increase in ER for a 1 °C increase in temperature. Temperatures in the North Slope of Alaska have already increased 2.9 °C since 1976 (Wendler *et al*. 2010), implying that ecosystem respiration rates are ~70 % greater than they were in 1976. Given that a major component of ER is organic matter decomposition, these increased ER rates will result not only in release of stored C as CO_2, but also mobilization of trapped nutrients. The current hypothesis for the increase in shrubs in the Arctic is associated with increased nutrient release as a result of warming soils (Sturm *et al*. 2005). My results and similar temperature responses determined by others suggest that a large proportional increase in ER and nutrient release has already

happened in the Arctic. However, much more remains to be learned, especially with regards to the proportion of ER contributed by plant respiration vs. that contributed by organic matter decomposition and whether the temperature responses of these different processes are the same.

In Chapter 3, I addressed winter water uptake, an important aspect of the water balance of tundra plants during the cold season. Winter water balance is important for tundra ecosystem carbon balance because some evergreens have been shown to be photosynthetically active under the snow, a finding that implies that plants may be losing water. Furthermore, in some years large numbers of shoots of both evergreens and deciduous species die over the winter, most likely from embolisms caused by freeze thaw action that are aggravated by plant water deficits. At snowmelt, evergreen shoots are exposed to bright, low humidity conditions while soils may be still frozen. How arctic evergreen plants rehydrate under these conditions is uncertain. In two different field experiments, I found that deuterium added as a tracer beneath snow was taken up by evergreen plants, although, the amount of label taken up was fairly small. However, in a lab experiment using cut shoots, I determined that evergreen plants have a high capacity to take up water through their leaves, suggesting that one way these plants rehydrate under the snow is via foliar uptake of snow meltwater. To my knowledge this study is the first demonstration of foliar leaf water uptake by tundra plants.

LITERATURE CITED

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Wendler, G., Shulski, M., and Moore, B. (2010). Changes in the climate of the Alaskan North Slope and the ice concentration of the adjacent Beaufort Sea. Theor Ap Clim 99(1-2):67-74.