# Exudation Rates and $\delta^{13}C$ Signatures of Bottomland Tree Root Soluble Organic Carbon: Relationships to Plant and Environmental Characteristics

**THESIS**

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Steven William Gougherty

Graduate Program in Evolution, Ecology and Organismal Biology

The Ohio State University

2015

Master's Examination Committee:

Dr. James Bauer, Advisor

Dr. Peter Curtis

Dr. Brian Lower

## ABSTRACT

Tree root exudation of soluble organic carbon (SOC) is often considered an important but under-assessed component of terrestrial net primary productivity that also strongly influences rhizosphere and soil biogeochemical processes. Although riparian and bottomland systems are often considered "hot spots" of biogeochemical activity that are potentially supported by root exudate SOC, *in situ* tree root exudation rates of SOC have not been previously reported for these systems. Additionally, there is an outstanding need to understand the $\delta^{13}C$ signatures of root exudates in relation to not only different plant components such as leaves and roots but also different ecosystem pools of C, such as $CO_2$ emitted from soil.

In the present study we used an *in situ* method to collect root exudate SOC in order to assess root exudation rates in a bottomland forest for *Acer saccharinum*, *Populus deltoides* and *Platanus occidentalis* trees over five sampling dates ranging from mid-summer to late-autumn. Leaves from *Acer negundo*, *Acer saccharinum*, *Lonicera maackii*, *Populus deltoides* and *Platanus occidentalis* were also collected. $\delta^{13}C$ values were determined for all of the root exudates, roots and leaves collected in this study. Exudation rates and $\delta^{13}C$ values were evaluated in relation to leaf and root morphology, leaf and root C and N contents and a number of environmental parameters (e.g. vapor pressure deficit) and net ecosystem exchange (NEE).

Findings indicate that exudation rates and $\delta^{13}C$ values of leaves and roots were significantly correlated to time-lagged measurements of NEE, suggesting a strong link

between exudation rates and $\delta^{13}C$ values of leaves and roots and photosynthetic rates. Various time lagged environmental parameters (e.g., vapor pressure deficit) were correlated to the $\delta^{13}C$ of exudates, leaves and roots—suggesting a rapid transfer of recent photosynthate from the canopy to roots and root exudates and relatively rapid turnover of C in leaves. When pooled together, the leaf $\delta^{13}C$ values for individual species were found to be significantly related to leaf nitrogen per unit leaf area—suggesting a strong leaf level control of N on leaf $\delta^{13}C$ values. We also observed that average exudate $\delta^{13}C$ values became progressively enriched across the first three sampling dates (-32.0 ± 1.0, -29.4 ± 0.7 and -27.9 ± 0.3, respectively), which then leveled off, potentially reflecting a shift in the relative contribution of two or more soluble plant pools that differed in $\delta^{13}C$ over the course of the study.

When the average net SOC exudation rate (14 ± 3 μmol C g $root^{-1}$ $d^{-1}$) is scaled to the entire sampling area, root exudation may account for as much as 3.2% of net C uptake. While this may not be major loss of C and energy from plants, this is the *net* rate, and therefore heterotrophic losses due to bacteria and fungi are not included; therefore, this represents a minimal loss rate. In contrast, this amount of root exudation may represent a potentially important flux of SOC to temperate bottomland soils and their heterotrophic communities.

We suggest that future studies examining $\delta^{13}C$ within plants or as a natural tracer of measurements of the $\delta^{13}C$ values of $CO_2$ would benefit by accounting for both the fluxes and remineralization of root exudates. This is because the findings from the present study suggest that root SOC exudation and $\delta^{13}C$ values are highly variable and

influenced by a number of environmental and plant-level processes that have yet to be fully elucidated.

## ACKNOWLEDGEMENTS

I would like to thank Dr. James Bauer for serving as my advisor and for his financial support and the feedback he provided on the work presented in this thesis. I would also like to thank Dr. Peter Curtis and Dr. Brian Lower for serving on my thesis committee and providing feedback at critical points in the study. I am indebted to Dr. John Pohlman for performing the isotopic analysis on the root exudate samples. The invaluable meteorological and net ecosystem exchange data used in this study were graciously provided by Dr. Gil Bohrer and Mr. Tim Morin of OSU's Department of Civil, Environmental and Geodetic Engineering. Mike Zianni is thanked for performing the DNA analysis on the roots collected for this study. Ziyue Chen is also thanked for statistics and data analysis consultation. Dr. Andrea Grottoli of the OSU School of Earth Sciences is thanked for allowing access to her laboratory to prepare solid samples for isotopic analysis. Dr. Bauer's lab manager, Amy Weber, generously contributed her time in showing me the ropes in the lab and answering many questions along the way. Jordan Myers is also thanked for assisting with the field work. Dr. Richard Phillips and his research group at Indiana University are also acknowledged for allowing me to visit his laboratory at Indiana University to learn about the root exudate collection method. Finally, I would like to thank my parents, Laura and Tom, for their support and encouragement throughout my studies.

## VITA

2011.....B.S. Environmental Science, Wayne State University

2012 to present .....Graduate Associate, Department of Evolution Ecology and Organismal Biology, The Ohio State University

## FIELDS OF STUDY

Major Field: Evolution, Ecology and Organizmal Biology

## TABLE OF CONTENTS

ABSTRACT ..... ii

ACKNOWLEDGEMENTS ..... v

VITA ..... vi

FIELDS OF STUDY ..... vi

TABLE OF CONTENTS ..... vii

LIST OF TABLES ..... x

LIST OF FIGURES ..... xii

INTRODUCTION ..... 1

MATERIALS AND METHODS ..... 7

Site description ..... 7

Root exudate collection ..... 8

Leaf collection ..... 10

Meteorological and net ecosystem exchange measurements ..... 11

Biomass estimates ..... 11

Leaf and root imaging and identification ..... 12

SOC and $\delta^{13}C$ measurements ..... 12

$\delta^{13}C$ and $\delta^{15}N$ measurements of leaves and roots ..... 13

Data analysis ..... 14

RESULTS ..... 15

Net root exudation rates ..... 15

$\delta^{13}C$ values of root exudates ..... 16

Root $\delta^{13}C$ values ..... 17

Leaf $\delta^{13}C$ values ..... 17

Comparison of silver maple leaf, root and root exudate $\delta^{13}C$ values ..... 18

DISCUSSION ..... 20

Relationship between photosynthesis and root exudation in bottomland trees. ..... 20

Drivers and implications of $\delta^{13}C$ variability of root exudates ..... 22

Sensitivity of root $\delta^{13}C$ to atmospheric conditions and photosynthetic rate ..... 25

Leaf properties and environmental drivers of leaf $\delta^{13}C$ ..... 26

Comparison of root exudation rates and extrapolation to stand scale ..... 28

Summary of leaf, root and exudate $\delta^{13}C$ and net exudation rates in a bottomland forest ..... 30

LITERATURE CITED ..... 31

APPENDIX A: TABLES ..... 39

APPENDIX B: FIGURES ..... 52

APPENDIX C: SUPPLEMENTAL INFORMATION ..... 62

Section 1. Root Exudate Collection Device Modifications ..... 63

Section 2. DNA barcoding to identify roots ..... 64

Section 3. Imputation and mixing model to estimate exudate $\delta^{13}C$..... 65

## LIST OF TABLES

**Table 1.** Definitions of the time lags calculated for environmental and NEE parameters used in the present study. All averages were computed over daylight values only (i.e., PAR>0). ..... 40

**Table 2.** Continuous and categorical parameters evaluated as potential predictors for net SOC exudation rates and for $\delta^{13}C$ values of bulk leaves, bulk roots, and root exudates in the present study. Parameters marked with an asterisk were assessed using various time lags as described in the Methods section. ..... 41

**Table 3.** Average (±SE) daily root exudate SOC fluxes and exudate SOC $\delta^{13}C$ values by sampling date and species. Within a species and measurement (i.e., exudate flux or $\delta^{13}C$), asterisks indicate significant differences at the $\alpha=0.05$ level. ND - not determined. ..... 42

**Table 4.** Average and observed ranges of leaf, root and exudate $\delta^{13}C$ values (all in ‰) arranged by species and sampling date. Values in parentheses represent ± 1 SE from the mean. Values with no SE value reported indicate sample size of n=1 for that observation. Asterisks indicate significant differences in leaf values between species at the $p<0.05$ level when assessed across all sampling dates. ND - not determined. ..... 44

**Table 5.** Adjusted $R^2$ values, residual standard error and significance level for the least squares regressions of various parameters as potential predictors of leaf $\delta^{13}C$. Significance levels are as follow: "ns"=not significant, "†" = significant at $\alpha \leq 0.10$, "*" = significant at $\alpha \leq 0.05$, "**" = significant at $\alpha \leq 0.01$, "***" = significant at $\alpha \leq 0.001$. NA - not applicable. ..... 45

**Table 6.** Adjusted $R^2$ values, residual standard error and significance level for least squares regressions of a various environmental parameters measured in the present study as potential predictors of leaf $\delta^{13}C$. Environmental parameter abbreviations and subscripts (lag times) are defined in the Methods section. Significance levels are as follows: "ns"=not significant, "†" = significant at $\alpha \leq 0.10$, "*" = significant at $\alpha \leq 0.05$, "**" = significant at $\alpha \leq 0.01$, "***" = significant at $\alpha \leq 0.001$..... 47

**Table 7.** ANOVA table comparing leaf and root $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study. ..... 49

**Table 8.** ANOVA table comparing leaf and root exudate $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study. ..... 49

**Table 9.** ANOVA table comparing root and root exudate $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study. ..... 49

**Table 10.** Average growing season NEE values for the nights (defined as when PAR<0) during which incubations took place compared to average daytime NEE for each root exudate incubation by sampling date. ..... 50

**Table 11.** Summary of net exudation rates reported for tree roots in this and other studies using a similar root exudate collection method. NR - not reported ..... 51

## LIST OF FIGURES

**Figure 1.** Daily SOC yields in individual incubations vs. a) root dry mass, b) root volume and c) root surface area. All plots include data from all root incubations and root samples from all collection dates in this study. Solid lines are least squares linear regressions and all relationships are significant at the $p<0.05$ level. ..... 53

**Figure 2.** Average (±SE) log transformed net root SOC exudation rates normalized to root dry mass vs. a) sampling date and b) average (±SE) $NEE_{inc}$ (NEE for the ~24 hour incubation period) for each sampling date. Both plots include data from all sampling dates and root samples measured in this study. Regression relationships for both plots are significant at the $p<0.05$ level. ..... 54

**Figure 3**. *A. saccharinum* leaf, root and root exudate $\delta^{13}C$ values for all sampling dates in the present study. Each point represents the means (±SE) for leaf (circles), root (squares) and root exudate (triangles) samples collected and measured on each date. ..... 55

**Figure 4.** Average (± SE) root exudate $\delta^{13}C$ values vs. a) Average $PAR_d$ (±SE) over the day during which the incubations were collected ($p<0.001$), b) Average VPD (±SE) over the two days prior to, and including the day of, the end of the incubations ($p<0.01$) and c) Average $T_{air}$ (±SE) over the three days prior to, and including the day of, the end of the incubations ($p<0.05$). The plots include data across all root incubations in this study. .. 56

**Figure 5.** Average (± SE) bulk root $\delta^{13}C$ values vs. a) Average NEE for the day of sample collection and the preceding six days prior to sampling ($p<0.5$) b) Average VPD for the day of sample collection and the preceding six days prior to sampling and c) Average RH for the day of sample collection and the preceding six days prior to sampling. The solid lines represent least squares linear regressions. All plots show average $\delta^{13}C$ values include for all roots collected from all tree species on each sampling date in this study. ..... 57

**Figure 6.** a) $\delta^{13}C$ values and b) $\delta^{15}N$ values of leaves of the tree species in the present study. Solid horizontal lines within each box represent median values and dotted horizontal lines represent mean values. For each plot, differing letters between species indicate significant differences at the $p<0.05$ level. ..... 58

**Figure 7.** Plots of leaf $\delta^{13}C$ vs. a) leaf carbon content ($C_{leaf}$), b) leaf nitrogen content ($N_{leaf}$), c) leaf % C, d) leaf % N, e) leaf area normalized C content ($C_{area\text{-}leaf}$), and f) leaf area normalized N content ($N_{area\text{-}leaf}$). AN=*A. negundo*, AS= *A. saccharinum*, LM=*L. maackii*, PD=*P. deltoides* and PO=*P. occidentalis*..... 59

**Figure 8.** Average (±SE) leaf $\delta^{13}C$ for each sampling date across all species vs. a) % daylight ($p<0.05$), b) $NEE_7$ ($p<0.10$) and c) $PARd_1$ ($p<0.05$). Solid lines are linear least squares regressions..... 60

**Figure 9.** Comparisons of glass bead root incubation substrates and their pre-treatment methods on contaminant SOC. a) A significant difference was found (t-test; $p= 0.004$) between the Labconco Ultra-Pure water and acid washed (10% HCl) and baked (400 $^{o}$C) soda lime beads. b) No significant difference (t-test; $p= 0.41$, $n=4$) was found between

Labconco Ultra-Pure water and baked (550 ºC) borosilicate beads. Error bars are ± 1 SD about the mean peak integration area. Peak integration areas are directly related to the concentration of OC in the samples. ..... 61

# INTRODUCTION

Organic carbon (OC) originating from plant roots is a major contributor to both reactive and stabilized OC pools in soils (Rasse *et al.*, 2005; Kramer *et al.*, 2010; Schmidt *et al.*, 2011). The transfer of soluble organic carbon (SOC) from plant roots to soils via exudation is one of the least understood and most difficult to measure components of terrestrial net primary productivity (NPP) and net ecosystem production (NEP) (Grayston *et al.*, 1997; Clark *et al.*, 2001; van Hees *et al.*, 2005; Chapin *et al.*, 2009). Root exudation has been estimated to account for 1-40% of NPP and the contributions of exudates to NEP is essentially unknown (Grayston *et al.*, 1997; Chapin *et al.*, 2011). Root exudation includes all active and passive loses of soluble organic matter (which includes C, N and P components) from living roots (Jones *et al.*, 2009) and the potentially significant loss of SOC by exudation has important implications for how the belowground component of the terrestrial carbon cycle is quantified and modelled (Heimann & Reichstein, 2008; Chapin *et al.*, 2009). For example, inputs of biologically reactive SOC to soils via root exudation may impact the size of the total soil OC pool (Schmidt *et al.*, 2011) and contribute to the so-called microbial 'priming effect', whereby the degradation of refractory soil OC is stimulated by heterotrophic microbial growth and metabolism (Kuzyakov, 2010).

Root exudation may also contribute to lateral exports of SOC from terrestrial ecosystems via hydrologic flow paths (Cole *et al.*, 2007; Marín-Spiotta *et al.*, 2014). At

the same time, these export losses from soils may be a source of reactive SOC to groundwater and streams and help support the net heterotrophy observed in nearly all inland waters (Raymond *et al.*, 2013; Lauerwald *et al.*, 2015). The idea that soil and root derived SOC may contribute to aquatic carbon budgets has been supported by a number of studies (e.g., Cole & Caraco, 2001; Sanderman *et al.*, 2009; Aufdenkampe *et al.*, 2011; Hossler & Bauer, 2013) and as a result is an important consideration for understanding the biogeochemistry of both terrestrial and aquatic ecosystems.

Within the plant-soil system, roots have also been observed to take up SOC from soil, but the overall magnitude of uptake relative to exudation is thought to be negligible (Jones *et al.*, 2005; Kuzyakov & Jones, 2006). Root exudation of SOC may benefit plants by increasing nutrient availability (reviewed by Dakora & Phillips, 2002). For example, several studies of upland systems suggest a link between net tree root SOC exudation rates and enhanced N availability in soils (Phillips *et al.*, 2011; Brzostek *et al.*, 2013; Yin *et al.*, 2014). Root exudates have also been suggested to be a source of labile SOC to denitrifying bacteria in wetlands (Burgin & Groffman, 2012; Zhai *et al.*, 2013) and stream sediments (Schade *et al.*, 2001).

Root exudation has been invoked as a source of SOC in forested riparian systems (O'Neill & Gordon, 1994; Martin *et al.*, 1999) and has recently been included in a model describing C and N cycling in riparian soils (Batlle-Aguilar *et al.*, 2012). Riparian forests have been observed to exhibit both high mineral N uptake by plants and high microbial denitrification rates, especially in zones immediately surrounding plant roots (Peterjohn

& Correll, 1984; Hill, 1996). Such observations have led to the designation of riparian systems as 'hot spots' of biogeochemical activity that may be critical for limiting anthropogenic nutrient loadings to inland waters (McClain *et al.*, 2003). However, to our knowledge, net exudation rates and chemical composition of root derived SOC for temperate bottomland hardwood trees have not yet been measured *in situ*.

Natural abundance $\delta^{13}C$ measurements have been used to assess SOC sources, inputs and cycling in terrestrial and aquatic systems, as well as to make inferences about plant physiological processes (Peterson & Fry, 1987; Farquhar *et al.*, 1989; Ehleringer *et al.*, 2000; Bowling *et al.*, 2008). Farquhar *et al.* (1982) modelled the isotopic fractionation of $CO_2$ ($\Delta$, the ‰ difference in $\delta^{13}C$ between source $CO_2$ and OC product) during photosynthesis in $C_3$ terrestrial plants. Within the Farquhar *et al.* (1982) model the partial pressure of $CO_2$ in the intercellular leaf space relative to that of the atmosphere, $p_i/p_a$, suggests that isotopic fractionation during photosynthesis is sensitive to the supply of and demand for $CO_2$ at the molecular level.

Empirical data support the model proposed by Farquhar *et al.* (1982). For example, leaves with greater photosynthetic capacity and demand for $CO_2$ (e.g., as indicated by light availability or high N content per leaf area (Niinemets, 1999; Poorter *et al.*, 2009)) have been found to be elevated in $\delta^{13}C$ relative to leaves with lower demand for $CO_2$ in a number of studies (e.g., Jackson *et al.*, 1993; Sparks & Ehleringer, 1997; Domínguez *et al.*, 2012). Time lagged environmental conditions (e.g., vapor pressure deficit) that influence stomatal conductance, and therefore the supply of $CO_2$ and $p_i/p_a$,

have also been reported to be correlated to the $\delta^{13}C$ of plant respired $CO_2$ or phloem sap on the order of hours to days (see reviews by Kuzyakov & Gavrichkova, 2010; Mencuccini & Hölttä, 2010; Brüggemann *et al.*, 2011).

Recently there has been an increasing focus on examining the factors responsible for observed variations of $\delta^{13}C$ in plant materials such as leaves and roots, and the $\delta^{13}C$ of autotrophically respired $CO_2$ (Ehleringer *et al.*, 2000; Badeck *et al.*, 2005; Cernusak *et al.*, 2009). A better understanding of both photosynthetic fractionation and post-photosynthetic fractionation will ultimately help explain variation of $\delta^{13}C$ in ecosystem pools (Bowling *et al.*, 2008). While post-photosynthetic fractionation processes that lead to differences in $\delta^{13}C$ within plant materials are less well understood than photosynthetic $CO_2$ fractionation, there is strong evidence that roots of terrestrial $C_3$ plants are consistently enriched in $^{13}C$ compared to leaf material (Badeck *et al.*, 2005; Bowling *et al.*, 2008; Cernusak *et al.*, 2009). For woody $C_3$ plants, available evidence suggests that $\delta^{13}C$ values of respiratory $CO_2$ from roots are also enriched relative to bulk leaf material (Ghashghaie & Badeck, 2014), but the effects of different methodologies used to capture root-respired $CO_2$ for $\delta^{13}C$ analysis is largely unknown (although see Snell *et al.*, 2015).

Although heterotrophic remineralization of SOC from root exudates may contribute as much as 20% of the total soil $CO_2$ flux to the atmosphere (van Hees *et al.*, 2005), there is currently a lack of information about the $\delta^{13}C$ signatures of root exudate SOC. Characterizing the $\delta^{13}C$ of root-derived SOC (and the $CO_2$ produced from the respiration of this SOC) and root respired $CO_2$ is of interest because of its potential use as

a natural tracer of heterotrophic and autotrophic respiration contributions to soil $CO_2$ efflux (Bowling *et al.*, 2008; Midwood *et al.*, 2008; Moyes *et al.*, 2010; Snell *et al.*, 2015). Previous studies have measured the $\delta^{13}C$ of laboratory-extracted SOC from roots and have suggested that it is similar to or slightly enriched by ~1‰ relative to root tissue measured on the same plant (Göttlicher *et al.*, 2006; Gessler *et al.*, 2007). There is a critical need for measurements of $\delta^{13}C$ values of root-exuded SOC collected *in situ* in order to evaluate it relative to bulk leaf and root $\delta^{13}C$ and further explore natural variation in $\delta^{13}C$ of major plant OC components (leaves, roots and root exudates) stored in and exported from both living plant biomass and terrestrial ecosystems in general.

The present study estimated net root exudation rates of SOC *in situ* and measured the $\delta^{13}C$ values of exudates, leaves and roots. Both exudation rates and $\delta^{13}C$ values were evaluated in relation to leaf and root morphology, leaf and root C and N contents and $\delta^{15}N$ values, and a variety of relevant environmental and meteorological parameters in a temperate hardwood bottomland forest. The overarching hypotheses were that 1) net root exudation rates in different tree species are controlled by time-lagged environmental conditions that influence photosynthetic rates and the amounts of potentially exchangeable root organic materials (e.g. as indicated by root mass), 2) $\delta^{13}C$ values of root exudates are related to time-lagged environmental conditions that influence $p_i/p_a$ during photosynthesis and 3) $\delta^{13}C$ values of leaves are related to both leaf-scale measurements that reflect photosynthetic capacity and time lagged-environmental parameters that influence photosynthetic $\delta^{13}C$ fractionation. Findings from this study

were further used in a comparative analysis of $\delta^{13}C$ signatures between leaf, root and root exudate OC and to scale net root exudation rates of SOC to the entire hardwood bottomland forest sampling area.

# MATERIALS AND METHODS

## Site description

The present study was conducted at the Wilma H. Schiermeier Olentangy River Wetland Research Park (ORWRP), located at The Ohio State University (OSU) in Columbus, Ohio. ORWRP is a 0.21 $km^2$ facility and contains a number of habitats (e.g., experimental wetlands, bottomland forest, oxbow lake) that have been used for research purposes since the establishment of the facility in the early 1990s (Mitsch & Wilson, 1996; Mitsch *et al.*, 2012).

Sampling was conducted in the 5,600 $m^2$ forested area between the two experimental wetlands located at the site. The sampling area shares characteristics of bottomland forest in the region (e.g., similar vegetation) and portions of the sampling area are occasionally inundated during large precipitation events (Brown & Peterson, 1983; Vadas & Sanger, 1997; Dudek *et al.*, 1998). The sampling area has undergone natural succession since the establishment of the site as a research park. The dominant tree species that have colonized the area include silver maple (*Acer saccharinum*), box elder (*Acer negundo*), red maple (*Acer rubrum*), eastern cottonwood (*Populus deltoides*) and black willow (*Salix nigra*). American sycamore (*Platanus occidentalis*) is also present at the site, but in lower numbers than the other species. The understory consists largely of bush honeysuckle (*Lonicera maackii*) in shaded areas and goldenrod (*Solidago* spp.) where the canopy is open.

## Root exudate collection

Tree root exudates were collected (n=6-8 per sample outing) five times throughout the summer and autumn 2014 on the following dates: 17-18 July, 31 Jul-1 Aug, 14-15 Aug, 28-29 September and 16-17 November. A modified version of the method initially described by Phillips *et al.* (2008) was used to collect root exudates and is described briefly below, with additional modifications to the method detailed in **Supplemental Information Section 1**.

*Root excavation and pre-conditioning.* Intact terminal fine roots (≤ 2 mm diameter) were excavated with forceps from the upper 10 cm of soil with extreme care taken to avoid root damage. Once excavated, each root system was carefully rinsed with distilled water, and any particulates adhering to the roots carefully removed. The goal of this step was to remove as much non-root material as possible while limiting root damage. Because the excavation process may stress the roots, the root system was reburied loosely in soil collected from the site for a for a 24-72 hour recovery period prior to the start of the incubations.

Following this recovery period, the root system was excavated again, carefully rinsed with distilled water and then placed into the exudate collection device which consisted of a 60 mL high density polyethylene syringe barrel with a stopcock fitting (both pre-soaked in 10% HCl) to allow for the introduction and recovery of the exudate solution. After the root was placed into the collection device it was then backfilled with pre-baked (500$^{o}$ C) 1 mm diameter borosilicate glass beads (Walter Stern, Inc.) using a

baked (500° C) glass funnel and then sealed with baked (500° C) aluminum foil and Parafilm. Both the form of the glass beads and the pre-baking procedure were found to be critical for removing all traces of contaminant soluble organic carbon (SOC) from the beads prior to use (**Supplemental Information Section 1**). Because we did not find a significant difference between SOC concentrations of incubated beads and Labconco Ultra-Pure water in preliminary tests there was no blank correction applied to our experimental incubations (**Supplemental Information Section 1**).

Prior to root exudate collection, a 24 hr pre-conditioning period was conducted by adding 20 ml of a 1 mM $CaCl_2$ solution through the bottom of the collection assembly to maintain root turgor and limit osmotic stress. Preliminary tests indicated that there was no measureable SOC contamination in the $CaCl_2$ solution compared to that of Labconco Ultra-Pure laboratory water. The incubation assembly was then wrapped in aluminum foil to avoid exposure to light and reburied in the soil. Landscaping cloth was installed over the device and covered with leaf litter in order to simulate soil conditions and limit exposure to light and animals at the site.

*Initiation of root incubations.* Following root pre-conditioning, the solution in the device was flushed three times via the stopcock using 1 mM $CaCl_2$ solution. The solution was drawn out of the incubation device under vacuum using a peristaltic pump and discarded. Each assembly was flushed a total of three times with 1 mM $CaCl_2$ to ensure a more complete removal of SOC prior to the initiation of the experimental incubations

(*sensu* Phillips et al. 2008). Following the third flush, 20 ml of fresh 1 mM $CaCl_2$ solution was added, and the device was reburied for the 24 hr experimental incubation.

*Termination of root incubation and sample collection.* Following incubation, the exudate collection assembly was filled to capacity with fresh 1 mM $CaCl_2$, and the solution from three separate flushes was collected into individual glass serum bottles (pre-baked at 500$^{o}$ C) that were wrapped in aluminum foil and fitted with crimp-top acid-rinsed Teflon-faced silicone septa. The samples were stored on ice in the dark for transport back to the laboratory. In the lab, samples were filtered through baked (500 $^{o}$C) glass fiber filters (Whatman GF/F, 0.7 μm nominal pore size), and frozen at -20 $^{o}$ C until analysis. The plant roots used in the incubations were detached from the plant after exudate collection and immediately frozen until laboratory processing.

## Leaf collection

Leaves were collected from trees in the study area on 17 July, 31 July, 14 August, 27 August and 28 September 2014. Leaves were not collected in November because trees had senesced and leaf fall had occurred by that time. Leaf material was collected from *Acer negundo*, *Acer saccharinum*, *Lonicera maackii*, *Platanus occidentalis* and *Populus deltoides* and frozen at -2 $^{o}$C until processing. For each tree species, four leaves were collected at ~2m height by nitrile gloved hand and ~7m (mid-canopy) height using a telescoping vegetation pruner for a total of 8 leaves per tree species for each sampling date. Upper canopy leaves (>7m) were not included in this study because they were not accessible at the time of sampling. *Acer negundo* leaves were not collected on 17 July

and only leaves from ~7m were collected on 31 July. Only 3-4 leaves were collected per sampling date for *Lonicera maackii* and no canopy position distinction was made because it is an understory plant.

### Meteorological and net ecosystem exchange measurements

The ORWRP is part of the Ameriflux network (http://ameriflux.ornl.gov/fullsiteinfo.php?sid=223) and gas flux measurements of $CO_2$ have been measured continuously to estimate net ecosystem exchange (NEE) since 2010 (Morin *et al.*, 2014a). Environmental measurements including direct and diffuse photosynthetically active radiation (PAR and PARd, respectively), air and soil temperature ($T_{air}$ and $T_{soil}$, respectively) and relative humidity (RH) were also measured continuously over the course of the study. From this data, other parameters such as vapor pressure deficit (VPD) were estimated (Morin *et al.*, 2014b). Details of the methodologies used for meteorological and NEE measurements are described in Morin *et al.* (2014b).

Time lags of various environmental parameters and NEE measurements were used in regression analyses of net SOC exudation rates, and $\delta^{13}C$ values of leaves, roots and exudates. The duration of each time lag is indicated as a subscript that follows each parameter (e.g., a 7 day time lag for NEE is termed $NEE_7$) and are defined in **Table 1**.

### Biomass estimates

Diameter at breast height (DBH), and genus for all trees ≥ 3 cm DBH (~1500 stems) within the sampling area, were recorded in spring 2015. The DBH measurements

were used to estimate fine root biomass using generalized biomass equations presented in Chonjacky *et al.* (2014). The resulting fine root biomass estimates do not account for trees with DBH<3 cm, shrubs (e.g., *L. maackii*) or herbaceous species (e.g., *Solidago* spp.) at the site. Fine root biomass estimates of trees >3cm DBH were used to scale net root exudation rates to the entire sampling area.

The areal size of the sampling area was estimated using the Polygon feature in Google Earth Pro (© 2015 Google Inc.), which allows the estimation of areas based on user generated polygons overlain on satellite imagery. This area estimate was used in conjunction with the biomass estimates and root level net exudation rate data in order to estimate SOC root exudation rates for the entire sampling area.

### Leaf and root imaging and identification

Leaves and roots were scanned to a computer using a Dell V105 at 600 dpi and images were then converted to black and white using GIMP (2.8.14). Leaf projected area was then determined using ImageJ (1.48v). Root images were analyzed in WinRhizo® to assess surface area, projected area, volume and number of root tips. In order to identify tree roots to genus or species, the DNA barcoding approach described by Kesanakurti *et al.*, (2011) was used. Details of this approach are described in **Supplemental Information Section 2**.

### SOC and $\delta^{13}C$ measurements

Concentration and $\delta^{13}C$ of SOC from *in situ* incubations were measured using a modified version of the method described by Osburn & St-Jean (2007). Briefly, an

Oceanographics International model 1030C DOC analyzer was interfaced with a modified Graden $CO_2$ trap and a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer (IRMS). $\delta^{13}C$ values (in ‰) are reported relative to the Vienna-PDB standard. All of the flush 1 samples from *in situ* root exudate incubations and a selected number of flushes 2 and 3 were analyzed for SOC concentration and $\delta^{13}C$. We used a statistical imputation method to estimate $\delta^{13}C$ and SOC concentration for flushes 2 and 3 that could not analyzed directly (i.e., analytical difficulties resulting from extremely low amounts of SOC) as described above and a mixing model was used to estimate the final exudate $\delta^{13}C$ from the combination of each of the three measured or modelled flushes (**Supplemental Information Section 3**).

### $\delta^{13}C$ and $\delta^{15}N$ measurements of leaves and roots

Bulk leaf and root samples were dried to a constant mass at 60°C, then ground with mortar and pestle (rinsed with Labconco Ultra-Pure water and methanol between samples). The ground samples were then acid-fumed using concentrated HCl to remove any inorganic carbon in the samples. The samples were then packed into tin boats and submitted to the Stable Isotope Facility at University of California Davis for analysis by elemental analysis (EA)-IRMS for C and N contents, and $\delta^{13}C$ and $\delta^{15}N$ values. $\delta^{13}C$ and $\delta^{15}N$ compositions (in ‰) are reported relative to the V-PDB and air standards, respectively.

## Data analysis

Linear regression analysis was used to test for significant relationships between continuous and categorical predictor variables listed and defined in **Table 2** and the response variables of net exudation rates, exudate SOC $\delta^{13}C$, bulk root $\delta^{13}C$ and bulk leaf $\delta^{13}C$. ANOVA was used to test for significant differences within $\delta^{13}C$ values of root exudate, roots and leaves across different sampling dates and species. ANOVA was also used to test for differences between $\delta^{13}C$ of exudate, roots and leaves across different sampling dates. When applicable and ANOVA results were significant, *post-hoc* t-tests with the Bonferroni correction for multiple comparisons were used to assess where significant differences arose. All statistics were performed in R version 3.2.0 (R Core Team, 2015).

## RESULTS

### Net root exudation rates

The net amount of root SOC collected in the field incubations showed significant positive relationships to dry root mass, root volume and root surface area ($p<0.05$ for each comparison) when assessed across all roots incubated and collected in this study (**Figure 1a-c**). In order to compare net root exudation rates across sampling dates, species and environmental conditions, individual incubations were standardized by dividing the daily (~24 hr) SOC yield by dry root mass.

Net exudation rates varied between 4 and 90 μmol g root$^{-1}$ day$^{-1}$ across all sampling dates and species (**Table 3**). ANOVA results indicate that net exudation rates were not equivalent across all sampling dates ($F_{4,29}=6.98$, $p<0.001$), however, the only pairwise comparisons that were significantly different were the greater net exudation rates on the 17-18 July sampling compared to all other sampling dates (**Table 3**). At the species level, net exudation rates for *A. saccharinum* and *P. occidentalis* were not significantly different from each other. *P. deltoides* net exudation rates tended to be higher than *A. saccharinum* and *P. occidentalis.* However the small sample size for *P. deltoides* (*n*=2 over the entire study) precluded the test for statistical differences in net exudation rates between *A. saccharinum* and *P. occidentalis*.

For all tree species combined, the average net exudation rate per sampling date decreased significantly ($p<0.05$) over the duration of the study (**Figure 2a**). Net root

exudation rates also showed a statistically significant positive relationship with NEE averaged over the daylight hours (i.e., PAR>0) of each 24 hr incubation (**Figure 2b**)

### $\delta^{13}C$ values of root exudates

Across all sampling dates and species root exudate $\delta^{13}C$ averaged (±SE) -28.8‰ (± 0.4) and the range of values observed varied between -35.0‰ and -25.7‰ (**Table 3** and **4**). There was no significant difference in exudate $\delta^{13}C$ values between species, but ANOVA results indicate a significant differences in exudate $\delta^{13}C$ between sampling dates ($F_{4,29}$=7.989; p<0.001) (**Table 3**). For *A. saccharinum* there was also a trend of progressive enrichment of average exudate $\delta^{13}C$ from -32.2 to -28.0‰ over the first through third sampling dates (17-18 Jul to 14-15 Aug) where a plateau was reached and sustained for the last two sampling dates (28-29 Sep = -27.4‰ and 16-17 Nov = -28.1‰) (**Table 3** and **Figure 3**). Similar enrichment then stabilization trends of exudate $\delta^{13}C$ were also observed for the other species (*P. deltoides* and *P. occidentalis*), but these species were not sampled during every collection period (**Table 3**).

Environmental parameters measured up to three days prior to sampling until termination of the incubation showed the largest number of significant relationships with exudate $\delta^{13}C$ compared to environmental variables calculated using different time intervals (up to 7 days prior to the termination of the incubation). For root exudate $\delta^{13}C$, significant (p<0.05) positive relationships with $VPD_{02}$ and $T_{air03}$ and negative relationships $PARd_0$ were found (**Figure 4a-c**).

## Root $\delta^{13}C$ values

The average (± SE) of root $\delta^{13}C$ values was -29.0‰ (± 0.1) and the range of observed root $\delta^{13}C$ values varied between -30.4 and -27.2 ‰, across all sampling dates and species (**Table 4**). ANOVA results indicate no significant difference in root $\delta^{13}C$ values between species ($F_{2,31}$=2.43, p>0.1) or sampling date ($F_{4,29}$=1.90, p>0.1) (**Figure 3** and **Table 4**). No significant relationships between root scale measurements (e.g., surface area, root nitrogen content) and root $\delta^{13}C$ were identified apart from a weak but significant negative relationship (p <0.05, adj. $R^2$=0.09) between $\delta^{15}N$ and $\delta^{13}C$ when all roots were assessed together (data not shown). Root $\delta^{13}C$ values showed a significant positive relationship with $NEE_7$ (p<0.05, adj. $R^2$=0.11) and $VPD_7$ (p<0.05, adj. $R^2$=0.08) (**Figure 5 a-b**). A significant negative relationship between root $\delta^{13}C$ and $RH_7$ was also identified (p<0.05, adj. $R^2$=0.13) (**Figure 5c**).

## Leaf $\delta^{13}C$ values

Leaf $\delta^{13}C$ values averaged (±SE) -30.8‰ (± 0.1) and varied between -33.5‰ and -26.7‰ across all sampling dates and species (**Table 4**). ANOVA test results indicate a significant difference in leaf $\delta^{13}C$ between species ($F_{4,159}$ = 21.64, $p$<0.001). *Post-hoc* multiple comparisons indicate that all pairwise comparisons between species were significantly different from each other, except for the *A. saccharinum—P. occidentalis* and *A. saccharinum—A.negudo* comparisons, which were not significantly different (**Figure 6a** and **Table 4)**. Average leaf $\delta^{15}N$ also showed significant differences ($F_{4,159}$ = 39.83, $p$<0.001) between species, as summarized in **Figure 6b**.

Results of simple linear regression between leaf $\delta^{13}C$ values and leaf properties assessed for both each species individually as well as for all species combined are shown in **Table 5** and **Figure 7**. The only predictor variables that showed significant relationships across all species were those that included a mass measurement standardized by leaf area (i.e., LMA, $C_{area\text{-}leaf,}$ $N_{area\text{-}leaf}$). These predictors also tended to display the highest $R^2$ values and lowest p-values. For the combined leaf data, all leaf scale measurements assessed in this study were positively and significantly related to leaf $\delta^{13}C$ except for leaf C:N which showed an inverse relationship and $\delta^{15}N$ which was not related to leaf $\delta^{13}C$ values (**Table 5**).

The 7-day average of PAR and % daylight prior to sampling was significantly related to *A. saccharinum*, *L. maackii* and *P. occidentalis* and combined leaf $\delta^{13}C$ (**Table 6** and **Figure 8a)**. *A. saccharinum* showed a number of significant relationships with the time averaged variables including $VPD_7$, $PAR_{d7}$, $RH_7$ and $NEE_7$. *P. occidentalis* leaves also showed significant relationships with $T_{air}$ and both *P. occidentalis* and *L. maackii* leaves were significantly related to $NEE_7$ (**Table 6**). $NEE_7$ and $PAR_d$ were also significantly related to combined leaf $\delta^{13}C$ (**Table 6** and **Figure 8b-c**). *A. negundo* and *P. deltoides* leaves did not show relationships with any of environmental parameters or with NEE.

### Comparison of silver maple leaf, root and root exudate $\delta^{13}C$ values

*A. saccharinum* was the only species for which leaves, roots and root exudates were collected with replication across all sampling dates. Because of this we focused the

comparison of $\delta^{13}C$ of different plant components (i.e., leaves, roots and exudates) on *A. saccharinum,* although the other species for which we have limited data qualitatively follow similar trends. Average (± SE) $\delta^{13}C$ values across sampling dates for *A. saccharinum* leaves, roots and exudates were -30.4‰ (± 0.2), -28.9‰ (± 0.2) and -28.8‰ (± 0.4), respectively (**Table 4**). ANOVA results indicate significant differences in $\delta^{13}C$ values between leaves and roots, but the differences are dependent on sampling date ($F_{5,46}$= 29.9, $p$<0.001) (**Figure 3** and **Table 7**). On average, $\delta^{13}C$ values of *A. saccharinum* roots were enriched by 1.5‰ compared to leaves.

Leaf and root exudate $\delta^{13}C$ values for *A. saccharinum* were also found to be significantly different from each other ($F_{5,46}$= 14.0, $p$<0.001) (**Figure 3** and **Table 8**). Root exudate $\delta^{13}C$ was enriched by an average of 1.6‰ compared to leaves. While the main effect of sampling date was not a significant factor, the interaction effect between plant component (i.e., leaf or exudate) and sampling date was significant.

The $\delta^{13}C$ values of roots and root exudates for *A. saccharinum* did not differ significantly from each other (**Figure 3** and **Table 9**). However, sampling date and the interaction effect between sampling date and plant component (i.e., exudate or root) was significant. The first sampling period (17-18 Jul) root exudate $\delta^{13}C$ was depleted relative to root material. For the second sampling period (31 Jul- 1 Aug) average root and root exudate $\delta^{13}C$ were nearly identical. For the last three sampling periods (14-15 Aug, 28-29 Sep and 16-17 Nov), root exudate $\delta^{13}C$ tended to be enriched in $\delta^{13}C$ by ~1 ‰ compared to the roots from which they were collected (**Figure 3**).

## DISCUSSION

### Relationship between photosynthesis and root exudation in bottomland trees.

A significant inverse relationship was found to exist in the present study between temperate hardwood tree net SOC exudation rates and NEE (**Figure 2b**). This relationship suggests that as photosynthetic rate increases (i.e., NEE decreases), there is a corresponding increase in translocation of tree SOC from aboveground to belowground where it is lost from roots through exudation. The variation in NEE during the growing season (i.e., prior to leaf senescence) in the present study was likely driven by differences in photosynthetic uptake of $CO_2$ rather than by ecosystem respiration because average night time (i.e., PAR<0) NEE varied by a maximum of 0.5 μmol C $m^{-2}$ $s^{-1}$ between sampling dates whereas the observed differences in average NEE during incubations across sampling dates were much larger (as high as 3 μmol C $m^{-2}$ $s^{-1}$) (**Figure 2b** and **Table 10**). Our finding of a link between photosynthetic rates and net root exudation rates provides support for the idea that temperate tree primary production is related to the magnitude of SOC lost via exudation to soil and rhizosphere microbial communities (Farrar & Jones, 2000; Kuzyakov & Gavrichkova, 2010). To our knowledge this is the first study to demonstrate such a relationship between tree photosynthesis (i.e., as NEE) and tree root exudation rates under field conditions.

We suggest that the higher exudation rates observed for the first sampling period in the present study may also have resulted from greater allocation of SOC belowground

to support the production of fine root biomass at the start of the growing season. Actively growing roots have been shown to exude more SOC than established roots in other studies (Rovira, 1969; Walker *et al.*, 2003). Therefore, sampling times with high root growth would be expected to have correspondingly high exudation rates. A recent report of *in situ* hardwood tree root growth measurements in Pennsylvania, USA showed that trees of the *Acer* genus (the same genus from which the majority of exudate samples were collected in this study) exhibit temporally concentrated fine root growth that typically peaked between April and early July (McCormack *et al.*, 2014). While root growth was not specifically assessed in the present study, it is likely that fine root biomass production already peaked by the time of the first sampling period. As a result, we may have captured root exudates from around the time when fine root production was decreasing or had ceased, thus contributing to the trend of progressively lower exudation rates over the course of the study (**Figure 2a**). The present study also provides information on root SOC exudation rates for temperate hardwood trees following leaf senescence (i.e., 16-17 Nov sampling). Interestingly, the average net root exudation rates for the 16-17 Nov sampling were not significantly lower than other sampling times apart for when compared to the initial sampling on 17-18 Jul (**Table 3**). The lack of significant differences in exudation rates between sampling dates pre- and post-leaf senescence in the present study (apart from the 16-17 Nov—17-18 Jul comparison) suggests that other factors beyond those assessed in the present study also influence net exudation rates.

## Drivers and implications of $\delta^{13}C$ variability of root exudates

For the only species we were able to measure the $\delta^{13}C$ of root exudate SOC for the entire study period, *A. saccharinum*, we observed a gradual increase in $\delta^{13}C$ over the first three sampling periods then a stabilization of root exudate $\delta^{13}C$ over the last two sampling periods (**Figure 3** and **Table 3**). While root exudates for the other species measured in the present study (*P. occidentalis* and *P. deltoides*) were not collected for every sampling period, similar general findings of increasing exudate $\delta^{13}C$ followed by stabilization of $\delta^{13}C$ values were observed for these species over sampling periods they were measured (**Table 3**).

We suggest that the apparent trend of gradual enrichment then stabilization of root exudate $\delta^{13}C$ observed over the course of the growing season in the present study is attributable to a shift in the relative contributions to root exudates of two or more plant SOC pools that differ in their $\delta^{13}C$ values. Specific SOC pools and their $\delta^{13}C$ values that may have contributed to the temporal changes in root exudate $\delta^{13}C$ reported in **Figure 3** and **Table 3** were beyond the scope of the present study. However, it is noteworthy that we found $\delta^{13}C$ values of bulk root material to be enriched by as much as 5.1‰, 3.2‰ and 4.5‰ compared to leaves for *A. saccharinum*, *P. deltoides* and *P. occidentalis*, respectively (**Table 4**). The same degree of enrichment in $^{13}C$ between bulk root and leaf tissue observed in the present study is predicted to also be reflected in the $\delta^{13}C$ measurements of root and leaf SOC pools (e.g., bulk tissue vs. extracted SOC $\delta^{13}C$ values reported by Göttlicher *et al.* (2006) differed by < ~1‰). Therefore the lower $\delta^{13}C$

exudate values observed during the first two sampling times (17-18 Jul and 31 Jul-1 Aug) for the present study may have resulted from a relatively larger allocation of $^{13}C$-depleted leaf-derived SOC and a relatively smaller contribution of $^{13}C$-enriched root-derived SOC compared to other sampling periods.

Additional support for leaf-derived SOC contributing greater relative amounts of $^{13}C$-depleted exudate SOC in the early sampling times and growing season of the present study comes from the significant positive trend between NEE and bulk root $\delta^{13}C$ (**Figure 5a**). We suggest that this pattern may arise from a greater allocation of $^{13}C$-depleted leaf SOC to roots when photosynthetic rates are high (**Figure 5a**). The association between higher net root exudation rates and higher photosynthetic rates in the present study also suggests that there is a quantitatively significant transfer of recently photosynthesized SOC (i.e., relatively depleted in $^{13}C$) belowground during the sampling periods in the earlier part of the growing season (**Figure 2b**). That we observed evidence of the same general trend of enrichment then stabilization of exudate $\delta^{13}C$ over the course of the study for all species (**Table 3**) suggests the temporal patterns in exudate $\delta^{13}C$ are not restricted to *A. saccharinum* and may be generalizable to other bottomland temperate hardwood trees and forests.

$\delta^{13}C$ exudate values were also found to be correlated to time-lagged environmental conditions (e.g., VPD, $T_{air}$)(**Figures 4a-c**). As VPD increases (as a function of RH and $T_{air}$) a predicted physiological response would be for the tree to close its leaf stomata, leading to lower $p_i/p_a$ in the leaf and greater enrichment of $\delta^{13}C$ of newly

synthesized photosynthate due to decreased kinetic fractionation as a result of limited diffusion of $CO_2$ into the leaf (Farquhar *et al.*, 1989; Mortazavi *et al.*, 2005). The weak but statistically significant positive relationships between exudate $\delta^{13}C$ and $VPD_{02}$ and $T_{air03}$ (**Figure 4b-c**) suggest that a portion of root exudate SOC is derived from recently synthesized OC that is transferred belowground on the order of several days after assimilation in the canopy. The significant relationships between root exudate $\delta^{13}C$ and time lagged VPD and $T_{air}$ supports the contention that $\delta^{13}C$ values of exudates are in part driven by environmental conditions that influence the leaf $p_i/p_a$ during photosynthesis. However, the generally weak relationships between exudate $\delta^{13}C$ and $VPD_{02}$ and $T_{air03}$ suggest additional factors that were not fully addressed in this study also interact with environmental conditions to influence exudate $\delta^{13}C$.

The identification of significant relationships between exudate $\delta^{13}C$ and time lagged environmental conditions is noteworthy because there have been a number of previous studies that have demonstrated correlations between $\delta^{13}C$ of soil $CO_2$ and time lagged environmental conditions (e.g, VPD) and have attributed these relationships solely to autotrophic respiration (Ekblad & Högberg, 2001; Kuzyakov & Gavrichkova, 2010; Brüggemann *et al.*, 2011). However, the findings in the present study of significant relationships between exudate $\delta^{13}C$ and time lagged environmental conditions (**Figure 4 a-c**) provides evidence that heterotrophic remineralization of root exudates also has the potential to contribute to the observed relationships between soil $\delta^{13}CO_2$ and environmental conditions such as VPD and $T_{air}$.

The $\delta^{13}C$ values of tree root exudates in the present study, and their comparison to bulk leaf and root $\delta^{13}C$ values are to our knowledge the first measured for any terrestrial system. We suggest that future studies using $\delta^{13}C$ as a natural tracer of soil $\delta^{13}CO_2$ measurements would benefit by accounting for the remineralization of root exudates because i) root SOC exudates may account for as much as 20% of soil respiration (van Hees *et al.*, 2005), ii) root SOC represents an as-yet poorly understood loss of tree NPP and source of NEP (Clark *et al.*, 2001; Chapin *et al.*, 2009), and iii) the findings from the present study suggest that root SOC exudation and $\delta^{13}C$ values are highly variable and influenced by a number of environmental and plant level processes that have yet to be fully elucidated.

### Sensitivity of root $\delta^{13}C$ to atmospheric conditions and photosynthetic rate

Bulk root $\delta^{13}C$ was found to be significantly correlated to time lagged $RH_7$ and $VPD_7$ (**Figure 5b-c**).These relationships suggest that a portion of bulk root OC is comprised of recently synthesized organic matter. Elevated RH and lower VPD are generally associated with open stomata, which leads to higher $p_i/p_a$ and a higher degree of isotopic fractionation against $^{13}C$ during photosynthesis. Ekblad & Högberg (2001) measured the $\delta^{13}C$ of $CO_2$ from soil respiration and found a significant decrease in $\delta^{13}C$ with increasing RH, a finding that closely matches the negative relationship between bulk root $\delta^{13}C$ and RH in the present study.

In contrast to bulk leaf $\delta^{13}C$, bulk root $\delta^{13}C$ was positively related to $NEE_7$ (**Figure 5a**). This trend was unexpected and the potential driving mechanism is not

entirely clear at present, although it may be related to greater allocation of SOC from $^{13}C$-depleted leaves to roots when photosynthetic uptake of C was high.

### Leaf properties and environmental drivers of leaf $\delta^{13}C$

Significant differences in leaf $\delta^{13}C$ values were observed between species in the present study (**Figure 6a** and **Table 4**). Differences in species specific leaf $\delta^{13}C$ at a single sampling site have also been observed in other studies of temperate hardwood forests (e.g., Garten & Taylor, 1992; Balesdent *et al.*, 1993). The shrub *L. maackii* exhibited the most depleted leaf $\delta^{13}C$ values ($\delta^{13}C$ = -32.5 ‰) in the present study (**Table 4**), which may have fixed relatively $^{13}C$-depleted soil $CO_2$ (typical $\delta^{13}C$ = -28‰ to -25‰) compared the average atmosphere $CO_2$ pool ($\delta^{13}C$= -8‰) due to the close proximity (< 1 m) of the leaves to the soil surface (Jackson *et al.*, 1993; Buchmann *et al.*, 2002). Other drivers of differences in leaf $\delta^{13}C$ values between species examined in the present study may have arisen from soil water availability at the study site. *P. detloides* was almost exclusively found in the persistently moist or saturated soils surrounding the experimental wetlands at the site, while most *A. negundo* trees were found in drier locations away from standing water. *P. occidentalis* and *A. saccharinum* were spatially more broadly distributed at the site. *A. negundo* had the most $^{13}C$ enriched leaves and *P. deltoides* had the most $^{13}C$ depleted leaves of any of the tree species measured at the study site. This finding is consistent with differing water availability to these two species (**Figure 6a** and **Table 4**). The elevated leaf $\delta^{15}N$ values in *P. deltoides* compared to *A. negundo* also suggests the latter species had restricted access to wetland water and N

because wetland N is likely to be enriched in $^{15}N$ due to microbial denitrification and anthropogenic inputs of N upstream (Alberts *et al.*, 2013; Hastings *et al.*, 2013) (**Figure 6b**). This further supports our contention of differing water availability to these two species controlling both $\delta^{13}C$ and $\delta^{15}N$ values of leaves and their soluble organic components.

LMA, $C_{area\text{-}leaf,}$ and $N_{area\text{-}leaf}$ have previously been considered as an indicator for photosynthetic potential (i.e., maximum photosynthetic rate) at the individual leaf level because the amount of material (i.e., total dry mass, and C and N contents) per unit area of leaf is likely related to the density of photosynthetic machinery (as reviewed by Poorter *et al.*, 2009). Higher photosynthetic potential, along with longer diffusion pathways from the atmosphere to the site of $CO_2$ fixation within leaves that have high LMA, $C_{area\text{-}leaf,}$ or $N_{area\text{-}leaf}$, may lead to low $p_i/p_a$ within leaves and result in positive relationships between leaf $\delta^{13}C$ and LMA, $C_{area\text{-}leaf,}$ or $N_{area\text{-}leaf}$ (Rao & Wright, 1994; Sparks & Ehleringer, 1997; Duursma & Marshall, 2006). While we observed positive correlations between leaf $\delta^{13}C$ and all three of these variables for all species in this study (**Table 5** and **Figure 7**), the specific mechanistic aspects of the drivers leading to these relationships were beyond the scope of the present study.

The negative relationship between the average $\delta^{13}C$ of all leaves grouped by sampling date and $NEE_7$ suggests that greater $CO_2$ fixation by photosynthesis (i.e., more negative NEE) causes a decrease in $p_i/p_a$ of leaves leading to a measureable increase $\delta^{13}C$ of bulk leaf material (**Figure 8b**). The positive relationship between average $\delta^{13}C$ of all

leaves grouped by sampling date and % daylight is interpreted in a similar manner (i.e., the longer the duration of photosynthesis per day, the lower $p_i/p_a$ becomes) (**Figure 8a**). Previous studies have reported seasonal variation in leaf $\delta^{13}C$, but to our knowledge this is the first study to show positive relationships with NEE and % daylight (Luo *et al.*, 2010; McKown *et al.*, 2012).

### Comparison of root exudation rates and extrapolation to stand scale

Across all sampling dates and species the average (±SE) net root exudation rate of SOC was estimated to be 14 (± 3) µmol C g root$^{-1}$ d$^{-1}$. When scaled to the entire 5,600 m$^2$ sampling area the flux was calculated to be 2.3 (± 0.5) mmol C m$^{-2}$ d$^{-1}$. On a yearly basis the flux estimate is calculated to be 0.83 (±0.18) mol C m$^{-2}$ yr$^{-1}$ (**Table 11**). Net C uptake at our study site is estimated to be 25.85 mol C m$^{-2}$ yr$^{-1}$ (Morin *et al.*, 2014b), therefore net root exudation rates reported in the present study may account for a maximum of 3.2% (±0.7) NEE at the study site. However, the actual contribution of root exudates to NEE at the site is probably lower than 3.2% because a significant portion of root exuded SOC is likely remineralized by soil microorganisms on time scale of hours to weeks (Uselman *et al.*, 2000; van Hees *et al.*, 2005).

To compare net root SOC exudation rates measured in the present study to other estimates reported in the literature, we compiled net hardwood and gymnosperm tree root exudation rate data from studies that used methods similar to those used here and originally outlined by Phillips *et al.* (2008) (**Table 11**). There is a high degree of variability in average root exudation rates reported between studies (12-200 µmol C g

root$^{-1}$ day$^{-1}$ ) and the average rate reported in this study (14 μmol C g root$^{-1}$ day$^{-1}$) is on the low side of this range (**Table 11**). Some of the variability in exudation rates may be attributable to differences between species or type of mycorrhizal association with roots (e.g., arbuscular or ectomycorrhizal fungi) (Yin *et al.*, 2014), but these variables are not found to be consistent drivers of net exudation rates across all studies (e.g., Brzostek *et al.*, 2013). An important methodological consideration in previous root exudation studies is that the exudate collection device and the materials used, especially the specific type of glass beads and the methods of cleaning them and the use of hydrocarbon-rich materials such as butyl rubber stoppers and Parafilm (Phillips *et al*., 2008), may have imparted significant SOC artifacts, and hence overestimated SOC concentrations and exudation rates (**Supplemental Information Section 1**). The use of ultra-clean materials and incubation conditions in the present study may therefore give a more accurate and realistic estimate of the amounts and net exudation rates of root-derived SOC *in situ*.

Overall, the net root exudation rates reported in the present study represent a relatively small portion (3.2% maximum) of net $CO_2$ uptake at the study site on an annual basis (**Table 11**). However, when assessed as an input of SOC to soil and groundwater, especially at longer and more ecologically relevant time scales (e.g., over the lifespan of a tree), exudate fluxes alone are likely to become a quantitatively important source of organic matter to bottomland soils that impact the biogeochemistry of both terrestrial and aquatic systems. For example a previous study in a temperate hardwood forest revealed that soil organic carbon contains C that was fixed centuries ago and estimated soil

organic carbon accumulation rates are on the order of 0.83-2.5 mol C $m^{-2}$ $yr^{-1}$ (Gaudinski *et al.*, 2000). If we compare our scaled root exudation rate estimate of 0.83 (±0.18) mol C $m^{-2}$ $yr^{-1}$ (**Table 11**) to the soil organic carbon accumulation rate reported by Gaudinski *et al.* (2000) (i.e., 0.83-2.5 mol C $m^{-2}$ $yr^{-1}$) it suggests that only a small proportion of root exuded SOC would need to persists in the soil for exudates to be a quantitatively important input of C to the soil organic carbon pool.

## Summary of leaf, root and exudate $\delta^{13}C$ and net exudation rates in a bottomland forest

Across the entire study NEE was found to be one of the primary drivers of variation in $\delta^{13}C$ of leaves, roots and exudation rates—suggesting a strong influence of photosynthetic rate on the isotopic composition of plant components and transfer and allocation of SOC within trees at our site. Component level measurements (e.g, LMA) were found to be important in explaining leaf $\delta^{13}C$ across all species in this study, but were of little use in explaining variation in root or root exudate $\delta^{13}C$. Our confirmation of root tissue being enriched in $^{13}C$ relative to leaf tissue is tempered by the fact that root exudate $\delta^{13}C$ was neither consistently enriched or depleted compared to both leaf and root tissue. Importantly, we did observe what appears to be a seasonal trend in exudate $\delta^{13}C$ along with relationships with time lagged environmental variables that should be explored in future studies.

## LITERATURE CITED


Alberts JM, Sullivan SMP, Kautza A (2013) Riparian swallows as integrators of landscape change in a multiuse river system: Implications for aquatic-to-terrestrial transfers of contaminants. *Science of The Total Environment*, **463–464**, 42–50.

Aufdenkampe AK, Mayorga E, Raymond PA et al. (2011) Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment*, **9**, 53–60.

Badeck F-W, Tcherkez G, Nogués S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon. *Rapid Communications in Mass Spectrometry*, **19**, 1381–1391.

Balesdent J, Girardin C, Mariotti A (1993) Site-Related $^{13}C$ of Tree Leaves and Soil Organic Matter in a Temperate Forest. *Ecology*, **74**, 1713–1721.

Batlle-Aguilar J, Brovelli A, Luster J, Shrestha J, Niklaus PA, Barry DA (2012) Analysis of carbon and nitrogen dynamics in riparian soils: Model validation and sensitivity to environmental controls. *Science of The Total Environment*, **429**, 246–256.

Bowling DR, Pataki DE, Randerson JT (2008) Carbon isotopes in terrestrial ecosystem pools and $CO_2$ fluxes. *New Phytologist*, **178**, 24–40.

Brown S, Peterson DL (1983) Structural Characteristics and Biomass Production of Two Illinois Bottomland Forests. *American Midland Naturalist*, **110**, 107–117.

Brüggemann N, Gessler A, Kayler Z et al. (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. *Biogeosciences*, **8**, 3457–3489.

Brzostek ER, Greco A, Drake JE, Finzi AC (2013) Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. *Biogeochemistry*, **115**, 65–76.

Buchmann N, Brooks JR, Ehleringer JR (2002) Predicting daytime carbon isotope ratios of atmospheric $CO_2$ within forest canopies. *Functional Ecology*, **16**, 49–57.

Burgin AJ, Groffman PM (2012) Soil $O_2$ controls denitrification rates and $N_2O$ yield in a riparian wetland. *Journal of Geophysical Research: Biogeosciences*, **117**, G01010.

Cernusak LA, Tcherkez G, Keitel C et al. (2009) Why are non-photosynthetic tissues generally $^{13}C$ enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. *Functional Plant Biology*, **36**, 199–213.

Chapin FSI, McFarland J, David McGuire A, Euskirchen ES, Ruess RW, Kielland K (2009) The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. *Journal of Ecology*, **97**, 840–850.

Chapin FS, III, Matson PA, Vitousek PM (2011) Principles of terrestrial ecosystem ecology.

Clark DA, Brown S, Kicklighter DW, Chambers JQ, Thomlinson JR, Ni J (2001) Measuring Net Primary Production in Forests: Concepts and Field Methods. *Ecological Applications*, **11**, 356–370.

Cole JJ, Caraco NF (2001) Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research*, **52**, 101–110.

Cole JJ, Prairie YT, Caraco NF et al. (2007) Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems*, **10**, 172–185.

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, **245**, 35–47.

Domínguez MT, Aponte C, Pérez-Ramos IM, García LV, Villar R, Marañón T (2012) Relationships between leaf morphological traits, nutrient concentrations and isotopic signatures for Mediterranean woody plant species and communities. *Plant and Soil*, **357**, 407–424.

Dudek DM, McClenahen JR, Mitsch WJ (1998) Tree Growth Responses of Populus deltoides and Juglans nigra to Streamflow and Climate in a Bottomland Hardwood Forest in Central Ohio. *The American Midland Naturalist*, **140**, 233–244.

Duursma RA, Marshall JD (2006) Vertical canopy gradients in $\delta^{13}C$ correspond with leaf nitrogen content in a mixed-species conifer forest. *Trees*, **20**, 496–506.

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon Isotope Ratios in Belowground Carbon Cycle Processes. *Ecological Applications*, **10**, 412–422.

Ekblad A, Högberg P (2001) Natural abundance of $^{13}C$ in $CO_2$ respired from forest soils reveals speed of link between tree photosynthesis and root respiration. *Oecologia*, **127**, 305–308.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual review of plant physiology*, **40**, 503–537.

Farrar JF, Jones DL (2000) The control of carbon acquisition by roots. *New Phytologist*, **147**, 43–53.

Garten CT, Taylor GE (1992) Foliar $\delta^{13}C$ within a temperate deciduous forest: spatial, temporal, and species sources of variation. *Oecologia*, **90**, 1–7.

Gaudinski JB, Trumbore SE, Davidson EA, Zheng S (2000) Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes. *Biogeochemistry*, **51**, 33–69.

Gessler A, Keitel C, Kodama N et al. (2007) δ13C of organic matter transported from the leaves to the roots in Eucalyptus delegatensis: short-term variations and relation to respired $CO_2$. *Functional Plant Biology*, **34**, 692–706.

Ghashghaie J, Badeck FW (2014) Opposite carbon isotope discrimination during dark respiration in leaves versus roots – a review. *New Phytologist*, **201**, 751–769.

Göttlicher S, Knohl A, Wanek W, Buchmann N, Richter A (2006) Short-term changes in carbon isotope composition of soluble carbohydrates and starch: from canopy leaves to the root system. *Rapid Communications in Mass Spectrometry*, **20**, 653–660.

Grayston SJ, Vaughan D, Jones D (1997) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**, 29–56.

Hastings MG, Casciotti KL, Elliott EM (2013) Stable Isotopes as Tracers of Anthropogenic Nitrogen Sources, Deposition, and Impacts. *Elements*, **9**, 339–344.

Van Hees PAW, Jones DL, Finlay R, Godbold DL, Lundström US (2005) The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biology and Biochemistry*, **37**, 1–13.

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature*, **451**, 289–292.

Hill AR (1996) Nitrate Removal in Stream Riparian Zones. *Journal of Environment Quality*, **25**, 743.

Hossler K, Bauer JE (2013) Amounts, isotopic character, and ages of organic and inorganic carbon exported from rivers to ocean margins: 1. Estimates of terrestrial losses and inputs to the Middle Atlantic Bight. *Global Biogeochemical Cycles*, **27**, 331–346.

Jackson PC, Meinzer FC, Goldstein G, Holbrook NM, Cavelier J, Rada F (1993) Environmental and physiological influences on carbon isotope composition of gap and understory plants in a lowland tropical forest. , pp. 131–140. Academic Press Inc.

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biology and Biochemistry*, **37**, 413–423.

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil*, **321**, 5–33.

Kesanakurti PR, Fazekas AJ, Burgess KS et al. (2011) Spatial patterns of plant diversity below-ground as revealed by DNA barcoding. *Molecular Ecology*, **20**, 1289–1302.

Kramer C, Trumbore S, Fröberg M et al. (2010) Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. *Soil Biology and Biochemistry*, **42**, 1028–1037.

Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry*, **42**, 1363–1371.

Kuzyakov Y, Gavrichkova O (2010) REVIEW: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Global Change Biology*, **16**, 3386–3406.

Kuzyakov Y, Jones DL (2006) Glucose uptake by maize roots and its transformation in the rhizosphere. *Soil Biology and Biochemistry*, **38**, 851–860.

Lauerwald R, Laruelle GG, Hartmann J, Ciais P, Regnier PAG (2015) Spatial patterns in $CO_2$ evasion from the global river network. *Global Biogeochemical Cycles*, 2014GB004941.

Luo T, Li M, Luo J (2010) Seasonal variations in leaf $\delta^{13}C$ and nitrogen associated with foliage turnover and carbon gain for a wet subalpine fir forest in the Gongga Mountains, eastern Tibetan Plateau. *Ecological Research*, **26**, 253–263.

Marín-Spiotta E, Gruley KE, Crawford J et al. (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. *Biogeochemistry*, **117**, 279–297.

Martin TL, Kaushik NK, Trevors JT, Whiteley HR (1999) Review: Denitrification in temperate climate riparian zones. *Water, Air, and Soil Pollution*, **111**, 171–186.

McClain ME, Boyer EW, Dent CL et al. (2003) Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic. *Ecosystems*, **6**, 301–312.

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2014) Variability in root production, phenology, and turnover rate among 12 temperate tree species. *Ecology*, **95**, 2224–2235.

McKown AD, Guy RD, Azam MS, Drewes EC, Quamme LK (2012) Seasonality and phenology alter functional leaf traits. *Oecologia*, **172**, 653–665.

Mencuccini M, Hölttä T (2010) The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phytologist*, **185**, 189–203.

Midwood AJ, Thornton B, Millard P (2008) Measuring the $^{13}C$ content of soil-respired CO2 using a novel open chamber system. *Rapid Communications in Mass Spectrometry*, **22**, 2073–2081.

Mitsch WJ, Wilson RF (1996) Improving the Success of Wetland Creation and Restoration with Know-How, Time, and Self-Design. *Ecological Applications*, **6**, 77–83.

Mitsch WJ, Zhang L, Stefanik KC et al. (2012) Creating Wetlands: Primary Succession, Water Quality Changes, and Self-Design over 15 Years. *BioScience*, **62**, 237–250.

Morin TH, Bohrer G, Naor-Azrieli L, Mesi S, Kenny WT, Mitsch WJ, Schäfer KVR (2014a) The seasonal and diurnal dynamics of methane flux at a created urban wetland. *Ecological Engineering*, **72**, 74–83.

Morin TH, Bohrer G, Frasson RP d. M, Naor-Azreli L, Mesi S, Stefanik KC, Schäfer KVR (2014b) Environmental drivers of methane fluxes from an urban temperate wetland park. *Journal of Geophysical Research: Biogeosciences*, **119**, 2014JG002750.

Mortazavi B, Chanton JP, Prater JL, Oishi AC, Oren R, Katul G (2005) Temporal variability in $^{13}C$ of respired $CO_2$ in a pine and a hardwood forest subject to similar climatic conditions. *Oecologia*, **142**, 57–69.

Moyes AB, Gaines SJ, Siegwolf RTW, Bowling DR (2010) Diffusive fractionation complicates isotopic partitioning of autotrophic and heterotrophic sources of soil respiration. *Plant, Cell & Environment*, **33**, 1804–1819.

Niinemets Ü (1999) Research review. Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist*, **144**, 35–47.

O'Neill GJ, Gordon AM (1994) The Nitrogen Filtering Capability of Carolina Poplar in an Artificial Riparian Zone. *Journal of Environment Quality*, **23**, 1218.

Osburn CL, St-Jean G (2007) The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. *Limnology and Oceanography: Methods*, **5**, 296–308.

Peterjohn WT, Correll DL (1984) Nutrient Dynamics in an Agricultural Watershed: Observations on the Role of A Riparian Forest. *Ecology*, **65**, 1466–1475.

Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics*, **18**, 293–320.

Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble root exudates in forest soils. *Functional Ecology*, **22**, 990–999.

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term $CO_2$ fumigation. *Ecology Letters*, **14**, 187–194.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, **182**, 565–588.

Rao RCN, Wright GC (1994) Stability of the Relationship between Specific Leaf Area and Carbon Isotope Discrimination across Environments in Peanut. *Crop Science*, **34**, 98.

Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, **269**, 341–356.

Raymond PA, Hartmann J, Lauerwald R et al. (2013) Global carbon dioxide emissions from inland waters. *Nature*, **503**, 355–359.

Rovira AD (1969) Plant root exudates. *The Botanical Review*, **35**, 35–57.

Sanderman J, Lohse KA, Baldock JA, Amundson R (2009) Linking soils and streams: Sources and chemistry of dissolved organic matter in a small coastal watershed. *Water Resources Research*, **45**, W03418.

Schade JD, Fisher SG, Grimm NB, Seddon JA (2001) The Influence of a Riparian Shrub on Nitrogen Cycling in a Sonoran Desert Stream. *Ecology*, **82**, 3363–3376.

Schmidt MWI, Torn MS, Abiven S et al. (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Snell HSK, Robinson D, Midwood AJ (2015) Sampling root-respired $CO_2$ in-situ for $^{13}C$ measurement. *Plant and Soil*, **393**, 259–271.

Sparks JP, Ehleringer JR (1997) Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. *Oecologia*, **109**, 362–367.

Uselman SM, Qualls RG, Thomas RB (2000) Effects of increased atmospheric $CO_2$, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (Robinia pseudoacacia L.). *Plant and Soil*, **222**, 191–202.

Vadas RL, Sanger JE (1997) Lateral Zonation of Trees along a Small Ohio Stream.

Walker TS, Bais HP, Grotewold E, Vivanco JM (2003) Root Exudation and Rhizosphere Biology. *Plant Physiology*, **132**, 44–51.

Yin H, Wheeler E, Phillips RP (2014) Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biology and Biochemistry*.

Zhai X, Piwpuan N, Arias CA, Headley T, Brix H (2013) Can root exudates from emergent wetland plants fuel denitrification in subsurface flow constructed wetland systems? *Ecological Engineering*, **61, Part B**, 555–563.

# APPENDIX A: TABLES

**Table 1.** Definitions of the time lags calculated for environmental and NEE parameters used in the present study. All averages were computed over daylight values only (i.e., PAR>0).

| Time lagged subscripts used with environmental and NEE parameters | Definition | Example use in present study |
|---|---|---|
| "1" | Average for the day of sample collection | $PARd_1$ |
| "7" | Average for the day of sample collection and the preceding six days | $NEE_7$, $VPD_7$, $RH_7$ |
| "inc" | Individually calculated average for the duration of each root exudate incubation | $NEE_{inc}$ |
| "inc0" | Individually calculated average over the day of sample collection for each root exudate incubation | $PARd_{inc0}$ |
| "inc02" | Individually calculated average for the duration of each root exudate incubation and the preceding two days | $VPD_{inc02}$ |
| "inc03" | Individually calculated average for the duration of each root exudate incubation and the preceding three days | $T_{air\text{-}inc03}$ |

**Table 2.** Continuous and categorical parameters evaluated as potential predictors for net SOC exudation rates and for $\delta^{13}C$ values of bulk leaves, bulk roots, and root exudates in the present study. Parameters marked with an asterisk were assessed using various time lags as described in the Methods section.

| Response Variable | Continuous parameters | Categorical parameters |
|---|---|---|
| Leaf $\delta^{13}C$ | Leaf area, dry leaf mass, $\delta^{15}N_{leaf}$, leaf %C, leaf %N, [a]$C_{-leaf}$,, [b]$N_{leaf}$,, [c]$C_{area-leaf}$ , [d]$N_{area-leaf}$, leaf C:N, [e]LMA, VPD*, PAR*, $PAR_d$*, $T_{air}$*, RH*, $T_{soil}$*, NEE*, % daylight | Canopy position, sampling date, tree species |
| Root $\delta^{13}C$ | Root projected area, root surface area, root volume, dry root mass, $\delta^{15}N_{root}$, root %C, root %N, [f]$C_{root}$, [g]$N_{root}$, [h]$C_{area-root}$, [i]$N_{area-root}$, root C:N, [j]RMA,, VPD*, PAR*, $PAR_d$*, $T_{air}$*, RH*, $T_{soil}$*, NEE*, % daylight | Sampling date, tree species |
| Net exudation rate and exudate $\delta^{13}C$ | Root projected area, root surface area, root volume, dry root mass, $\delta^{15}N_{root}$, $\delta^{13}C_{root}$, root %C, root %N, $C_{root}$,), $N_{root}$,, $C_{area-root}$ , $N_{area-root}$, root C:N, RMA,, VPD*, PAR*, $PAR_d$*, $T_{air}$*, RH*, $T_{soil}$*, NEE*, % daylight | Sampling date, tree species |

[a]$C_{leaf}$: mass of C in leaf sample; [b]$C_{leaf}$: mass of N in leaf sample; [c]$C_{area-leaf}$: mass of C per unit area of leaf; [d]$N_{area-leaf}$ : mass of N per unit area of leaf; [e]LMA: mass per unit area of leaf; [f]$C_{root}$: mass of C in root sample; [g]$N_{root}$: mass of N in root sample; [h]$C_{area-root}$: mass of C per unit surface area of root; [i]$N_{area-root}$: mass of N per unit surface area of root; [j]RMA: mass per unit surface area of root.

**Table 3.** Average (±SE) daily root exudate SOC fluxes and exudate SOC $\delta^{13}C$ values by sampling date and species. Within a species and measurement (i.e., exudate flux or $\delta^{13}C$), asterisks indicate significant differences at the $\alpha$=0.05 level. ND - not determined.

| Sampling Date | **17-18 Jul** | | | **31 Jul- 1 Aug** | | | **14-15 Aug** | | |
|---|---|---|---|---|---|---|---|---|---|
| Species | *n* | Flux (µmol C g root$^{-1}$ d$^{-1}$) | Exudate $\delta^{13}C$ (‰) | *n* | Flux (µmol C g root$^{-1}$ d$^{-1}$) | Exudate $\delta^{13}C$ (‰) | *n* | Flux (µmol C g root$^{-1}$ d$^{-1}$) | Exudate $\delta^{13}C$ (‰) |
| *A. saccharinum* | *4* | 32 (±10)$^{*}$ | -32.2 (±1.5)$^{*}$ | *7* | 8 (±1)$^{**}$ | -29.5 (±0.8)$^{*, **}$ | *5* | 12 (±1)$^{**}$ | 28.0 (±0.3)$^{**}$ |
| *P. occcidentalis* | *1* | 32 | -30.2 | ND | ND | ND | *1* | 12 | -27.4 |
| *P. deltoides* | *1* | 90 | -32.7 | *1* | 16 | -28.5 | ND | ND | ND |
| **Avg.** | ***6*** | **41 (±12)$^{*}$** | **-32.0 (±1.0)$^{*}$** | ***8*** | **9 (±1)$^{**}$** | **-29.4 (±0.7)$^{*, **}$** | ***6*** | **12 (±1)$^{**}$** | **-27.9 (±0.3)$^{**}$** |

Continued

**Table 3** continued

| Sampling date | | **28-29 Sep** | | | **16-17 Nov** | |
|---|---|---|---|---|---|---|
| Species | *n* | Flux (µmol C g root$^{-1}$ d$^{-1}$) | Exudate $\delta^{13}C$(‰) | *n* | Flux (µmol C g root$^{-1}$ d$^{-1}$) | Exudate $\delta^{13}C$ (‰) |
| *A. saccharinum* | *8* | 7 (±1)** | -27.4 (±0.3)** | *4* | 8 (±1)** | -28.1 (±0.9)** |
| *P. occcidentalis* | ND | ND | ND | *2* | 5 (±1) | -27.3 (±1.2) |
| *P. deltoides* | ND | ND | ND | ND | ND | ND |
| **Avg.** | ***8*** | **7 (±1)**** | **-27.4 (±0.3)**** | ***6*** | **7 (±1)**** | **-27.8 (±0.7)**** |

**Table 4.** Average and observed ranges of leaf, root and exudate $\delta^{13}C$ values (all in ‰) arranged by species and sampling date. Values in parentheses represent ± 1 SE from the mean. Values with no SE value reported indicate sample size of n=1 for that observation. Asterisks indicate significant differences in leaf values between species at the $p<0.05$ level when assessed across all sampling dates. ND - not determined.

| Species | Component | 17-18 Jul | 31 Jul-1 Aug | 14-15 Aug | 27 Aug[a] | 28-29 Sep | 16-17 Nov | Average | Observed Range |
|---|---|---|---|---|---|---|---|---|---|
| ***A. negundo*** | Leaf * | ND | -29.7 (0.4) | -29.9 (0.3) | -29.9 (0.2) | -29.9 (0.4) | ND | **-29.9 (0.2)** | -31.0 to -28.0 |
| | Root | ND | ND | ND | ND | ND | ND | **ND** | ND |
| | Exudate | ND | ND | ND | ND | ND | ND | **ND** | ND |
| ***A. saccharinum*** | Leaf *, ** | -30.0 (0.4) | -28.9 (0.7) | -31.0 (0.3) | -30.7 (0.4) | -31.2 (0.1) | ND | **-30.4 (0.2)** | -32.3 to -26.7 |
| | Root | -28.7 (0.4) | -29.4 (0.2) | -28.6 (0.4) | ND | -28.6 (0.4) | -29.0 (0.4) | **-28.9 (0.2)** | -30.4 to -27.2 |
| | Exudate | -32.2 (1.5) | -29.5 (0.8) | -28.0 (0.3) | ND | -27.4 (0.3) | -28.1 (0.9) | **-28.8 (0.4)** | -35.0 to -25.7 |
| ***L. maackii*** | Leaf *** | -31.0 (0.3) | -32.1 (0.2) | -32.7 (0.3) | -33.0 (0.3) | -32.8 (0.2) | ND | **-32.3 (0.2)** | -33.5 to -30.3 |
| | Root | ND | ND | ND | ND | ND | ND | **ND** | ND |
| | Exudate | ND | ND | ND | ND | ND | ND | **ND** | ND |
| ***P. deltoides*** | Leaf **** | -31.1 (0.4) | -31.3 (0.3) | -31.6 (0.2) | -31.6 (0.2) | -31.1 (0.2) | ND | **-31.4 (0.1)** | -33.0 to -29.5 |
| | Root | -29.8 | -30.3 | ND | ND | ND | ND | **-30.0 (0.2)** | -30.3 to -29.8 |
| | Exudate | -32.7 | -28.5 | ND | ND | ND | ND | **-30.6 (2.1)** | -32.7 to -28.5 |
| ***P. occidentalis*** | Leaf **, ***** | -29.6 (0.4) | -30.7 (0.3) | -30.7 (0.5) | -30.9 (0.3) | -31.1 (0.2) | ND | **-30.6 (0.2)** | -32.8 to -27.9 |
| | Root | -29.5 | ND | -28.3 | ND | ND | -29.9 (0.4) | **-29.4 (0.4)** | -30.4 to -28.3 |
| | Exudate | -30.2 | ND | -27.4 | ND | ND | -27.3 (1.2) | **-28.0 (0.9)** | -30.2 to -26.1 |
| **Average** | **Leaf** | **-30.4 (0.2)** | **-30.6 (0.3)** | **-31.0 (0.2)** | **-31.0 (0.2)** | **-31.0 (0.2)** | **ND** | **-30.8 (0.1)** | -33.5 to -26.7 |
| | **Root** | **-29.0 (0.3)** | **-29.5 (0.2)** | **-28.6 (0.5)** | **ND** | **-28.6 (0.4)** | **-29.3 (0.3)** | **-29.0 (0.1)** | -30.4 to -27.2 |
| | **Exudate** | **-32.0 (1.0)** | **-29.4 (0.7)** | **-27.9 (0.3)** | **ND** | **-27.4 (0.3)** | **-27.8 (0.7)** | **-28.8 (0.4)** | -35.0 to -25.7 |

[a]Aug 27: During this sampling period only leaves were collected, roots and root exudates were not assessed.

**Table 5.** Adjusted $R^2$ values, residual standard error and significance level for the least squares regressions of various parameters as potential predictors of leaf $\delta^{13}C$. Significance levels are as follow: "ns"=not significant, "†" = significant at $\alpha \leq 0.10$, "*" = significant at $\alpha \leq 0.05$, "**" = significant at $\alpha \leq 0.01$, "***" = significant at $\alpha \leq 0.001$. NA - not applicable.

| | ***A. negundo*** | | | ***A. saccharinum*** | | | ***L. maackii*** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level |
| Canopy position[a] | 0.02 | 0.82 | ns | 0.18 | 1.20 | ** | NA | NA | NA |
| Leaf Area ($cm^2$) | -0.03 | 0.84 | ns | 0.02 | 1.32 | ns | -0.02 | 0.88 | ns |
| Leaf Mass (g) | -0.04 | 0.84 | ns | 0.14 | 1.23 | * | -0.04 | 0.89 | ns |
| $\delta^{15}N$ (‰) | 0.05 | 0.81 | ns | -0.03 | 1.35 | ns | -0.02 | 0.88 | ns |
| Leaf %C | 0.00 | 0.84 | ns | 0.01 | 1.32 | ns | 0.11 | 0.82 | † |
| Leaf %N | 0.03 | 0.82 | ns | 0.03 | 1.31 | ns | -0.03 | 0.89 | ns |
| $C_{leaf}$ (mg C) | -0.04 | 0.84 | ns | 0.14 | 1.23 | * | -0.04 | 0.89 | ns |
| $C_{area\text{-}leaf}$ (mg C $cm^{-2}$) | 0.18 | 0.75 | * | 0.45 | 0.98 | *** | 0.42 | 0.66 | ** |
| $N_{leaf}$ (mg N) | -0.03 | 0.84 | ns | 0.10 | 1.26 | * | -0.05 | 0.90 | ns |
| $N_{area\text{-}leaf}$ (µg N $cm^{-2}$) | 0.28 | 0.71 | ** | 0.32 | 1.10 | *** | 0.35 | 0.71 | ** |
| Leaf C:N | 0.02 | 0.82 | ns | 0.04 | 1.30 | ns | -0.06 | 0.90 | ns |
| LMA (mg $cm^{-2}$)[b] | 0.26 | 0.71 | ** | 0.45 | 0.99 | *** | 0.47 | 0.64 | *** |

Continued

**Table 5** continued

| | *P. occidentalis* | | | *P. deltoides* | | | Combined leaf data | | |
|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level |
| Canopy position[a] | 0.09 | 1.05 | * | 0.08 | 0.76 | * | 0.26 | 1.08 | *** |
| Leaf Area ($cm^2$) | 0.03 | 1.08 | ns | 0.10 | 0.75 | * | 0.08 | 1.21 | *** |
| Leaf Mass (g) | 0.33 | 0.90 | *** | 0.12 | 0.74 | * | 0.13 | 1.17 | *** |
| $\delta^{15}N$ (‰) | 0.00 | 1.10 | ns | -0.01 | 0.80 | ns | 0.00 | 1.26 | ns |
| Leaf %C | 0.23 | 0.97 | ** | 0.13 | 0.74 | * | 0.10 | 1.19 | *** |
| Leaf %N | -0.01 | 1.10 | ns | 0.02 | 0.79 | ns | 0.09 | 1.20 | *** |
| $C_{leaf}$ (mg C) | 0.34 | 0.89 | *** | 0.14 | 0.74 | * | 0.14 | 1.17 | *** |
| $C_{area\text{-}leaf}$ (mg C $cm^{-2}$) | 0.74 | 0.56 | *** | 0.30 | 0.66 | *** | 0.01 | 1.19 | *** |
| $N_{leaf}$ (mg N) | 0.26 | 0.94 | *** | 0.14 | 0.73 | ** | 0.15 | 1.16 | *** |
| $N_{area\text{-}leaf}$ (µg N $cm^{-2}$) | 0.60 | 0.69 | *** | 0.27 | 0.68 | *** | 0.16 | 1.16 | *** |
| Leaf C:N | 0.05 | 1.07 | † | -0.02 | 0.80 | ns | 0.04 | 1.23 | ** |
| LMA (mg $cm^{-2}$)[b] | 0.75 | 0.55 | *** | 0.25 | 0.69 | *** | 0.06 | 1.22 | *** |

[a]Canopy position is defined as height in the canopy from which leaves were collected, lower canopy (~2m aboveground) or mid-canopy (~7m aboveground). t-tests were used to test for statistical differences of leaf $\delta^{13}C$ collected from different canopy positions. [b]LMA: Leaf mass per unit area.

**Table 6.** Adjusted $R^2$ values, residual standard error and significance level for least squares regressions of a various environmental parameters measured in the present study as potential predictors of leaf $\delta^{13}C$. Environmental parameter abbreviations and subscripts (lag times) are defined in the Methods section. Significance levels are as follows: "ns"=not significant, "†" = significant at $\alpha \leq 0.10$, "*" = significant at $\alpha \leq 0.05$, "**" = significant at $\alpha \leq 0.01$, "***" = significant at $\alpha \leq 0.001$.

| | ***A. negundo*** | | | ***A. saccharinum*** | | | ***L. maackii*** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level |
| $VPD_1$ | -0.03 | 0.84 | ns | 0.16 | 1.22 | ** | 0.10 | 0.83 | † |
| $PAR_1$ | -0.04 | 0.84 | ns | -0.03 | 1.34 | ns | -0.04 | 0.89 | ns |
| $PAR_{d1}$ | -0.34 | 0.84 | ns | 0.29 | 1.12 | *** | 0.26 | 0.75 | * |
| $T_{air1}$ | -0.04 | 0.84 | ns | 0.03 | 1.30 | ns | -0.04 | 0.89 | ns |
| $RH_1$ | -0.35 | 0.84 | ns | 0.15 | 1.23 | * | -0.01 | 0.88 | ns |
| $T_{soil1}$ | -0.04 | 0.84 | ns | 0.02 | 1.31 | ns | -0.01 | 0.88 | ns |
| $NEE_1$ | -0.04 | 0.84 | ns | 0.17 | 1.21 | ** | 0.15 | 0.81 | † |
| $VPD_7$ | -0.03 | 0.84 | ns | 0.16 | 1.22 | ** | -0.05 | 0.89 | ns |
| $PAR_7$ | -0.03 | 0.84 | ns | 0.28 | 1.12 | *** | 0.50 | 0.62 | *** |
| $PAR_{d7}$ | -0.04 | 0.84 | ns | 0.21 | 1.18 | ** | 0.13 | 0.82 | † |
| $Tair_7$ | -0.04 | 0.84 | ns | -0.03 | 1.34 | ns | 0.09 | 0.83 | ns |
| $RH_7$ | -0.04 | 0.84 | ns | 0.21 | 1.19 | ** | -0.04 | 0.89 | ns |
| $Tsoil_7$ | 0.04 | 0.84 | ns | 0.04 | 1.30 | ns | 0.10 | 0.83 | † |
| $NEE_7$ | -0.04 | 0.84 | ns | 0.24 | 1.15 | ** | 0.18 | 0.79 | * |
| % daylight | -0.04 | 0.84 | ns | 0.16 | 1.22 | ** | 0.71 | 0.34 | ** |

Continued

**Table 6** continued

| | *P. occidentalis* | | | *P. deltoides* | | | *Combined leaf data* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level | Adj. $R^2$ | Res. Std. Error | Sig. level |
| $VPD_1$ | 0.04 | 1.08 | ns | -0.01 | 0.80 | ns | 0.01 | 1.25 | ns |
| $PAR_1$ | 0.01 | 1.09 | ns | 0.02 | 0.79 | ns | -0.01 | 1.26 | ns |
| $PAR_{d1}$ | 0.00 | 1.09 | ns | -0.01 | 0.80 | ns | 0.02 | 1.24 | * |
| $T_{air1}$ | -0.02 | 1.11 | ns | 0.00 | 0.79 | ns | 0.00 | 1.26 | ns |
| $RH_1$ | -0.02 | 1.11 | ns | 0.00 | 0.79 | ns | 0.00 | 1.26 | ns |
| $T_{soil1}$ | 0.02 | 1.09 | ns | 0.01 | 0.79 | ns | 0.00 | 1.26 | ns |
| $NEE_1$ | 0.04 | 1.07 | ns | -0.02 | 0.80 | ns | 0.01 | 1.25 | † |
| $VPD_7$ | -0.01 | 1.10 | ns | -0.02 | 0.80 | ns | -0.01 | 1.26 | ns |
| $PAR_7$ | 0.08 | 1.05 | * | -0.01 | 0.80 | ns | 0.04 | 1.24 | ** |
| $PAR_{d7}$ | 0.02 | 1.08 | ns | -0.02 | 0.80 | ns | 0.01 | 1.25 | † |
| $Tair_7$ | 0.09 | 1.05 | * | -0.01 | 0.80 | ns | 0.00 | 1.25 | ns |
| $RH_7$ | -0.02 | 1.11 | ns | -0.01 | 0.80 | ns | 0.00 | 1.26 | ns |
| $Tsoil_7$ | 0.07 | 1.06 | . | -0.01 | 0.79 | ns | 0.00 | 1.26 | ns |
| $NEE_7$ | 0.04 | 1.08 | ns | -0.02 | 0.80 | ns | 0.02 | 1.25 | † |
| % daylight | 0.11 | 1.04 | * | -0.02 | 0.80 | ns | 0.02 | 1.25 | * |

**Table 7.** ANOVA table comparing leaf and root $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study.

| *Source of variation* | *df* | *Sum of squares* | *Mean square* | *F* | *P-val* | *Significance* |
|---|---|---|---|---|---|---|
| Leaf vs. Root | 1 | 29.243 | 29.243 | 29.906 | <0.05 | *** |
| Sampling Date | 3 | 5.451 | 1.817 | 1.858 | >0.05 | ns |
| (Leaf vs. Root) * Sampling Date | 3 | 19.973 | 6.658 | 6.809 | <0.05 | *** |
| Residuals | 46 | 44.981 | 0.978 | | | |

**Table 8.** ANOVA table comparing leaf and root exudate $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study.

| *Source of variation* | *df* | *Sum of squares* | *Mean square* | *F* | *P-val* | *Significance* |
|---|---|---|---|---|---|---|
| Leaf vs. Exudate | 1 | 28.340 | 28.241 | 13.973 | <0.05 | *** |
| Sampling Date | 3 | 12.264 | 4.088 | 2.015 | >0.05 | ns |
| (Leaf vs. Exudate) * Sampling Date | 3 | 80.860 | 26.954 | 13.290 | <0.05 | *** |
| Residuals | 46 | 93.296 | 2.028 | | | |

**Table 9.** ANOVA table comparing root and root exudate $\delta^{13}C$ values for *A. saccharinum* across different sampling dates in the present study.

| *Source of variation* | *df* | *Sum of squares* | *Mean square* | *F* | *P-val* | *Significance* |
|---|---|---|---|---|---|---|
| Root vs. Exudate | 1 | 0.175 | 0.175 | 0.096 | >0.05 | ns |
| Sampling Date | 4 | 41.566 | 10.392 | 5.671 | <0.05 | ** |
| (Leaf vs. Root) * Sampling Date | 4 | 33.756 | 8.441 | 4.607 | <0.05 | ** |
| Residuals | 50 | 93.296 | 2.028 | | | |

**Table 10.** Average growing season NEE values for the nights (defined as when PAR<0) during which incubations took place compared to average daytime NEE for each root exudate incubation by sampling date.

| Sampling Period | Average Night NEE (μmol C $m^{-2}$ $s^{-1}$) | Average NEE during incubations (μmol C $m^{-2}$ $s^{-1}$) |
|---|---|---|
| 17-18 Jul | 3.9 | -13.9 |
| 31 Jul- 1 Aug | 4.3 | -12.2 |
| 14-15 Aug | 3.8 | -14.0 |
| 28-29 Sep | 3.8 | -11.1 |

**Table 11.** Summary of net exudation rates reported for tree roots in this and other studies using a similar root exudate collection method. NR - not reported

| Source | Species sampled | Estimated fine root biomass | Mass based exudation rate (μmol C g root$^{-1}$ day$^{-1}$) | Yearly based exudation rate (mol C m$^{-2}$ y$^{-1}$) |
|---|---|---|---|---|
| Aoki et al., 2012 | *Lithocarpus, Syzygium, Tristaniopsis, Dacrycarpus, Dacrydium* genera | 665-874 g dry wt m$^{-2}$ | 12-27 | 2.52-8.40 |
| Brzostek et al., (2013) | *Acer saccharum, Fraxinus Americana, Tsuga canadensis, Fagus grandifolia* | 367-504 g dry wt m$^{-2}$ | 57-200 | NR |
| Drake et al., (2011) | *Pinus taeda* | 127 g C m$^{-2}$ | NR | 1.91-2.91 |
| Phillips et al., (2008) | *Pinus taeda* | 250 g dry wt m$^{-2}$ | NR | 0.78 |
| Phillips et al., (2011) | *Pinus taeda* | 235 g dry wt m$^{-2}$ | 16-34 | 1.93 |
| Yin et al., (2013) | *Picea asperata* | NR | 33-58 | NR |
| Yin et al., (2014) | *Quercus alba, Fagus grandifolia, Acer saccharum, Liriodendron tulipifera* | NR | 15-41 | 0.67-2.16 |
| This study | *Acer saccharinum, Populus deltoides, Platanus occidentalis* | 162 g dry wt m$^{-2}$ | 14 | 0.83 |

## APPENDIX B: FIGURES

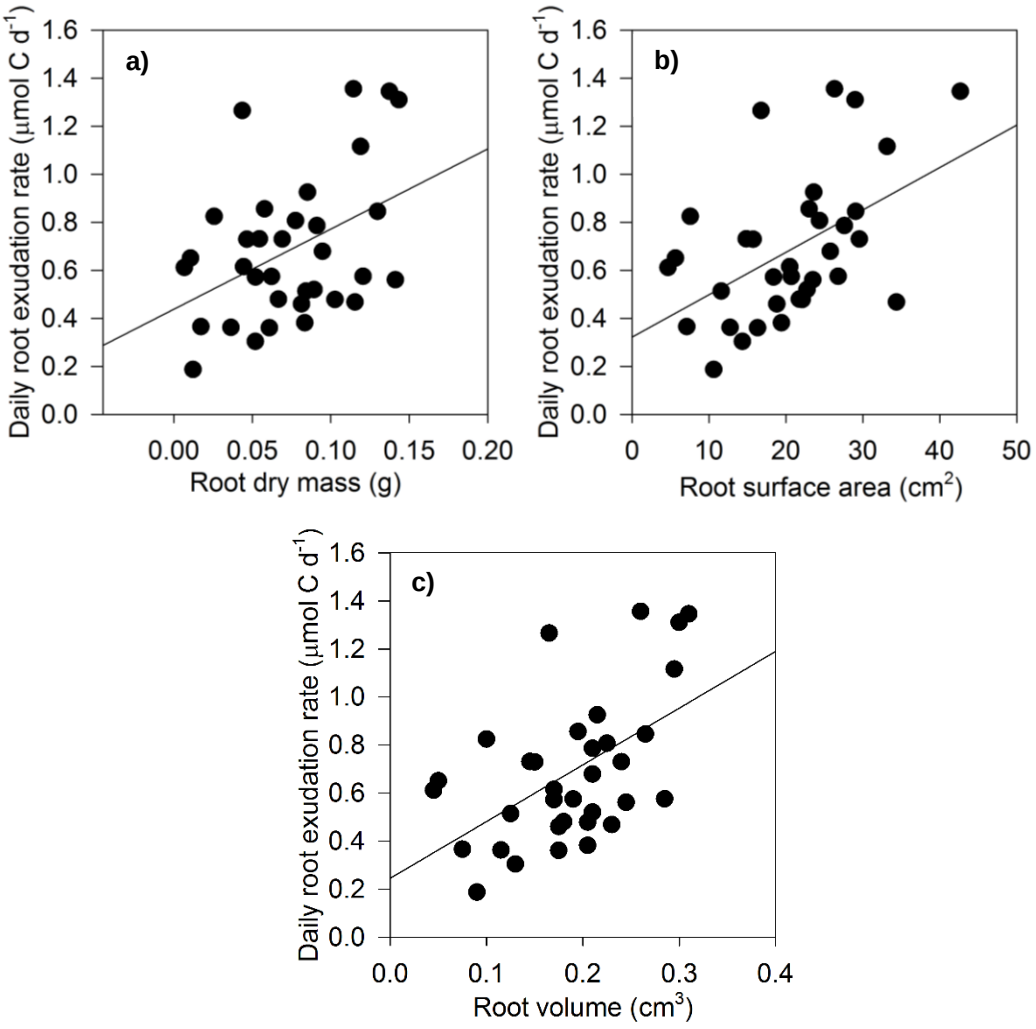


**Figure 1.** Daily SOC yields in individual incubations vs. a) root dry mass, b) root volume and c) root surface area. All plots include data from all root incubations and root samples from all collection dates in this study. Solid lines are least squares linear regressions and all relationships are significant at the $p<0.05$ level.

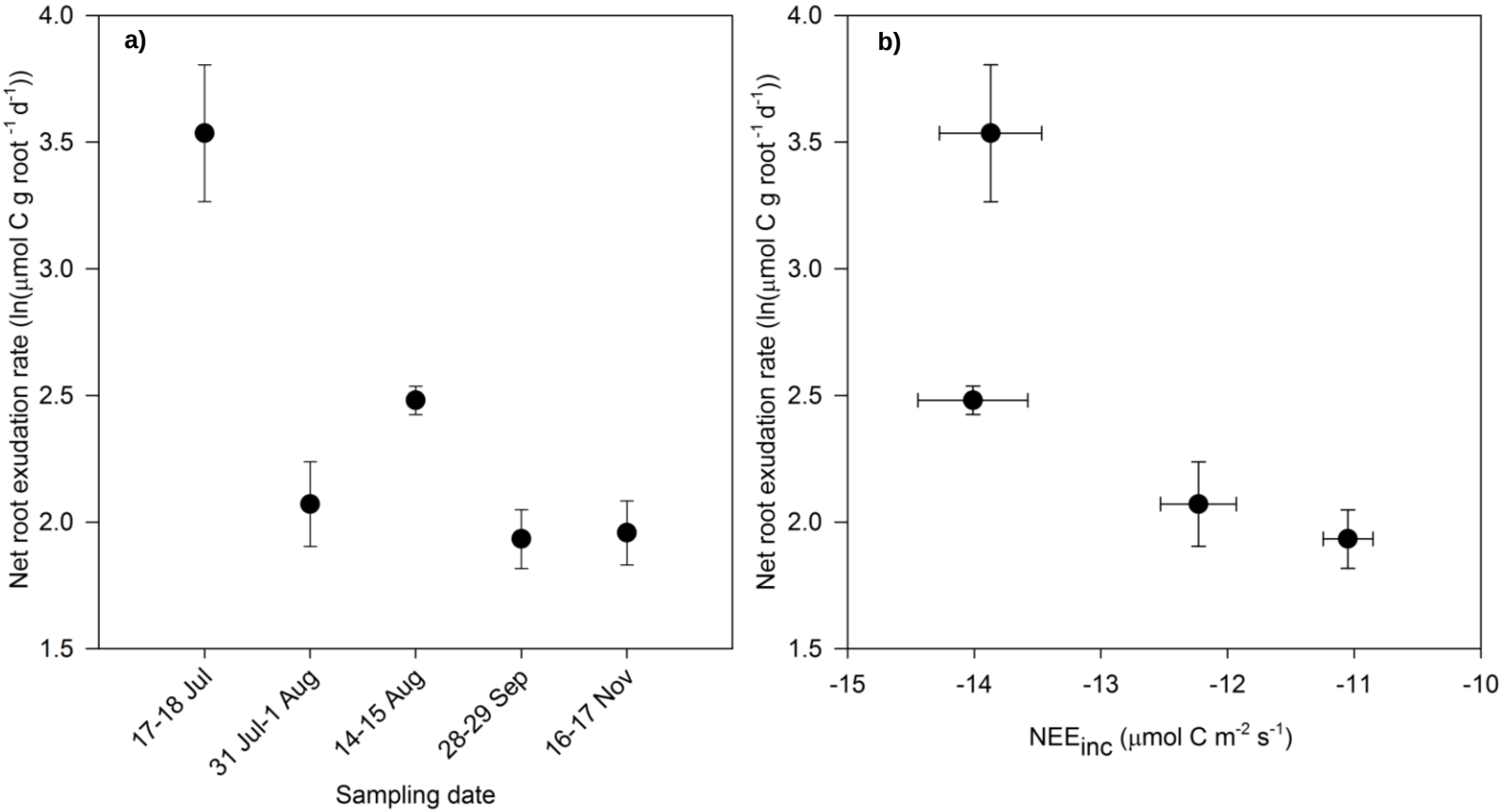


**Figure 2.** Average (±SE) log transformed net root SOC exudation rates normalized to root dry mass vs. a) sampling date and b) average (±SE) $NEE_{inc}$ (NEE for the ~24 hour incubation period) for each sampling date. Both plots include data from all sampling dates and root samples measured in this study. Regression relationships for both plots are significant at the $p<0.05$ level.

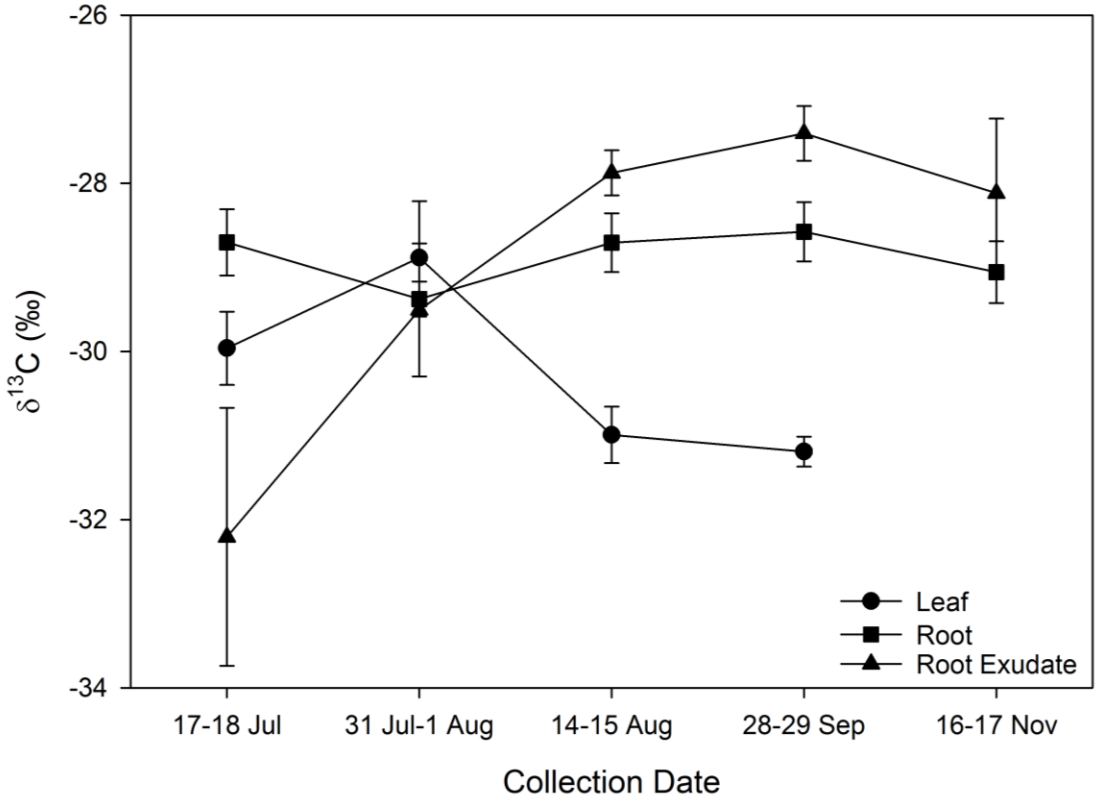


**Figure 3**. *A. saccharinum* leaf, root and root exudate $\delta^{13}C$ values for all sampling dates in the present study. Each point represents the means (±SE) for leaf (circles), root (squares) and root exudate (triangles) samples collected and measured on each date.

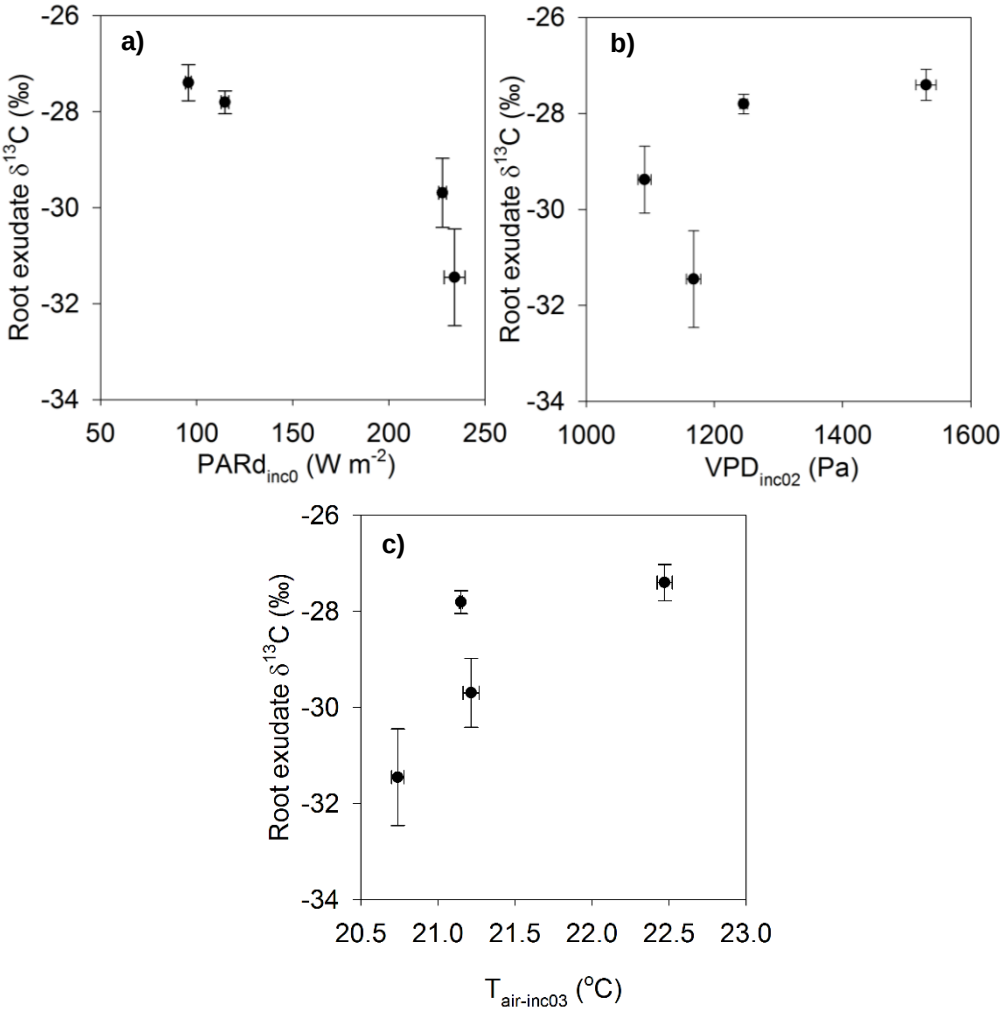


**Figure 4.** Average (± SE) root exudate $\delta^{13}C$ values vs. a) Average $PAR_d$ (±SE) over the day during which the incubations were collected ($p<0.001$), b) Average VPD (±SE) over the two days prior to, and including the day of, the end of the incubations ($p<0.01$) and c) Average $T_{air}$ (±SE) over the three days prior to, and including the day of, the end of the incubations ($p<0.05$). The plots include data across all root incubations in this study.

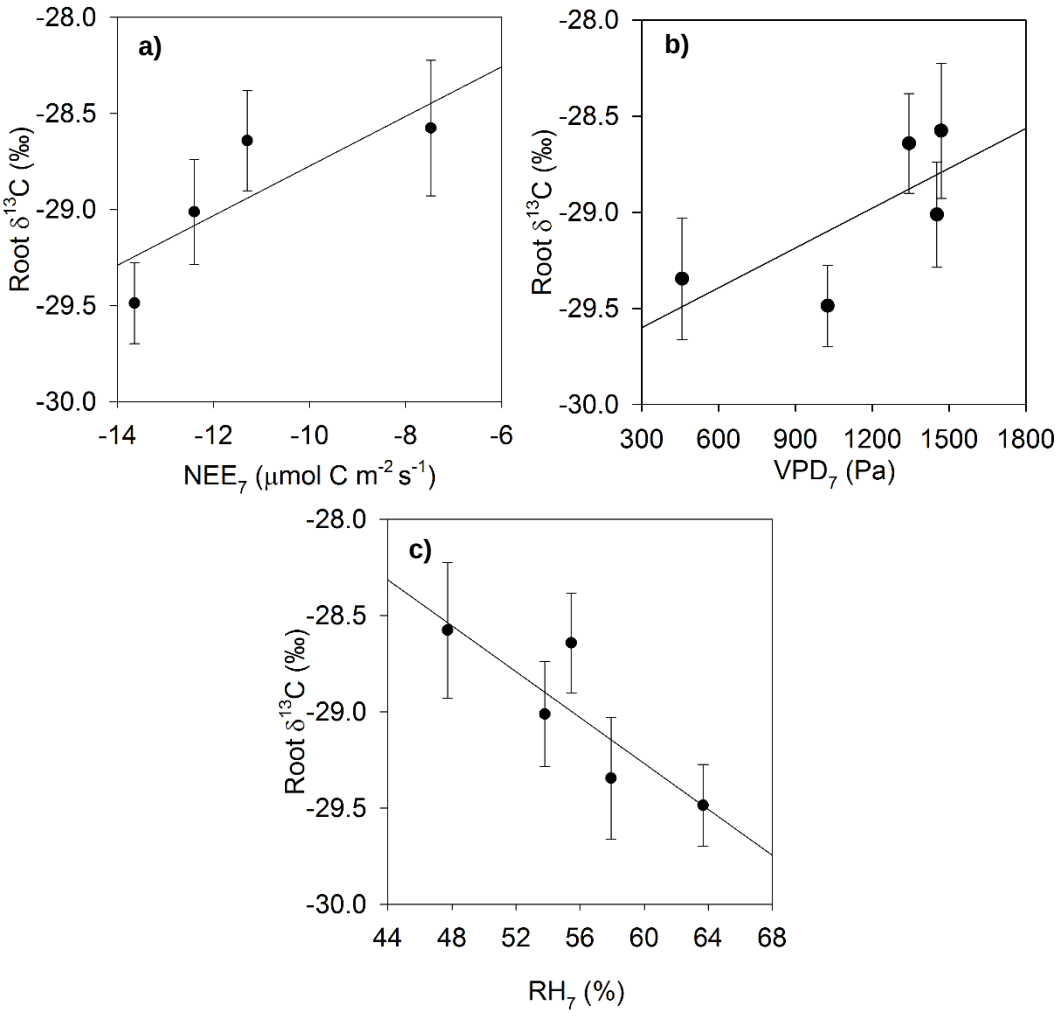


**Figure 5.** Average (± SE) bulk root $\delta^{13}C$ values vs. a) Average NEE for the day of sample collection and the preceding six days prior to sampling ($p<0.5$) b) Average VPD for the day of sample collection and the preceding six days prior to sampling and c) Average RH for the day of sample collection and the preceding six days prior to sampling. The solid lines represent least squares linear regressions. All plots show average $\delta^{13}C$ values include for all roots collected from all tree species on each sampling date in this study.

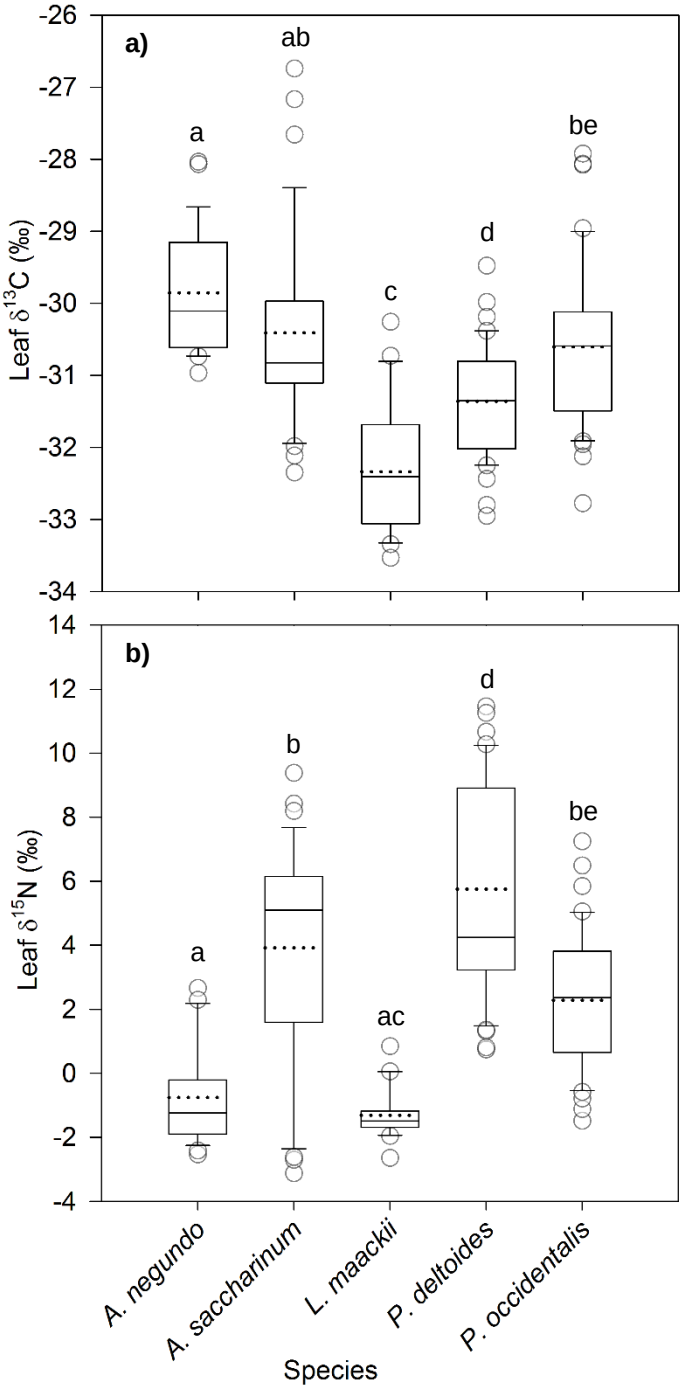


**Figure 6.** a) $\delta^{13}C$ values and b) $\delta^{15}N$ values of leaves of the tree species in the present study. Solid horizontal lines within each box represent median values and dotted horizontal lines represent mean values. For each plot, differing letters between species indicate significant differences at the $p<0.05$ level.

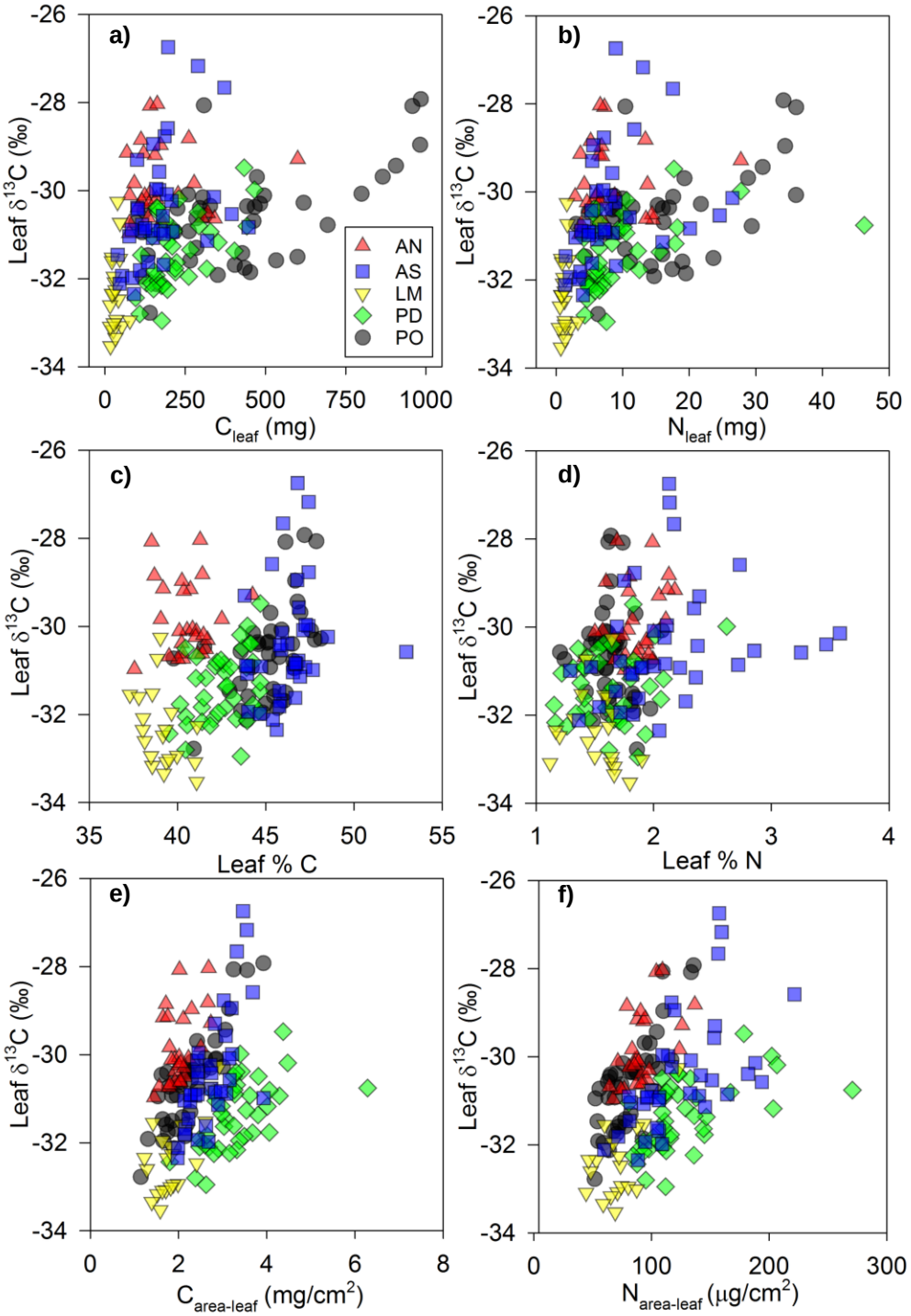


**Figure 7.** Plots of leaf $\delta^{13}C$ vs. a) leaf carbon content ($C_{leaf}$), b) leaf nitrogen content ($N_{leaf}$), c) leaf % C, d) leaf % N, e) leaf area normalized C content ($C_{area\text{-}leaf}$), and f) leaf area normalized N content ($N_{area\text{-}leaf}$). AN=*A. negundo*, AS= *A. saccharinum*, LM=*L. maackii*, PD=*P. deltoides* and PO=*P. occidentalis*.

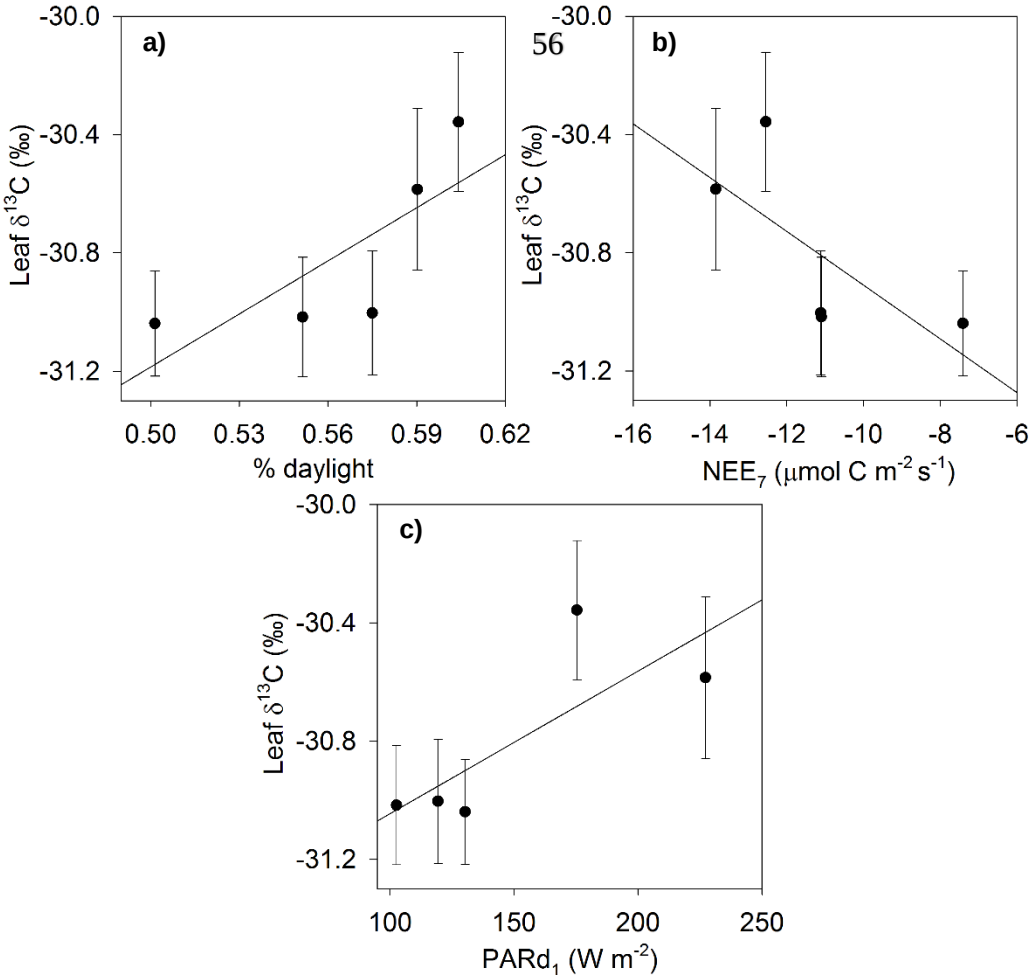


**Figure 8.** Average (±SE) leaf $\delta^{13}C$ for each sampling date across all species vs. a) % daylight ($p<0.05$), b) $NEE_7$ ($p<0.10$) and c) $PARd_1$ ($p<0.05$). Solid lines are linear least squares regressions.

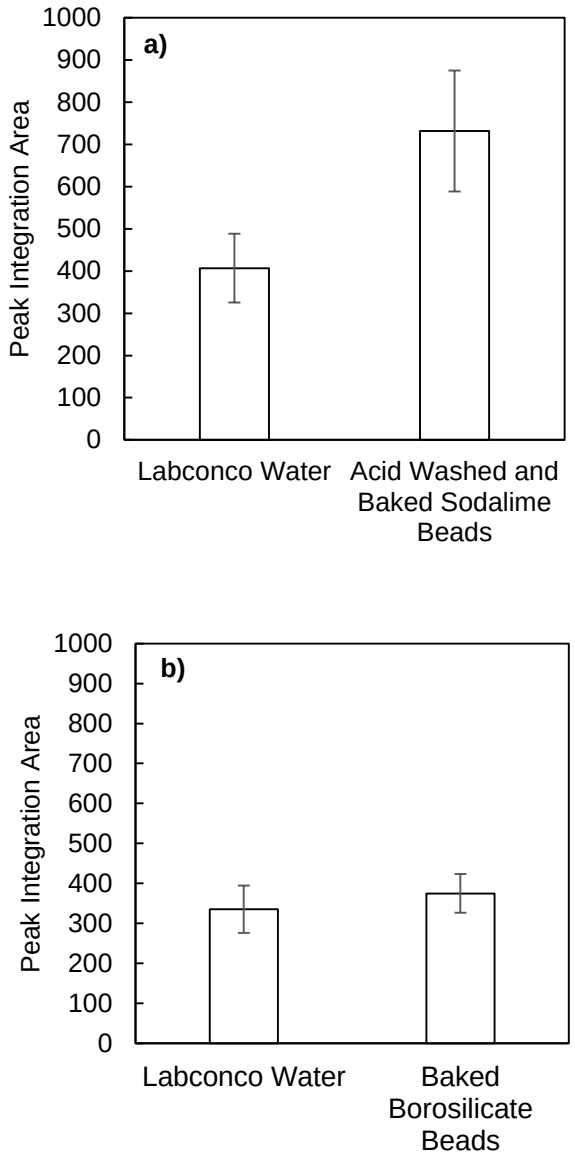


**Figure 9.** Comparisons of glass bead root incubation substrates and their pre-treatment methods on contaminant SOC. a) A significant difference was found (t-test; p= 0.004) between the Labconco Ultra-Pure water and acid washed (10% HCl) and baked (400 $^{o}$C) soda lime beads. b) No significant difference (t-test; p= 0.41, n=4) was found between Labconco Ultra-Pure water and baked (550 $^{o}$C) borosilicate beads. Error bars are ± 1 SD about the mean peak integration area. Peak integration areas are directly related to the concentration of OC in the samples.

# APPENDIX C: SUPPLEMENTAL INFORMATION

## Section 1. Root Exudate Collection Device Modifications

The specific modifications to the root exudate collection device and methodology described in Phillips *et al.* (2008) used for this study were as follows:

1. Use of 1 mm borosilicate glass beads. Preliminary tests indicated that soda lime glass beads similar to those used by Phillips et al. (2008) were a significant source of potential SOC contamination, even after 24 hrs of soaking in fresh 10% HCl and pre-baking at 400-450$^{o}$ C for up to 24 hours (**Figure 9a**). This baking temperature was used because the temperatures of transformation (i.e., changes in mechanical properties) and softening of soda lime glass beads are 440$^{o}$ C and 505$^{o}$ C, respectively. Pre-baking at 400-450$^{o}$ C of other forms of glass (e.g., borosilicate, Pyrex and quartz) has been found to completely eliminate all contaminant OC, but not soda lime glass. Therefore, borosilicate beads were pre-baked at 500$^{o}$ C, thus assuring full oxidation of residual OC. Preliminary tests indicated no significant differences between the SOC concentrations obtained from Labconco Ultra-Pure water and pre-baked (500-550 $^{o}$C) borosilicate beads that were subsequently allowed to extract in Labconco Ultra-Pure water for 24 hours (**Figure 9b**).

2. Instead of the butyl rubber stoppers used by Phillips et al. (2008) as the caps for the exudate collection devices, a pre-baked (500$^{o}$ C) sheet of aluminum foil was used in the present study. Rubber stoppers are known to leach significant amounts of SOC (J. Bauer,

personal communication), and the proposed modification avoided this additional form of sample contamination.

3. Instead of a fine mesh cloth cone at the base of the exudate collection device as employed by Phillips et al. (2008), a pre-baked (500$^{o}$ C ) glass fiber filter (Whatman GF/F, 0.7 μm), was inserted to prohibit beads from being lost from the device during sample collection. Since GF/F filters can be pre-baked, this further eliminates another potential source of SOC contamination to the samples that may be contributed by a fine meshed cloth, which was also not pre-cleaned in any way.

## Section 2. DNA barcoding to identify roots

In order to identify tree roots to genus or species, a DNA barcoding approach was used (Kesanakurti *et al.*, 2011). Root material was dried at 60$^{o}$C and then ground with mortar and pestle and a ~50 mg subsample transferred to a centrifuge tube. DNA was then extracted using the PowerPlant Pro DNA Isolation Kit (Mo Bio Laboratories, Inc.) and amplicons for the rbcL and psbA-trnH regions of the chloroplast genome were amplified using PCR, purified and then sequenced at the Plant Microbe Genomics Facility located at The Ohio State University. Sequence data was then analyzed using the Basic Local Alignment Search Tool (Altschul *et al.*, 1990) to assign a genus or species level designation. This method was not able to distinguish between silver and red maple—a matter which is even more challenging because these two species are known to naturally hybridize and produce fertile offspring. For this reason this study does not

differentiate between *Acer rubrum* and *Acer saccharinum*, and instead we refer to *Acer saccharinum* in the text because this species was present in far greater relative abundances at the site than *A. rubrum*.

### Section 3. Imputation and mixing model to estimate exudate $\delta^{13}C$

To estimate the unknown (i.e., unmeasurable) $\delta^{13}C$ values from flushes 2 and 3 from each incubation we used a stochastic regression imputation approach (Little & Rubin, 2014) . First, a linear regression model was used to regress those flush 2 $\delta^{13}C$ values that were measureable against measured flush 1 $\delta^{13}C$ values from incubations we had complete measurements for both flushes 1 and 2 (n=4). The unknown flush 2 $\delta^{13}C$ values were predicted from the regression equation and random normal noise was added to the final predicted value based on the residual standard error of the regression model between flush 2 $\delta^{13}C$ values and flush 1 $\delta^{13}C$ values. Flush 3 $\delta^{13}C$ values were estimated in the same manner as for flush 2 $\delta^{13}C$ values.

Similarly, SOC concentrations for flushes 2 and 3 that could not be measured due to their low OC contents were estimated by assuming that flushes 2 and 3 contained 15.6 and 9.0%, respectively of the total amount of SOC collected from the sum of the three flushes. We based these estimates on samples for which we had complete sets (n=17) (i.e., SOC measurements for flush 1,2 and 3 were measured). The mean (±SE) percentage of the total yield from three flushes for the 17 samples for which all three flushes were measured was 75.4 (±4.3), 15.6 (±2.3) and 9.0 (±2.5).

In order to estimate root exudate SOC $\delta^{13}C$ values for each incubation we used a mixing model as described below:

$$Exudate\ \delta^{13}C_{total} = \sum_{i=1}^{3} f_{flush\ (i)} * \delta^{13}C_{flush\ (i)}$$

$f_{flush(i)}$ represents the measured or estimated fraction of organic carbon for a particular flush relative to the yield of all three flushes combined and $\delta^{13}C_{flush(i)}$ represents the measured or estimated $\delta^{13}C$-SOC for a particular flush.