

# Exudation rates and $\delta^{13}\text{C}$ signatures of tree root soluble organic carbon in a riparian forest

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**Abstract** Tree root exudation (TRE) of water soluble organic carbon (WSOC) is an important but under-assessed component of net primary production, and is thought to strongly influence rhizosphere biogeochemistry. Riparian systems in particular are often viewed as biogeochemical hot spots fueled partially by root exudate WSOC. However, TRE rates have not been previously reported for these systems. The  $\delta^{13}\text{C}$  signatures of exudates may provide important insights into plant physiology and inform isotope-based methods to identify sources of soil  $\text{CO}_2$  fluxes, but this information is also generally lacking. In the

present study, root exudate WSOC was collected in situ to assess both net exudation rates and exudate  $\delta^{13}\text{C}$  values in a temperate riparian forest. Net TRE rates were found to be most strongly related to a combination of tree species, root characteristics and net ecosystem exchange (Adj.  $R^2 = 0.73$ ;  $p < 0.001$ ). In contrast, exudate  $\delta^{13}\text{C}$  values were correlated to time-lagged vapor pressure deficit (Adj.  $R^2 = 0.21$ ;  $p < 0.05$ ) and air temperature (Adj.  $R^2 = 0.43$ ;  $p < 0.05$ ), suggesting a rapid transfer of photosynthate from the canopy to the rhizosphere. Extrapolation of mean net TRE rates ( $13 \mu\text{mol C g root}^{-1} \text{ day}^{-1}$ ) from a root mass basis to the entire sampling area suggests that TRE may account for as much as 3% of net annual C uptake and represents an important input of organic matter to riparian soils. Our findings of predictable TRE rates and exudate  $\delta^{13}\text{C}$  values in the present study suggest that future studies examining  $\delta^{13}\text{C}$  values of different plant components, soil organic matter and respired soil  $\text{CO}_2$  would benefit by accounting for the impact of root exudates.

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## Introduction

Organic carbon (OC) originating from plant roots is a major contributor to both the reactive and stabilized OC pools contained within soils (Rasse et al. 2005;

Kramer et al. 2010; Schmidt et al. 2011). The transfer of water soluble organic carbon (WSOC) from plants to soils via tree root exudation (TRE) 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). As a result, there is a need to better understand both the specific drivers and temporal dynamics related to plant allocation of OC supporting TRE and the general variability in TRE rates across different terrestrial ecosystems.

Tree root exudation encompasses all active and passive losses of WSOC from living tree roots (Jones et al. 2009). It has been estimated that TRE may account for as much as 40% of NPP (Grayston et al. 1997), however, the methodological challenges of studying belowground plant processes make exudate contributions to NEP uncertain (Chapin et al. 2011). TRE rates are thought to depend on a number of factors ranging from the physical environment (e.g., light and water availability), root traits (e.g., root surface area), and biotic interactions (e.g., the type of mycorrhizae associated with the plant) (Rovira 1969; Phillips et al. 2008; Badri and Vivanco 2009; Yin et al. 2014). Losses of WSOC from plants by TRE have important ramifications for how belowground terrestrial C cycling is quantified and modelled (Heimann and Reichstein 2008; Chapin et al. 2009). For example, inputs of WSOC to soils via TRE may impact the size, composition and turnover times of total soil OC pools (Schmidt et al. 2011). In addition, TRE-derived WSOC has the potential to subsidize microbially driven OC sequestration in soils, ecosystem respiration processes, and dissolved organic carbon exports from terrestrial systems (Kuzyakov 2010; Finzi et al. 2015; Snell et al. 2016; Dodds et al. 2017). It is therefore essential to evaluate the amounts and characteristics of WSOC supplied to soils via TRE in order to better understand the overall role of root exudates in belowground C cycling.

Natural abundance  $\delta^{13}\text{C}$  measurements of phloem sap (a precursor of root exudate WSOC) and of plant and soil respired  $\text{CO}_2$  have been reported to correlate with numerous time-lagged environmental factors that influence stomatal conductance (e.g., vapor pressure deficit, relative humidity) and, as a result, isotopic fractionation of  $\text{CO}_2$  during photosynthesis on the order of hours to days (see reviews by Kuzyakov and

Gavrichkova 2010; Mencuccini and Hölttä 2010; Brüggemann et al. 2011). Such temporally delayed relationships convey important insights about the rates and extents to which recently synthesized OC in leaves is either translocated within or remineralized by the plant and its associated soil environment. Because WSOC in plants originates in leaves and is transported throughout the plant via phloem, many plant components are likely to have  $\delta^{13}\text{C}$  signatures that are related to time-lagged canopy conditions at the time of photosynthesis (Kuzyakov and Gavrichkova 2010). This isotopic signature has also been measured in respired soil  $\text{CO}_2$  and suggested to be indicative of transport and autotrophic remineralization of recently generated photosynthetic products (e.g., Ekblad and Höglberg 2001; Mortazavi et al. 2005). Tree root exudate  $\delta^{13}\text{C}$  values may also be related to time-lagged environmental conditions such as vapor pressure deficit and provide information regarding the timing between  $\text{CO}_2$  fixation, and WSOC translocation within trees and loss from roots to soils. Previous work based on  $\delta^{13}\text{C}$  of soil and root respiration suggests the time lags associated with exudate  $\delta^{13}\text{C}$  of and environmental variables (e.g., vapor pressure deficit) should be between 1 and 5 days (Mencuccini and Hölttä 2010 and references therein). At the ecosystem level, knowledge of TRE rates and exudate  $\delta^{13}\text{C}$  values may lead to better understanding of belowground terrestrial C cycling, including linkages between belowground NPP and NEP, and the differentiation between the autotrophic and soil heterotrophic respiration ( $R_a$  and  $R_h$ , respectively) terms of regional and global C budgets (Subke et al. 2006).

The present study collected root exudates in situ for measurement of net TRE rates and exudate  $\delta^{13}\text{C}$ , as well as the  $\delta^{13}\text{C}$  values of plant leaves and roots, in a temperate mixed hardwood riparian forest of an experimental wetland. Both net TRE rates and exudate  $\delta^{13}\text{C}$  values were evaluated in relation to various plant characteristics and relevant environmental and meteorological parameters. The overarching goals of the study were to (1) assess net TRE rates in riparian tree species in relation to both time-lagged environmental parameters and root traits and, (2) determine the relevant time lags (if any) between the  $\delta^{13}\text{C}$  values of root exudates and environmental factors that influence isotopic fractionation of  $\text{CO}_2$  during photosynthesis. Findings from this study were further used to evaluate relationships between exudate  $\delta^{13}\text{C}$  and bulk leaf and

root materials, and to estimate net TRE rates for the entire riparian hardwood forest.

## Materials and methods

### Study site description

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

Sampling was conducted in the 5600 m<sup>2</sup> forested area between the two experimental wetlands located at the site (see Supplemental Information [SI] Section 1). The ORWRP has undergone natural succession since the establishment of the site as an experimental research wetland. The sampling area has similar plant diversity to other established riparian forests in the region (Brown and Peterson 1983), and portions of the sampling area are occasionally inundated during large precipitation events (Vadas and Sanger 1997; Dudek et al. 1998). The dominant tree species 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 but in smaller relative abundances compared to other tree 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

Riparian hardwood tree root exudates were collected during in situ incubations conducted throughout the summer and autumn of 2014 on the following dates: 17–18 July, 31 July–1 August, 14–15 August, 28–29 September and 16–17 November. A modified version of the method initially developed and described by Phillips et al. (2008) was used to collect exudates in situ. Briefly, intact fine root systems (n = 6–8 per sampling time) were excavated at the bases (within 1 m of the bole) of randomly selected target trees (*A.*

*saccharinum* and *P. deltoides*), cleaned of soil particles, incubated in collection devices for 24 h in situ, and then rinsed three times with 20 mL of 1 mM CaCl<sub>2</sub> to collect root exudate WSOC. Details of the exudate collection method are presented in SI Section 2. To ensure that within-site variability was captured in our sampling, the site was split into ~ 1400 m<sup>2</sup> quadrants, and samples were collected randomly in each quadrant on each sampling date.

### Root imaging and identification

Because the tree species that the roots belonged to could not be identified by visual inspection at the time of excavation, we utilized a DNA barcoding approach described by Kesanakurti et al. (2011) to identify tree roots to genus or species (details are described in SI Section 3). While the DNA barcoding approach provides more definitive information on which particular taxa were sampled, this analysis can only be done a posteriori. Because species identification of roots at the time of field sampling was not possible, this resulted in a sample size imbalance for the tree species assessed in this study. As a result, *A. saccharinum* dominated our samples, though we also collected several samples from each *P. occidentalis* and *P. deltoides*. Images of roots used in in situ incubations were transferred to a computer using a Dell V105 scanner at 600 dpi and images were then converted to black and white using GIMP (2.8.14). Root images were analyzed in WinRhizo<sup>®</sup> to assess surface area, volume and number of root tips.

### Leaf collection

Live leaves were collected from *A. saccharinum* (AS), *P. occidentalis* (PO) and *P. deltoides* (PD) trees on 17 July, 31 July, 14 August, 27 August and 28 September 2014 and frozen at – 20 °C until processing. Leaves were not collected in November because trees had senesced and leaf fall had occurred by that time. For each tree species, four leaves were randomly collected at ~ 2 and ~ 7 m (mid-canopy) height using a telescoping vegetation pruner for a total of eight leaves per tree species for each sampling date.

## Meteorological and net ecosystem exchange measurements

The ORWRP is part of the Ameriflux network (<http://sites.ameriflux.lbl.gov/US-ORv/>), and CO<sub>2</sub> flux measurements have been measured continuously since 2010 to estimate quarter-hourly net ecosystem exchange (NEE, a measure of the balance between CO<sub>2</sub> uptake and release by the entire system relative to atmospheric CO<sub>2</sub>; Morin et al. 2014a). Continuous environmental measurements made over the course of the study include direct and diffuse photosynthetically active radiation (PAR and PAR<sub>d</sub>, respectively), air and soil temperature (T<sub>air</sub> and T<sub>soil</sub>, respectively) and relative humidity (RH). From these measurements, other parameters such as vapor pressure deficit (VPD) are calculated (Morin et al. 2014b). VPD describes the pressure difference between the amount of water vapor air can potentially hold and the amount of atmospheric water vapor at a given time (Anderson 1936). 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 against net TRE rates and δ<sup>13</sup>C values of roots and exudates. Time lags, similar to those employed in the present study, have been applied in a number of previous studies and have demonstrated correlations between environmental parameters (e.g., VPD) and δ<sup>13</sup>C values of plant or soil respired CO<sub>2</sub> and phloem sap (as reviewed by Mencuccini and Hölttä 2010). The time lags used in the present study (< 1–5 days prior to sample collection) to assess relationships between environmental parameters and net TRE rates and exudate δ<sup>13</sup>C values fall within the range used in previous studies (Mencuccini and Hölttä 2010). For bulk root material, we also explored time lags of greater duration (up to 17 days) because of the greater expected turnover times of OC in roots compared to root exudates. Our approach of averaging environmental variables over daytime hours (using the definition of daytime as when PAR > 0.0 W m<sup>-2</sup>) is consistent with the approach of Mortazavi et al. (2005). The durations of time lags in days are indicated as subscripts following each parameter (e.g., a 7-day time lag for NEE is termed NEE<sub>7</sub>) and are defined in SI Section 3.

## Scaling up of net TRE rates

Diameter at breast height (DBH) and genera of all trees ≥ 3 cm DBH (~ 1500 stems in total were measured) within the sampling area were recorded in spring 2015. The DBH measurements were used to estimate fine root biomass using the relationships developed by Chojnacky et al. (2014). Fine root biomass was subsequently used to scale net TRE rates to the entire sampling area. The 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, all of which combined account for less than ~ 10% of plant biomass at the site. Hence, both our fine root biomass and system-scale net root exudation rate estimates are conservative.

## δ<sup>13</sup>C measurements of root exudates

Concentrations and δ<sup>13</sup>C values of root exudate WSOC were analyzed by high temperature combustion-isotope ratio mass spectrometry (HTC-IRMS) at the US Geological Survey (Woods Hole, MA, USA) Dissolved Carbon Isotope Lab. The HTC-IRMS system was a modification of the system described by LaLonde et al. (2014) and consisted of an OI Analytical 1030C Total Carbon Analyzer and a Graden molecular sieve trap interfaced to a Thermo-Finnigan DeltaPlusXP IRMS via a modified ConFlo IV (see SI Section 2 for details).

Following each in situ root incubation, exudates were collected in individual pre-cleaned glass serum bottles by flushing the incubation chambers with 20 mL of 1 mM CaCl<sub>2</sub> solution a total of three times (see SI Section 2 for details). All of the initial flush (“flush 1”) collections, and a selected number of repeat flushes (“flush 2” and “flush 3”) were collected and analyzed for WSOC concentration and δ<sup>13</sup>C. We used a statistical imputation method to estimate WSOC δ<sup>13</sup>C values and concentrations for flush 2 and flush 3 collections in cases where their WSOC concentrations were too low to analyze directly (see SI Section 2 for details). A mixing model was then used to estimate total exudate WSOC δ<sup>13</sup>C from the combination of each of the three measured or estimated flush values for each incubation (see SI Section 2 for details).

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

Bulk leaf and root samples were dried to a constant mass at 60 °C, then ground using a mortar and pestle that was cleaned with methanol between samples. Ground samples were acid-fumed for 24 h using fresh concentrated HCl (12.1 N) in a glass desiccator to remove any inorganic carbon. Samples were then packed into tin boats and submitted to the Stable Isotope Facility at University of California Davis for elemental analysis (EA)-IRMS for C and N contents and  $\delta^{13}\text{C}$  values. Leaf and root  $\delta^{13}\text{C}$  values are reported relative to the V-PDB standard.

## Data analysis

The Kruskal–Wallis test by ranks was used to test for significant differences in net TRE rates across sampling date and species. Simple linear regression was used to identify relationships between the potential continuous and categorical predictors and the response variables of net TRE rates, and the  $\delta^{13}\text{C}$  values of exudates and bulk root materials. We restricted the use of NEE and metrological variables in regression analyses to sampling dates prior to leaf senescence because the mechanistic link with TRE exudation rates and/or  $\delta^{13}\text{C}$  values were anticipated to be mediated by photosynthetic activity in leaves. Candidate predictor variables in the present study are defined and described in detail in SI Section 5. When appropriate, ANOVA and Tukey post hoc pairwise comparisons were used to assess differences in exudate  $\delta^{13}\text{C}$ , leaf  $\delta^{13}\text{C}$  and root  $\delta^{13}\text{C}$  values across time and species. Exudate  $\delta^{13}\text{C}$ , leaf  $\delta^{13}\text{C}$  and root  $\delta^{13}\text{C}$  values were also compared using ANOVA.

Multiple linear regression (MLR) was used to fit models to predict net TRE rates that contained multiple predictor variables. Candidate models were generated by utilizing a step-wise selection procedure based on akaike information criterion (AIC). Although, *P. occidentalis* and *P. deltooides* root exudate samples were collected less frequently than *A. saccharinum*, we included species as a candidate predictor in the MLR models. Austin and Steyerberg (2015) demonstrate that even with categories that contain just two measurements the results of multiple linear regression, and specifically, adjusted  $R^2$ , as we report in the present study, are valid and results are not

meaningfully biased. All statistics were performed in R version 3.2.0 (R Core Team 2015).

## Results

### Net tree root exudation rates

#### *Assessment by sampling date and species*

Net TRE rates averaged ( $\pm$  SE) 13 ( $\pm$  3)  $\mu\text{mol C g root}^{-1} \text{ day}^{-1}$  and varied between 3 and 73  $\mu\text{mol C g root}^{-1} \text{ day}^{-1}$  across all sampling dates and species (Table 1). Kruskal–Wallis H test results indicate that net TRE rates were not equivalent across all sampling dates ( $H = 17.65$ ,  $p < 0.01$ ), however, the only significantly different pairwise comparisons were the higher net exudation rates measured on 17–18 July compared to each of the last two sampling dates (28–29 Sep and 16–17 Nov) (Table 1). At the species level, net TRE rates for *A. saccharinum*, *P. occidentalis* and *P. deltooides* were not significantly different from each other ( $H = 4.99$ ,  $p > 0.05$ ). No significant differences in net TRE rates were observed between *A. saccharinum* and *P. occidentalis* across sampling dates. The small sample size for *P. deltooides* ( $n = 2$  over the entire study) precluded tests for significant differences in net TRE rates across different sampling dates for this species.

#### *Relationships between TRE rates, environmental conditions, and root traits*

Simple linear regression results indicate net TRE rates were inversely related to both  $\text{NEE}_{\text{inc0}}$  (Adj.  $R^2 = 0.35$ ,  $p < 0.001$ ) and  $\text{T}_{\text{air-inc0}}$  (Adj.  $R^2 = 0.13$ ,  $p < 0.05$ ) for all tree species assessed prior to leaf senescence (see SI Section 6 for summary of  $\text{NEE}_{\text{inc0}}$  and  $\text{T}_{\text{air-inc0}}$  values). Net TRE rates were also found to be inversely related to the following log-transformed root traits: (i) mass per unit surface area of root (RMA) (Adj.  $R^2 = 0.46$ ,  $p < 0.001$ ), (ii) carbon content per unit surface area of root ( $\text{C}_{\text{area-root}}$ ) (Adj.  $R^2 = 0.42$ ,  $p < 0.001$ ), and (iii) nitrogen content per unit surface area of root ( $\text{N}_{\text{area-root}}$ ) (Adj.  $R^2 = 0.35$ ,  $p < 0.001$ ) prior to leaf senescence (see SI Section 6 for a summary of root trait values). The AIC-based stepwise variable selection procedure for multiple linear regression analysis indicated that a model with

**Table 1** Average ( $\pm$  SE) net tree root exudation (TRE) rates and  $\delta^{13}\text{C}$  values of exudates, fine tree roots and leaves by 2014 sampling date and species

Species	17–18 Jul			31 Jul–1 Aug			14–15 Aug								
	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{days}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{days}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{days}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)
AS	4	22.6 <sup>a</sup> (9.5)	-32.2 <sup>a</sup> (1.0)	-28.7 (0.4)	-30.0 (0.4)	7	5.6 <sup>a</sup> (1.0)	-29.5 <sup>a</sup> (0.8)	-29.4 (0.2)	-28.9 (0.7)	5	9.9 <sup>a</sup> (0.8)	-28.0 <sup>a</sup> (0.3)	-28.7 (0.4)	-30.0 (0.34)
PO	2	25.0 <sup>a</sup> (1.5)	-29.3 <sup>a</sup> (0.9)	-29.2 (0.3)	-29.6 (0.4)	0	nd	nd	nd	-30.7 (0.3)	2	41.6 <sup>a</sup> (31.0)	-27.6 <sup>a</sup> (0.2)	-28.5 (0.1)	-30.7 (0.5)
PD	1	69.3	-32.7	-29.8	-31.2 (0.4)	1	12.6	-28.5	-30.3	-31.3 (0.3)	0	nd	nd	nd	-31.6 (0.21)
Average	7	30.0 <sup>a</sup> (8.3)	-31.5 <sup>a</sup> (1.0)	-29.0 (0.3)	-30.2 (0.5)	8	6.5 <sup>ab</sup> (1.2)	-29.4 <sup>ab</sup> (0.7)	-29.5 (0.2)	-30.8 (0.5)	7	18.9 <sup>3ab</sup> (9.0)	-27.8 <sup>ab</sup> (0.2)	-28.6 (0.4)	-30.4 (0.5)
Species	28 Aug			28–29 Sep			16–17 Nov								
	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{day}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{day}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)	n	TRE rate ( $\mu\text{mol C}$ $\text{g root}^{-1}$ $\text{day}^{-1}$ )	Exudate $\delta^{13}\text{C}$ (‰)	Root $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)
AS	0	nd	nd	nd	-30.7 (0.4)	8	5.9 <sup>a</sup> (0.8)	-27.4 <sup>a</sup> (0.3)	-28.6 (0.4)	-31.2 (0.2)	4	5.7 <sup>a</sup> (1.3)	-28.1 <sup>a</sup> (0.9)	-29.1 (0.4)	nd
PO	0	nd	nd	nd	-30.9 (0.3)	0	nd	nd	nd	-31.1 (0.2)	2	3.3 <sup>a</sup> (0.3)	-27.3 <sup>a</sup> (1.2)	-29.9 (0.4)	nd
PD	0	nd	nd	nd	-31.6 (0.2)	0	nd	nd	nd	-31.1 (0.2)	0	nd	nd	nd	nd
Average	0	nd	nd	nd	-31.2 (0.4)	8	5.9 <sup>b</sup> (0.8)	-27.4 <sup>b</sup> (0.3)	-28.6 (0.4)	-31.5 (0.2)	6	4.9 <sup>b</sup> (1.0)	-27.8 <sup>ab</sup> (0.7)	-29.4 (0.3)	nd

Different superscripted letters (a, b) indicate significant differences at the  $\alpha=0.05$  level within a species and measurement type (i.e., TRE rate or  $\delta^{13}\text{C}$ ). TRE rates are expressed per g dry weight of root material. Sample size (n) refers to the level of replication for net TRE rates, exudate  $\delta^{13}\text{C}$  and root  $\delta^{13}\text{C}$ . The sample size for all reported leaf measurements is n = 8

AS: *Acer saccharinum*

PO: *Platanus occidentalis*

PD: *Populus deltoides*

nd: not determined

multiple predictors greatly improves the overall fit to explain net TRE rates using a range of potential models (Table 2a, b). The model that includes  $NEE_{inc0}$ ,  $N_{area-root}$  and species explained a significant amount of the observed variation in net TRE rates (Adj.  $R^2 = 0.73$ ,  $p < 0.001$ ), while requiring relatively few predictors (Fig. 1a, b).

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

The grand mean ( $\pm$  SE) root exudate  $\delta^{13}C$  value was  $-28.8 \pm 0.4\%$  and varied between  $-35.0$  and  $-25.7\%$  across all sampling dates and species (Table 1). Kruskal–Wallis H test results ( $H = 13.11$ ,  $p < 0.05$ ) indicate that the only significant difference in exudate  $\delta^{13}C$  values between sampling dates were the lower values observed on 17–18 Jul (average  $\pm$  SE =  $-31.5 \pm 1.0\%$ ) compared to the higher values observed on 28–29 Sep (average  $\pm$  SE =  $-27.4 \pm 0.3\%$ ) (Table 1). No significant differences in exudate  $\delta^{13}C$  values were observed between either different tree species or within a single species across multiple sampling dates. Mean exudate  $\delta^{13}C$  values became progressively higher over the course of the first three sampling periods and were

then maintained for the remainder of the study (Table 1). Exudate  $\delta^{13}C$  values also showed a quadratic relationship with  $T_{air-inc03}$  (Adj.  $R^2 = 0.43$ ,  $p < 0.001$ ) and a positive relationship with  $VPD_{inc02}$  (Adj.  $R^2 = 0.21$ ,  $p < 0.05$ ) (Fig. 2a, b). Exudate  $\delta^{13}C$  also showed a positive relationship with  $VPD_{inc03}$  ( $R^2 = 0.10$ ;  $p < 0.05$ ), but the relationship was not as strong as the relationship with  $VPD_{inc02}$ .

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

The overall average ( $\pm$  SE) root tissue  $\delta^{13}C$  value was  $-28.9 \pm 0.1\%$  across all species and sampling dates and ranged between  $-30.4$  and  $-27.2\%$  (Table 1). Root tissue  $\delta^{13}C$  was found to be positively related to both  $NEE_7$  (Adj.  $R^2 = 0.11$ ,  $p < 0.05$ ) and  $VPD_7$  (Adj.  $R^2 = 0.11$ ,  $p < 0.05$ ), and negatively related to  $RH_7$  (Adj.  $R^2 = 0.13$ ,  $p < 0.05$ ) (Fig. 3a–c; see SI Section 6 for a summary of the  $NEE_7$ ,  $VPD_7$ , and  $RH_7$  values). Significant linear relationships were not observed when comparing root tissue  $\delta^{13}C$  to shorter time lags for either VPD or RH. However, 3- and 4-day time lags for NEE were found to be significantly positively related to root tissue  $\delta^{13}C$  (data not shown). Unlike VPD and RH, we had no a priori expectation

**Table 2** (a) Results from the AIC-based stepwise variable selection procedure to predict log transformed mass-based net root WSOC exudation rates. (b) Results from the AIC-based

stepwise variable selection procedure for alternative models that include variables that are correlated to the predictor variables in the stepwise selection procedure

(a)

Predictors in model	Step	AIC	Residual sum of squares	Adj. $R^2$	p value
None	Add log(RMA)	– 6.88	21.35	–	–
log(RMA)	Add species	– 23.64	11.18	0.46	< 0.001
log(RMA) + species	Add $NEE_{inc0}$	– 38.58	5.82	0.69	< 0.001
log(RMA) + species + $NEE_{inc0}$	None	– 40.02	5.17	0.72	< 0.001

(b)

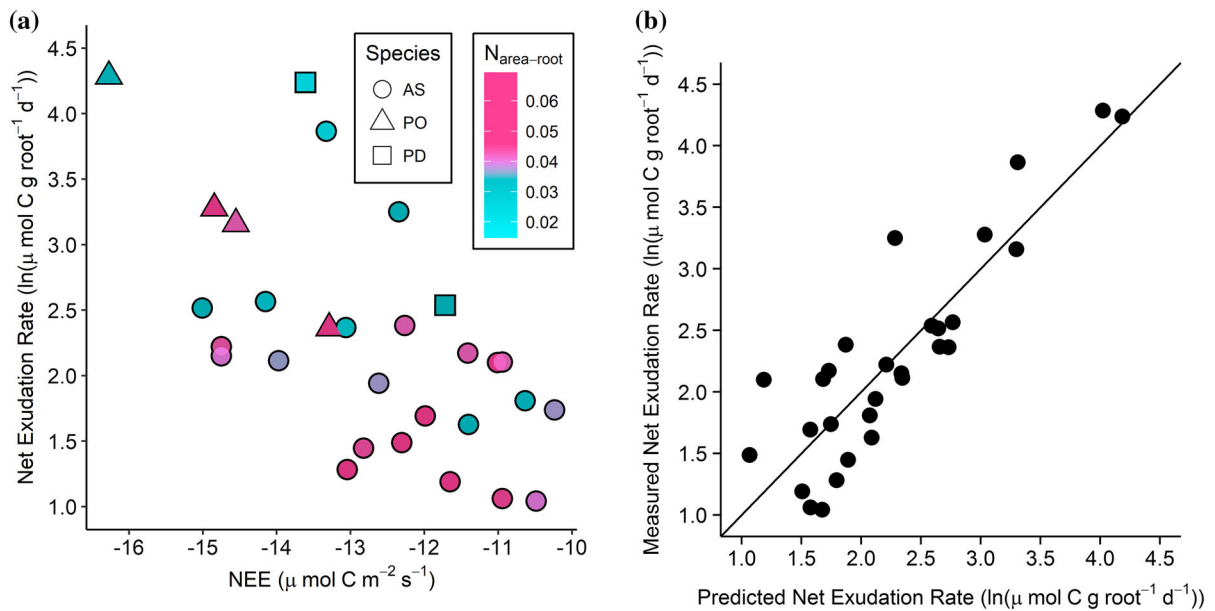
Alternative models

Predictors in model <sup>a</sup>	Adj. $R^2$	p-value
$\log(N_{area-root}) + \text{species} + NEE_{inc0}$	0.73	< 0.001
$\log(RMA) + \text{species} + T_{air-inc0}$	0.72	< 0.001
$\log(N_{area-root}) + \text{species} + T_{air-inc0}$	0.73	< 0.001

See SI Section 6 for summary of RMA,  $N_{area-root}$ ,  $NEE_{inc0}$ ,  $T_{air-inc0}$  values used in this study

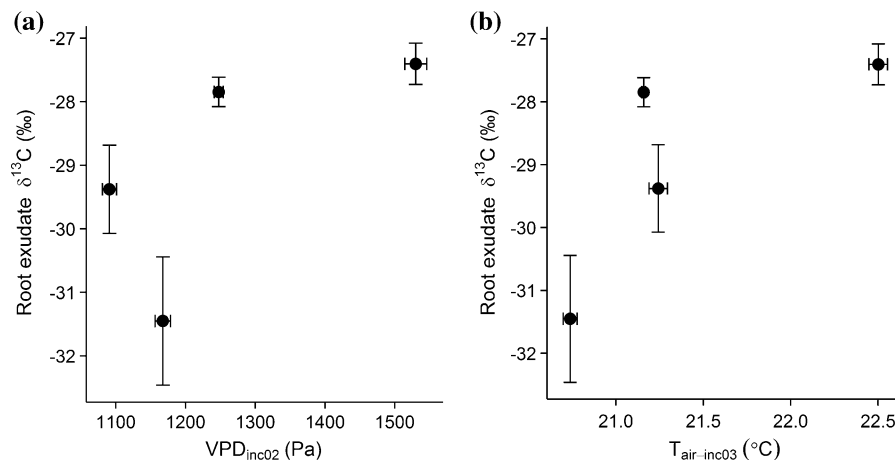
AIC akaike information criterion

<sup>a</sup> $T_{air-inc0}$  is defined as the air temperature averaged over the entire day of each root incubation



**Fig. 1** **a** Net root WSOC exudation (i.e., TRE) rate versus average  $\text{NEE}_{\text{inc0}}$  for different tree species in the present study (AS: *Acer saccharinum*, PO: *Platanus occidentalis*, PD: *Populus deltoides*). Color coding indicates  $N_{\text{area-root}}$  ( $\text{mg N cm}^{-2}$ ). Adj.  $R^2 = 0.73$  ( $p < 0.001$ ) for the full model that includes NEE,  $N_{\text{area-root}}$  and species as predictors of net root exudation rates. Adj.  $R^2 = 0.35$  ( $p < 0.001$ ) for the reduced

model that includes only  $\text{NEE}_{\text{inc0}}$  as a predictor of net root exudation rates (see text for details). **b** Measured net root WSOC exudation rate versus predicted net WSOC exudation rate based on the model containing NEE,  $N_{\text{area-root}}$  and species as predictors (see text for details). The solid line represents the 1:1 relationship. (Color figure online)



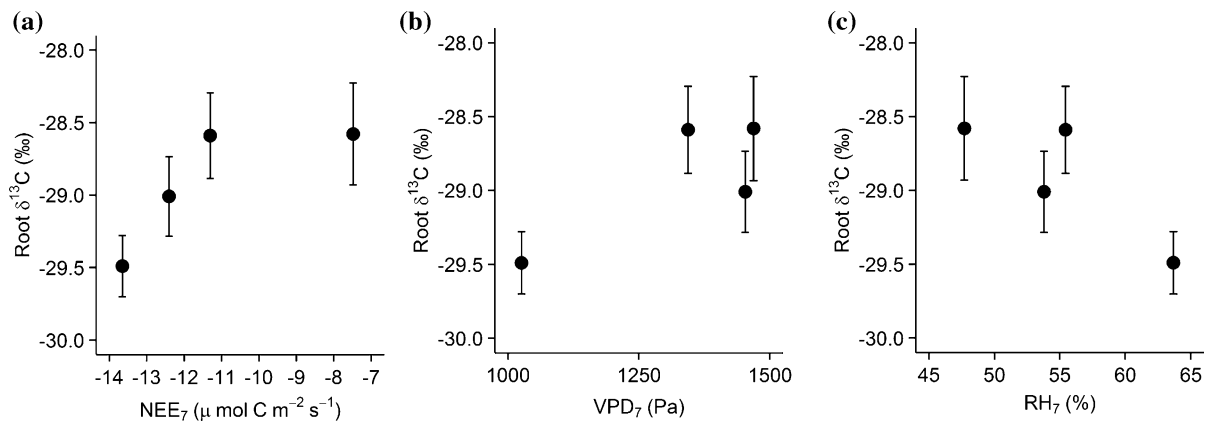
**Fig. 2** Average ( $\pm$  SE) root exudate WSOC  $\delta^{13}\text{C}$  values versus **a** average ( $\pm$  SE) VPD over the 2 days prior to (and including the day of) the end of the incubations (Adj.  $R^2 = 0.21$ ;  $p < 0.05$ ) and **b** average ( $\pm$  SE)  $T_{\text{air}}$  over the 3 days prior to

(and including the day of) the end of the incubations (Adj.  $R^2 = 0.43$ ;  $p < 0.05$ ). The plots include data across all root incubations for individual sampling dates prior to leaf senescence in the present study.  $n = 7\text{--}8$  per data point

for a relationship between root  $\delta^{13}\text{C}$  and NEE, therefore we focus on the longer 7-day time lag rather than the 3- and 4-day time lags. Because non-structural carbon (NSC) in bulk root tissue has the potential

to turnover more slowly than 7 days, we also looked for relationships between root tissue  $\delta^{13}\text{C}$  and 10 day and 17 day time lags for NEE, VPD and RH. The only relationship that we found to be significant for the





**Fig. 3** Average ( $\pm$  SE) bulk root  $\delta^{13}\text{C}$  values versus **a** average NEE for the day of sample collection and the preceding 6 days prior to sampling (Adj.  $R^2 = 0.11$ ,  $p < 0.05$ ), **b** average VPD for the day of sample collection and the preceding 6 days prior to sampling (Adj.  $R^2 = 0.11$ ,  $p < 0.05$ ) and **c** average RH for

longer time lags was the 10 day lag for RH (Adj.  $R^2 = 0.11$ ,  $p < 0.05$ ) (data not shown). No significant differences in root tissue  $\delta^{13}\text{C}$  values were found between species or sampling date (Table 1). In addition, root tissue  $\delta^{13}\text{C}$  was not found to be significantly related to exudate  $\delta^{13}\text{C}$  values or any root traits (e.g., RMA) assessed in this study.

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

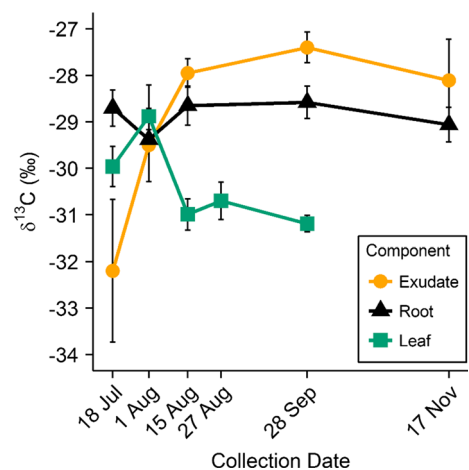
Leaf tissue  $\delta^{13}\text{C}$  values of different tree species averaged ( $\pm$  SE) across the entire study were  $-30.6 \pm 0.2$ ,  $-31.4 \pm 0.1$ , and  $-30.4 \pm 0.2\text{‰}$  for *P. occidentalis*, *P. deltooides* and *A. saccharinum*, respectively. ANOVA results ( $F_{2,114} = 8.3$ ,  $p < 0.001$ ) and subsequent Tukey pairwise comparisons indicate that *P. deltooides* leaf  $\delta^{13}\text{C}$  was significantly lower compared to both *A. saccharinum* ( $p < 0.01$ ) and *P. occidentalis* ( $p < 0.01$ ) (Table 1) but that *A. saccharinum* and *P. occidentalis* leaf  $\delta^{13}\text{C}$  values were not significantly different from each other ( $p > 0.05$ ).

#### Comparison of *A. saccharinum* leaf and root tissue, and root exudate $\delta^{13}\text{C}$ values

*A. saccharinum* was the only species for which leaves, roots and exudates were collected with replication across all sampling dates. For this reason we focused the comparison of  $\delta^{13}\text{C}$  of these different plant

the day of sample collection and the preceding 6 days prior to sampling (Adj.  $R^2 = 0.13$ ,  $p < 0.05$ ). All plots show average  $\delta^{13}\text{C}$  values for all roots collected from all tree species for individual sampling dates prior to leaf senescence in this study.  $n = 7\text{--}8$  per data point

components on *A. saccharinum*, although the other species for which we have limited data qualitatively followed similar trends (Table 1). Average ( $\pm$  SE)  $\delta^{13}\text{C}$  values across sampling dates for *A. saccharinum* leaves, roots and exudates were  $-30.4 \pm 0.2$ ,  $-28.9 \pm 0.4$ , and  $-28.8 \pm 0.4\text{‰}$ , respectively (Table 1). ANOVA results indicate significant differences in  $\delta^{13}\text{C}$  values between leaves and roots, but the differences were dependent on sampling date ( $F_{7,46} = 8.0$ ,  $p < 0.001$ ) (Fig. 4). On average,  $\delta^{13}\text{C}$



**Fig. 4**  $\delta^{13}\text{C}$  values of root WSOC exudate, root OC, and leaf OC for *A. saccharinum* for all sampling dates in the present study. Each point represents the mean ( $\pm$  SE) for samples collected on each date. Leaf samples were not collected on 17 Nov. because they had already undergone senescence and leaf fall by this time. (Color figure online)

values of *A. saccharinum* roots were enriched by 1.5‰ compared to leaves.

Leaf and root exudate  $\delta^{13}\text{C}$  values for *A. saccharinum* were also found to be significantly different from each other ( $F_{7,46} = 8.6$ ,  $p < 0.001$ ) (Fig. 4). Exudate  $\delta^{13}\text{C}$  values were 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 ( $p < 0.001$ ). The  $\delta^{13}\text{C}$  values of roots and exudates for *A. saccharinum* did not differ significantly from each other (Fig. 4). However, sampling date ( $p < 0.001$ ) and the interaction effect between sampling date and plant component (i.e., exudate or bulk root) were significant ( $p < 0.01$ ).

## Discussion

Relationship between photosynthesis, root traits and root exudation in riparian trees

### *Rates of exudation versus NEE*

A significant inverse relationship was observed between TRE rates and NEE in the present study (Fig. 1a). To our knowledge this is the first demonstration of a relationship between NEE and TRE under field or laboratory conditions. Because NEE varies as a function of both photosynthesis and respiration, we are unable to definitively test whether the net TRE rate-NEE relationship would also hold for TRE rate versus gross primary production (GPP). However, in our root exudate collections conducted during the growing season, average night time (i.e.,  $\text{PAR} < 0$ ) NEE had a maximum difference of  $0.5 \mu\text{mol C m}^{-2} \text{s}^{-1}$  between

sampling dates; in contrast, average day time NEE differences across sampling dates were much larger (as high as  $3.3 \mu\text{mol C m}^{-2} \text{s}^{-1}$ ) (Table 3). These day-night differences suggest that day time NEE variations prior to autumn leaf senescence were dominated by differences in photosynthetic uptake of  $\text{CO}_2$  rather than by ecosystem respiration. The relationship between net TRE rates and NEE observed in the present study further suggests that increases in the net photosynthetic rate lead to increases in the total amount of tree NSC that is passively or actively transported from root cells to the environment through exudation (Subke et al. 2006; Jones et al. 2009).

### *Root exudation as a loss of non-structural plant carbon*

Subke et al. (2006) suggested that when tree NSC is not limiting to tree growth or metabolic requirements, TRE rate may increase with photosynthetic rate due to the accumulation and ultimate release of NSC in roots during periods of relatively high photosynthetic activity. In contrast, when tree metabolic and growth demands for NSC are high (i.e., NSC is potentially limiting), these workers suggested that the anticipated increase in tree NSC during high rates of photosynthesis would likely be allocated and used to satisfy  $R_a$  and growth demands. Under such conditions, net TRE rates would not be expected to scale with photosynthetic rates (Subke et al. 2006; Dietze et al. 2013). When interpreted in the context of NSC demand and allocation within individual trees, the inverse relationship observed between net TRE rates and NEE in the present study (Fig. 1a) suggests that the trees in our study system were not limited by NSC over the course of the growing season. Additionally, episodic high internal NSC demand within trees, such as during

**Table 3** Average ( $\pm$  SE) 2014 growing season NEE values for nighttime (defined as  $\text{PAR} < 0$ ) and daytime periods for root incubations to collect exudates

Sampling period	Average nighttime NEE ( $\mu\text{mol C m}^{-2} \text{s}^{-1}$ )	Average daytime NEE ( $\mu\text{mol C m}^{-2} \text{s}^{-1}$ )
17–18 Jul	3.9 (0.1)	– 12.9 (0.3)
31 Jul–1 Aug	4.3 (0.2)	– 12.7 (0.2)
14–15 Aug	3.8 (0.1)	– 12.3 (0.1)
28–29 Sep	3.8 (0.1)	– 9.6 (0.5)

major phenological events (e.g., flowering, or leaf expansion in spring) that may not have been captured by our sampling frequency, may result in altered (i.e., lower or higher) net TRE rates due to different allocation patterns among tree tissues and organs throughout periods of growth and dormancy (Dietze et al. 2013).

One particularly important belowground phenological event for trees that is expected to directly affect both net TRE rates and NSC allocation is the production of fine roots, which predominantly occurs in late spring and early summer for temperate hardwood trees in the study region (McCormack et al. 2014). While the timing of root growth was not specifically assessed in the present study, it is likely that fine root biomass production had already peaked by the time of the first sampling period (17–18 Jul) (McCormack et al. 2014) (Table 1). However, in other studies, actively growing roots tips have been observed to exude more WSOC than established roots, and this is thought to be driven by a number of factors, including the maintenance of contact between the root and soil particles (Rovira 1969; Walker et al. 2003). Periods with high rates of fine root production, and thus demand for NSC, would be predicted to have correspondingly high net TRE rates compared to periods with relatively lower fine root production.

#### *Modelling net tree exudation rates*

Despite the large number and type of potential plant-specific determinants of net TRE rates, the present study demonstrates that TRE rates are best predicted by a combination of ecosystem C fluxes (i.e., NEE) and plant-specific properties (i.e., species and  $N_{\text{area-root}}$ ; Table 2, Fig. 1a). Other environmental and biological factors that have been reported to influence net TRE rates include nutrient availability, soil temperature, atmospheric  $p\text{CO}_2$  and the specific types of root-mycorrhizae relationships (e.g., Rovira 1969; Phillips et al. 2011; Aoki et al. 2012; Yin et al. 2013). Our finding of a relationship between TRE rates and individual root traits (i.e., negative correlations with both RMA and  $N_{\text{area-root}}$ ) for riparian trees across the entire study (Table 2, Fig. 1a) is similar to previous studies in perennial grasses that suggest roots with low mass per unit length exhibit increased exudation rates (Adkins et al. 2016). The present findings are relevant because root traits have been suggested to

be linked to particular functions such as nutrient acquisition (Eissenstat et al. 2015). Roots with lower RMA and root mass per unit length are generally thought to increase plant nutrient uptake to a greater extent than roots with greater RMA or root mass per unit length (Bardgett et al. 2014). Root exudates have also been proposed to be important in increasing nutrient availability to plants (Grayston et al. 1997; Meier et al. 2017). As a result, the increased plant nutrient uptake associated with lower RMA may be partially attributable to the impacts of increased root exudation and soil nutrient availability. Temperate deciduous trees are also known to exhibit both interspecific and intraspecific variation in root traits (Eissenstat et al. 2015) and should therefore be further considered within the context of TRE and the belowground portion of the C cycle.

Potential drivers and implications of  $\delta^{13}\text{C}$  variability of root exudates

#### *Mechanistic link between VPD and exudate $\delta^{13}\text{C}$*

The  $\delta^{13}\text{C}$  values of tree root exudates were found to be significantly and positively correlated to time-lagged environmental parameters (i.e., VPD and  $T_{\text{air}}$ ) (Fig. 2a, b). We suggest that the observed positive relationships are a result of isotopic fractionation of  $\text{CO}_2$  during photosynthesis (Farquhar et al. 1989; Kuzyakov and Gavrichkova 2010; Brüggemann et al. 2011). Specifically, as VPD increases (i.e., as a function of RH and  $T_{\text{air}}$ ), a predicted physiological response is for the tree to decrease leaf stomatal conductance, leading to lower  $\text{CO}_2$  in leaf intercellular space ( $p_i$ ) relative to that of the atmosphere ( $p_a$ ) (i.e., lower  $p_i/p_a$ ) during photosynthesis in the leaf and concomitantly greater enrichment in  $^{13}\text{C}$  of newly synthesized photosynthate, and hence root exudates, due to decreased kinetic fractionation (Farquhar et al. 1989; Mortazavi et al. 2005). While this is the first study to our knowledge to demonstrate positive relationships between exudate  $\delta^{13}\text{C}$  and VPD, the mechanism we propose is consistent with other studies that have found correlations between VPD and either the  $\delta^{13}\text{C}$  of phloem sap in trees (e.g., Keitel et al. 2003) or with  $\delta^{13}\text{C}$  signatures of  $\text{CO}_2$  respired from soils (e.g., Mortazavi et al. 2005) in forested systems.

### Estimating rates of translocation of NSC from leaves to root exudates

The statistically significant positive correlations observed between exudate  $\delta^{13}\text{C}$  values and  $\text{VPD}_{\text{incO}_2}$  and  $T_{\text{air-incO}_3}$  (Fig. 2a, b) suggest that a portion of root exudate WSOC is derived from recently synthesized NSC that is transferred belowground on timeframes of several days after production in the canopy. Additionally, the 2–3 day time lags we observed between exudate  $\delta^{13}\text{C}$  and VPD and  $T_{\text{air}}$  in the present study (Fig. 2a, b) fall within the range of phloem transport rates ( $\sim 2$ –5 days for dicot trees) compiled by Mencuccini and Hölttä (2010) that integrate the time from fixation of  $\text{CO}_2$  by leaves in the canopy to transport of NSC to belowground pools. It is also noteworthy that the time lags used to predict exudate  $\delta^{13}\text{C}$  (2–3 days in VPD and  $T_{\text{air}}$ ) and net TRE rates (0 days for NEE) are different. This suggests that the increasing rates of net root exudation associated with increasing net photosynthetic rate (Fig. 1a) are at least partially due to exudate WSOC that is comprised of NSC produced 2–3 days prior to its release from roots (Fig. 2a, b). While it is not possible to definitively confirm this mechanism in the present study, future tests in either field or greenhouse settings could be designed to directly manipulate the levels of photosynthesis (e.g., using shade cloth) and utilize an isotopic tracer (i.e.,  $^{13}\text{C}$ -labeled  $\text{CO}_2$ ) to track the allocation of ‘new’ versus ‘old’ NSC belowground to root exudation.

### Relationships between $\delta^{13}\text{C}$ of exudates, roots and leaves and net root exudation rates

Bulk root  $\delta^{13}\text{C}$  was found to be significantly positively correlated to time-lagged  $\text{VPD}_7$  and negatively correlated to  $\text{RH}_7$  (Fig. 3b, c). Elevated RH and lower VPD are generally associated with increased stomatal aperture, which leads to higher  $p_i/p_a$  and greater fractionation against  $^{13}\text{C}$  during photosynthesis (i.e.,  $\delta^{13}\text{C}$  is lower in the resultant photosynthate) (Farquhar and Sharkey 1982). The correlations between bulk root  $\delta^{13}\text{C}$  values and RH and VPD in the present study suggest that a significant amount of recently fixed OC is allocated to and detectable in tree roots.

The observed relationships between bulk root  $\delta^{13}\text{C}$  values and RH and VPD are also consistent with our contention that root exudate consists largely of WSOC

produced 2–3 days prior to exudation (Fig. 2a, b). It is reasonable to expect that the NSC that is not exuded is also utilized in one or more ways, either for new growth or as NSC stored in root tissues. The time lags of the environmental factors controlling bulk root  $\delta^{13}\text{C}$  (i.e., time lags of 7 days for VPD and RH) and exudate  $\delta^{13}\text{C}$  (i.e., time lags of 2–3 days for VPD and  $T_{\text{air}}$ ) were different (Figs. 2a, b and 3a, b). This difference suggests that the signature of recent photosynthate in roots would likely be more difficult to discern in bulk root materials compared to exudates because of the relatively large dilutional effect of existing structural OC in bulk root material compared to the soluble NSC pool that contributes directly to exudate WSOC. Whether the observed correlations between bulk root  $\delta^{13}\text{C}$  and VPD and RH resulted primarily from increasing concentrations of recently produced NSC (e.g., sugars or starch) in root material or from the incorporation of recently produced NSC into structural components of new fine root growth, or both, was beyond the scope of the present study but merits further investigation.

Bulk root  $\delta^{13}\text{C}$  was also positively related to NEE in the present study (Fig. 3a). Our original expectation was that bulk root  $\delta^{13}\text{C}$  and NEE would be negatively related because greater levels of photosynthesis at a constant stomatal aperture would result in decreased  $p_i/p_a$  and increased  $\delta^{13}\text{C}$  value of photosynthate (Farquhar et al. 1989). One potential explanation for the observed versus expected relationship between root  $\delta^{13}\text{C}$  and  $\text{NEE}_7$  is the greater allocation of relatively  $^{13}\text{C}$ -depleted (i.e., lower  $\delta^{13}\text{C}$  values) NSC from leaves to roots when photosynthesis is high. The observed  $^{13}\text{C}$  enrichment (i.e., higher  $\delta^{13}\text{C}$  values) of bulk root material compared to leaves (Fig. 4) supports this as a possible mechanism by suggesting that NSC recently translocated from leaves would be isotopically depleted relative to bulk roots. In addition, our findings of (i) greater net TRE rates with lower NEE (Fig. 1) and (ii) exudate  $\delta^{13}\text{C}$  being influenced by canopy conditions 2–3 days prior (Fig. 2a, b) further suggest a greater allocation of recently fixed NSC from the canopy to roots when photosynthetic rates are high. While the reasons for the discrepancy between the observed and predicted relationship between root  $\delta^{13}\text{C}$  and  $\text{NEE}_7$  are not entirely understood at present, these findings suggest that the positive relationship between bulk root  $\delta^{13}\text{C}$  and  $\text{NEE}_7$  may be related to greater allocation of NSC

from  $^{13}\text{C}$ -depleted leaves to roots when photosynthetic uptake of C was high.

Extrapolation of root exudation rates to stand-scale and comparison to other studies

The overall average ( $\pm$  SE) net TRE rate of WSOC was  $13 \pm 3 \mu\text{mol C g root}^{-1} \text{ days}^{-1}$  across all sampling dates and tree species examined in the present study. When scaled to the entire  $5600 \text{ m}^2$  riparian forest area of the ORWRP the TRE flux is calculated to be  $2.1 (\pm 0.5) \text{ mmol C m}^{-2} \text{ day}^{-1}$ , or  $0.77 (\pm 0.18) \text{ mol C m}^{-2} \text{ year}^{-1}$  (Table 4). Average annual net  $\text{CO}_2$  uptake from 2011 to 2013 using eddy flux towers at our study site has been estimated to be  $25.85 \text{ mol C m}^{-2} \text{ year}^{-1}$  (Morin et al. 2014b). Therefore, net root exudation rates of WSOC reported in the present study account for as much as  $\sim 3\%$  net C uptake at this riparian mixed forest site on an annual basis. This is a conservative estimate of total root exudation because it is based only on trees with  $\text{DBH} > 3 \text{ cm}$  and does not include other plants found in this system such as woody shrubs (e.g., *L. maackii*) or herbaceous species (e.g., *Solidago* spp.). The contribution of root exudates to the overall OC accumulation in soils, however, is likely lower than  $3\%$  because some portion (up to  $\sim 60\%$  as estimated by Uselman et al. 2000) of exudate WSOC is remineralized by soil microorganisms on time scale of hours to weeks, and/or lost from the system through hydrological flow in soils (van Hees et al. 2005).

In order to compare net TRE rates measured in the present study to previous estimates, we compiled net hardwood and gymnosperm TRE rate data from all known studies that used field approaches and methods similar to those used here and originally outlined by Phillips et al. (2008) (Table 4). A high degree of variability in average root mass-normalized exudation rates is found between different studies ( $12\text{--}600 \mu\text{mol C g root}^{-1} \text{ day}^{-1}$ ), with the average rate reported in the present study ( $13 \mu\text{mol C g root}^{-1} \text{ day}^{-1}$ ) at the low end of this range. Some of the variability in net TRE rates may be attributable to differences between species or the types of mycorrhizal association with roots (e.g., arbuscular or ectomycorrhizal fungi) (Yin et al. 2014), but these variables have not been found to be consistent drivers of net TRE rates across all studies (e.g., Brzostek et al. 2013). Other biologically relevant factors that could

drive the large variability of reported net TRE rates include soil and biome type, soil moisture and nutrient availability and belowground competition with other plants (Rovira 1969; Phillips et al. 2011; Aoki et al. 2012; Yin et al. 2013).

An important methodological consideration that could lead to higher estimates of net TRE rates in previous studies is the exudate collection device and the materials used. In particular, the specific type of glass beads used, the methods of cleaning them of contaminant OC, and the use of OC-rich and leachable materials such as butyl rubber stoppers and Parafilm (Phillips et al. 2008), may impart significant WSOC artifacts, as demonstrated by preliminary methodological tests conducted as part of the present study (see SI Section 2). The use of ultra-clean materials and incubation conditions and development of a novel method for calculating the concentrations of analytical blanks in the present study should therefore provide more accurate estimates of in situ TRE.

Importance of root exudate fluxes to ecosystem carbon budgets

While net root WSOC exudation rates reported in the present study represent a relatively small component ( $\sim 3\%$ ) of annual net  $\text{CO}_2$  fixation (Table 4), when assessed as an input to soils, especially at longer time scales (e.g., over the lifespan of a tree), exudate fluxes are likely to become a quantitatively important source of OC to riparian soils and to both terrestrial and associated aquatic C and organic matter inputs and cycling. To illustrate this point, we compiled a number of C fluxes using literature estimates of soil OC accumulation rates, heterotrophic soil respiration fluxes, WSOC losses from soils (both organic and mineral soil horizons), and amounts of terrestrial soil- (including root-) derived OC exported to streams and rivers (Table 5).

When we consider the fate of OC originating in soil as root exudates, based on the relative magnitudes of the fluxes in Table 5 it is likely that a large portion of root exudate WSOC is used to support the microbially driven heterotrophic soil respiration efflux of  $\text{CO}_2$ . For example, Uselman et al. (2000) report a  $60\%$  loss of exudate WSOC over a 7 day incubation. However, the recent recognition that a significant proportion of soil OC may be microbially processed or ‘repackaged’ (Schmidt et al. 2011), suggests that root exudate

**Table 4** Summary of net WSOC exudation rates reported for fine tree roots in the present study and from other published field studies using similar root exudate collection methods

System type and source	Species sampled	Estimated fine root biomass <sup>a</sup>	Mass-based exudation rate ( $\mu\text{mol WSOC g root}^{-1} \text{ day}^{-1}$ )	Annual exudation rate ( $\text{mol WSOC m}^{-2} \text{ year}^{-1}$ )
Temperate mixed forest Abramoff and Finzi (2016)	<i>Quercus rubra</i> , <i>Fraxinus Americana</i> , <i>Tsuga canadensis</i>	245 g C m <sup>-2</sup>	69–109	3.83–4.58 <sup>b</sup>
Tropical rainforest Aoki et al. (2012)	<i>Lithocarpus</i> , <i>Syzygium</i> , <i>Tristaniopsis</i> , <i>Dacrycarpus</i> , <i>Dacrydium</i> genera	665–874 g dry wt m <sup>-2</sup>	12–27	2.52–8.40
Temperate mixed hardwood forest Brzostek et al. (2013)	<i>Acer saccharum</i> , <i>Fraxinus americana</i> , <i>Tsuga canadensis</i> , <i>Fagus grandifolia</i>	367–504 g dry wt m <sup>-2</sup>	57–200	NR
Temperate Pine Forest Drake et al. (2011)	<i>Pinus taeda</i>	127 g C m <sup>-2</sup>	NR	1.91–2.91
Temperate pine forest Phillips et al. (2008)	<i>Pinus taeda</i>	250 g dry wt m <sup>-2</sup>	NR	0.78
Temperate pine forest Phillips et al. (2011)	<i>Pinus taeda</i>	235 g dry wt m <sup>-2</sup>	16–34	1.93
Temperate mixed deciduous-evergreen forest Sun et al. (2017)	<i>Quercus serrata</i> , <i>Ilex pedunculosa</i> , <i>Quercus glauca</i> , <i>Ilex macropoda</i>	60 g dry wt m <sup>-2</sup>	250–600 <sup>c</sup>	4.56–13.68 <sup>c</sup>
Temperate beech forest Tückmantel et al. (2017)	<i>Fagus sylvatica</i>	248 g dry wt m <sup>-2</sup>	138	5.66
Boreal forest Yin et al. (2013)	<i>Picea asperata</i>	NR	33–58	NR
Temperate mixed hardwood forest Yin et al. (2014)	<i>Quercus alba</i> , <i>Fagus grandifolia</i> , <i>Acer saccharum</i> , <i>Liriodendron tulipifera</i>	NR	15–41	0.67–2.16
Temperate riparian mixed hardwood forest (present study)	<i>Acer saccharinum</i> , <i>Populus deltoides</i> , <i>Platanus occidentalis</i>	162 g dry wt m <sup>-2</sup>	13	0.77

<sup>a</sup>All mass based rates are reported on a mass dry weight basis with the exception of Abramoff and Finzi (2016) and Drake et al. (2011) who only reported the amount of OC in fine root biomass and not root biomass on a dry weight basis

<sup>b</sup>Estimate reported in original publication is for growing season only

<sup>c</sup>Estimate based on hourly or daily flux rate and extracted from figure in original publication

NR values were not reported in the source publication

**Table 5** Summary of flux rates from the literature and the present study for root WSOC exudation, heterotrophic soil respiration, WSOC losses from organic and mineral soil horizons, soil OC accumulation, and exports of WSOC from terrestrial to riverine systems

Flux type	Source	System type	Flux (mol C m <sup>-2</sup> year <sup>-1</sup> )
Root exudate fluxes	Mean from Table 4	Various <sup>a</sup>	1.4–2.8
Heterotrophic soil respiration	Carbone et al. (2016)	Temperate-boreal forest	71.19
	Subke et al. (2006) and sources therein	Temperate deciduous forest	42.16
Organic soil horizon WSOC loss	Aitkenhead-Peterson et al. (2003) and sources therein	Cool deciduous forest	2.10
	Kindler et al. (2011) and sources therein	U.K. and European forests	1.09
Mineral soil horizon WSOC loss	Aitkenhead-Peterson et al. (2003) and sources therein	Cool deciduous forest	1.49
	Kindler et al. (2011) and sources therein	U.K. and European forests	0.29
Soil OC accumulation	Ricker et al. (2014)	Riparian	24.15
	Ricker et al. (2012)	Riparian	6.74
	Gaudinski et al. (2000)	Temperate forest	0.83–2.5
Terrestrial WSOC export to streams and rivers	Hossler and Bauer (2013)	Northeast U.S. rivers	6.53

<sup>a</sup>System types include boreal, temperate, riparian, and tropical rain forests

WSOC may also contribute significantly to soil OC content and/or the amount of WSOC lost from soils via hydrologic transport. In fact, emerging views of soil OC also suggest that sorption of simple low molecular weight organic compounds (like those found in root exudates) to soil mineral surfaces is a significant factor that stabilizes OC in soil (Lehmann et al. 2008; Schmidt et al. 2011).

Quantitative information about the fates and characteristics of OC that originates in soil as exudates under field conditions is generally lacking, and therefore speculative at present. If we assume 40% of root exudate WSOC is not heterotrophically remineralized (consistent with the observations by Uselman et al. 2000), exudates may contribute as much as 1.00–1.82 mol C m<sup>-2</sup> year<sup>-1</sup> to other fluxes listed in Table 5 (i.e., organic and mineral soil WSOC leaching, soil OC accumulation, and transfer to riverine systems). If this potential flux of WSOC derived from exudates is then distributed equally (i.e., 0.25–0.46 mol C m<sup>-2</sup> year<sup>-1</sup>) to the remaining fluxes in Table 5, root exudate fluxes have the concurrent potential to contribute 12–42% of organic soil WSOC losses, 17–159% of mineral soil WSOC

losses, 1–55% of soil OC accumulation, and 4–7% of terrestrial WSOC exports to riverine systems. This simplistic exercise demonstrates that WSOC originating as exudates has the potential to comprise substantial portions of various belowground OC fluxes, even after accounting for heterotrophic remineralization. We also suggest that riparian vegetation, including plant root-derived WSOC, should be viewed as a potentially important component of terrestrial-aquatic transfers of organic matter that may help explain the terrestrial-like  $\delta^{13}\text{C}$  signatures and relatively young  $^{14}\text{C}$  ages of riverine dissolved OC, and the dominance of net heterotrophy observed in the vast majority of inland waters (Table 5) (Cole et al. 2007; Hossler and Bauer 2013; Raymond et al. 2013; Dodds et al. 2017).

## Conclusions and future research directions

Over the duration of the present study, NEE,  $N_{\text{area-root}}$ , and tree species were found to be primary drivers of variation in root WSOC exudation rates, implying a strong influence of both photosynthetic rates and plant traits on the transfer and allocation of NSC within

riparian trees at our site. We suggest that studies on the phenomenon of root exudation should seek to identify important predictors of net exudation rates in a variety of vegetated ecosystems and over longer temporal scales, with the ultimate goal of more fully integrating belowground plant processes in C cycling models and budgets. The significant correlations between exudate  $\delta^{13}\text{C}$  values and both VPD and  $T_{\text{air}}$  observed in the present study further suggest that such relationships should be considered when interpreting natural abundance  $\delta^{13}\text{C}$  values of soil  $\text{CO}_2$ . Finally, studies of the chemical composition, post-exudation modifications, and fates of root exudate WSOC (i.e., decomposition/remineralization, storage in soils, hydrologic export) are needed to better evaluate its role both within terrestrial systems and in exchanges between terrestrial and adjoining (e.g., aquatic) systems.

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