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Entitled
Do the availability and accessibility of soil saccharides and nutrients vary with the phenology of *Acer rubrum* and *Lonicera maackii*?

By
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Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Biology

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An Abstract of

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Stable soil organic carbon (SOC) is a globally important carbon (C) pool with the potential to significantly alter atmospheric greenhouse gas concentrations, and it is believed to originate primarily from microbial necromass. However, we are not always capable of accurately predicting the magnitude and occasionally the direction of C fluxes into and out of the soil, in part due to a lack of understanding of how C moves from plants through the microbial community. C moves from the atmosphere into the soil primarily through plant uptake and incorporation followed by senescence and decomposition, or by exudation directly from roots, which especially stimulates production of readily stabilized microbial biomass in mineral soils. Root exudation rates depend on the plant species, but variations among plant species in the timing and rate of exudation have not yet been fully characterized. The primary question this study addresses is: How different are concentrations of root exudates for plant species with varying phenology? We hypothesized that plants varying in seasonal leaf expansion/senescence times also vary in seasonal concentrations of root exudation
because of the difference in the timing of leaf presence and C fixation. To test this hypothesis, we measured root exudation between two species with varying leaf expansion and senescence times from April-December 2015 by measuring soil pore water saccharides as a proxy for root exudation. We also measured nitrate, ammonium, and phosphate concentrations in soil pore water and 0.5 M K₂SO₄ extracts to determine their distribution within the soil matrix. We observed microbial responses to root exudation by measuring soil microbial respiration, biomass, and ecoenzyme (enzymes existing/functioning outside of cells) activities. Two woody plants with different light acquisition strategies were chosen within the same temperate deciduous forest stand and soil type (coarse-loamy, mixed, nonacid, mesic Aeric Haplaquepts): Honeysuckle (Lonicera maackii), whose leaves expand early and senesces late, and red maple (Acer rubrum), which has its leaves for a much shorter time but obtains more light and shades L. maackii when its canopy is open due to its height. These were compared with plant-free control plots that were trenched in order to reduce root inputs.

Saccharide concentrations varied less in soil pore water than expected, and did not track leaf expansion and senescence as predicted, with the exception of May 18, when saccharide concentrations significantly increased in L. maackii soils just before leaf expansion was complete, at which time the L. maackii would become maximally shaded by the A. rubrum canopy (A. rubrum leaf expansion was complete one week later). Soil microbial biomass and activity also varied little between the two plant species, but all ecoenzyme activities varied significantly by date due to a decrease in December, apparently associated with seasonal temperature decline. Furthermore, although leaf litter was removed, the legacy of L. maackii’s high quality litter was apparent, with higher
nutrient (nitrate and phosphate) concentrations from March to May (when leaf expansion was complete). Overall, however, nutrient dynamics were not clearly related to exudation or phenological events.

Furthermore, the distribution of saccharides within the soil matrix is still uncharacterized. A comparison of the concentrations we observed in soil pore water and salt extracts indicates that most dissolved saccharides are physically separated from the mobile pool, spatially inaccessible within the soil matrix and isolated from organisms. To our knowledge, no other studies have measured both soil pore water and extractable saccharides, but separate measurements suggest that spatially inaccessible saccharide concentrations likely range from 10-500 times greater than pore water, or “mobile”, saccharides. This suggests that our estimate of 10 times more saccharides in the spatially inaccessible than the mobile pool may be on the low side.

Overall, we conclude that concentrations of soil saccharides did not track phenological events as expected. However, while we did not observe an overall difference in soil saccharides between species, our results still suggest that the relationships between exudation and phenological events, such as leaf expansion and senescence, are likely species dependent. For example, there was an apparent pulse of soil saccharides only around L. maackii just before leaf expansion was complete. Furthermore, we conclude that there is a spatially inaccessible pool of soil saccharides that is likely much larger ($\geq$ 10 times) than the mobile pool.
I would like to thank my mom and dad, Michele and Gordon McMillan.

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List of Abbreviations

BG .........................β-1,4-glucosidase
BX ............................β-1,4-xylosidase
DIN ..........................Dissolved inorganic nitrogen
IRGA .........................Infrared gas analyzer
LAP ...........................Leucine amino peptidase
ANOVA ........................Analysis of variance
MBC ..........................Microbial biomass carbon
MBN ..........................Microbial biomass nitrogen
MC ............................Methylcoumarin
MUB ..........................Modified universal buffer
MUF ...........................4-methylumbelliferone
NAG ..........................β-1,4-N acetyl-glucosaminidase
PAHBAH ........................Para-hydroxybenzoic acid hydrazide
PEROX ........................Peroxidase
PHENOX ........................Phenol oxidase
PHOS ..........................Acid phosphatase
TOC-V_{cpn} ....................Shimadzu total organic carbon and nitrogen analyzer
TRS ............................Total reducing sugar
USA ............................United States of America
USGS ..........................United States Geological Survey
List of Symbols

C .........................Carbon
CaCl ........................Calcium chloride
HCl ..........................Hydrochloric acid
K₂SO₄ ..........................Potassium sulfate
KNO₃ ..........................Potassium nitrate
N .............................Nitrogen
Na₃C₆H₅O₇ ...............Trisodium citrate
NaOH ........................Sodium hydroxide
NH₄⁺-N ......................Nitrogen from ammonium
NO₃⁻-N ........................Nitrogen from nitrate
P ..........................Phosphorus
PO₄³⁻-P ..........................Phosphorus from phosphate
VCl₃ ..........................Vanadium chloride
Chapter 1

Introduction

Globally, soil organic matter (SOM) contains more than twice as much C as the atmosphere (Stocker 2013, Smith 2004, Lal 2008), and SOM is a dynamically active C pool. Because of its size and responsiveness (Lal 2008), it is important to understand the dynamics of SOM and how C flows between the soil and atmosphere. However, we are often unable to predict the magnitude, and occasionally direction of changes in these fluxes in response to disturbances such as warming (Stocker 2013). This partially comes from a lack of understanding about plant C inputs into soil and their fates. Recent research suggests that stabilized SOM actually originates from the tissues of decomposer microorganisms (Grandy and Neff 2008, Schmidt et al. 2011, Cotrufo et al. 2013), rather than recalcitrant pant material. Thus, microbes both add to and decompose SOM, and understanding what stimulates microbial production in soils is therefore necessary for understanding SOM cycling.

The microbial necromass that ultimately becomes SOM (Grandy and Neff 2008, Schmidt et al. 2011, Cotrufo et al. 2013) is primarily derived from plant inputs of easily-
degraded soluble C (Cotrufò et al. 2013), such as root exudates, which stimulate microbial production (Bais et al. 2006). Thus, the increase in microbial biomass in response to root exudation may increase SOM by increasing inputs of microbial necromass. On the other hand, inputs of easily degraded C may stimulate soil microorganisms, increasing SOM decomposition and CO$_2$ production rates (Parnas 1976, Kuzyakov et al. 2000). CO$_2$ production from the mineralization of root exudates does not cause a net increase in the atmospheric C pool, as this C was recently fixed and may be considered a component of autotrophic soil respiration (Kuzyakov 2006); net C losses from the soil result from stimulating decomposition of extant SOM.

Root exudates primarily consist of water, ions, free oxygen, enzymes, mucilage, and a wide variety of C compounds (Nardi et al. 2000). Low molecular weight (LMW) compounds (e.g. amino acids, organic acids, saccharides, phenolics) account for most of the chemical diversity found within exudates (Rougier 1981, Marschner 2011). The LMW components decompose rapidly, with an estimated residence time of 1-10 hours (van Hees et al. 2005). The high molecular weight compounds (polysaccharides and proteins) are more abundant by mass but have longer residence times (hours to days) (Abbott and Murphy 2003). These exudates are a significant C cost for plants (Marschner 1995). Estimates of the quantity of photosynthate exuded range widely, from 1-40% (Kaiser et al. 2015, Meharg 1994, Walker et al. 2003). However, most studies focus on agricultural crops and there have been few studies in natural systems (Horowitz et al. 2009), and most focus the impact of elevated CO$_2$ on root exudation (Phillips et al. 2011). The quantity and composition of root exudates depends on the plant species, but their controls are still not well understood. It is widely believed that photosynthate moves
through plants rapidly (e.g. Horowitz et al. 2009, Hogberg et al. 2001), suggesting that the timing of plant phenological events, such as leaf expansion and senescence, should have significant effects on the temporal dynamics of exudation rates. For example, an isotopic pulse chase study found that 30% of total labeled C taken up by Lolium perenne was transported below ground within 24 hours (Rattray et al. 1995), and a large-scale tree girdling experiment in a boreal mixed coniferous forest estimated that photosynthate fixed in the canopy of 20 m trees becomes available in the rhizosphere in 1 to 4 days (Högberg 2001). As temperatures rise in the spring, plants begin to break their buds and expand their leaves, but the timing of leaf-out varies by species. Because photosynthate is exuded from the roots relatively quickly, it would make sense if plants with different light acquisition strategies have different seasonal patterns of root exudation, but to our knowledge, this is not yet known. The primary question this study addresses is: How different are concentrations of root exudates from plant species with varying phenology? We hypothesized that plants varying in seasonal leaf expansion/senescence times also vary in seasonal concentrations of root exudation because of the difference in the timing of leaf presence and C fixation. We predicted that root exudation would closely follow light acquisition, increasing when leaves were out and obtaining full sun.

Root exudates are likely to be a significant contributor of saccharides to the soil dissolved organic C (DOC) pool. For example, it has been estimated that 22-30% of extractable DOC is saccharides (Hishi et al. 2004, Fischer et al. 2007, Tian et al. 2010). Most soil saccharides are in a polymeric form, requiring extracellular degradation by microbial ecoenzymes (Gunina and Kuzyakov 2015, Tian et al. 2010). Of the monosaccharides found in DOC, it has been found that the main saccharide present is
glucose (Hütsch et al. 2002, Fischer et al. 2007). In Zea mays, for example, glucose can
comprise as much as 40-50% of root exudate saccharides, with fructose and sucrose the
next most prevalent (~23%) (Hütsch et al. 2002).

Although most research is based on glucose (Gunina and Kuzyakov 2015), it has
been shown that other monosaccharides behave similarly when moving through the soil
mineral/biotic matrix (Derrien et al. 2007, Gunina et al. 2014). Thus, we assume that
these monosaccharides have similar fates and availability within the soil matrix.
However, the distribution of saccharides within the soil matrix is not well understood.
The most immediately available in the soil matrix is the mobile pool of saccharides in
soil pore water (Darrouzet-Nardi and Weintraub 2014). Charged dissolved solutes bound
to cation exchange sites are considered to be in an adsorbed pool (Strahm and Harrison
2008, Rothstein 2010), but we hypothesize that an adsorbed soil saccharide pool does not
occur due to their lack of charge. A third pool is comprised of solutes that are physically
separated from the mobile pool, spatially inaccessible within the soil matrix and isolated
from organisms: the spatially inaccessible pool. These three pools defined in Darrouzet-
Nardi and Weintraub (2014) are quantified using different methods: the mobile pool is
sampled using tension micro-lysimetry (see methods) to collect available pore water
directly from the soil; the adsorbed and spatially inaccessible pools are obtained by
extracting soil in water and in salt solutions, respectively: shaking with water is intended
to break apart soil structure (Kramer and Boyer 1995) and extract spatially inaccessible
nutrients and dissolved organic matter, while leaving most charged ions adsorbed to the
soil matrix; the salt extraction is performed the same way, but also extracts adsorbed ions
from cation exchange sites (Strahm and Harrison 2008, Rothstein 2010). The observation
of a relatively large spatially inaccessible amino acid pool in a moist acidic tundra soil in N. Alaska USA, and a review of data from other ecosystems, strongly suggest the existence of other spatially inaccessible DOC pools (Darrouzet-Nardi and Weintraub 2014). Because the proposed mechanism of entrainment is an inherent property of the soil matrix itself, there is also likely to be a spatially inaccessible pool of saccharides, as this mechanism should not exclude such molecules. Thus, we predict that the likely fates of root exudates (prior to microbial uptake) are the mobile or spatially inaccessible pools, measurable by comparing concentrations in soil pore water to salt or water extracted samples.

Inevitably, once accessible, soil saccharides are taken up. Fischer et al. (2010) concluded that microbial uptake of monosaccharides in a Haplic Luvisol (a silt loam) is twice as fast as or faster than any kind of sorption. They also found that glucose took roughly 400 min. to reach an equilibrium state within sterile soil solution between the mobile and adsorbed pools, with only 7-10% in a sorbed form at equilibrium, while microbial uptake of monosaccharides only took a few minutes to reach near 100% (Fischer et al. 2010). Given that saccharides are utilized rapidly by microbes, extractable concentrations of soil saccharides are surprisingly high, ranging from 18 to 151 µg glucose equiv. g⁻¹ dry soil in many studies (Sripriya et al. 1997, Zhang et al. 2000) (the highest extractable saccharide concentration we observed was 75 µg glucose equiv. g⁻¹ dry soil). Given their high uptake rates, such large concentrations do not make sense unless a substantial proportion of extractable saccharides are spatially inaccessible. However, to our knowledge, the distribution of saccharides among these pools has never been studied in any soil. Thus, the question arises: what are the relative sizes of the
mobile and spatially inaccessible saccharide pools? We hypothesize that the spatially inaccessible pool of soil saccharides will be larger than the mobile pool, because of rapid microbial uptake from the mobile pool. We also predict that the spatially inaccessible saccharide pool will remain more constant through the season, as inputs and uptake of saccharides are presumed to go through the mobile pool; thus, we also expect to see larger relative changes over time in the mobile pool.

Once exuded, the majority of saccharides are either incorporated into microbial biomass, or respired to the atmosphere. For example, 69-86% of the C in root exudates was mineralized to CO$_2$ within one week in _Z. mays_ plants grown in pots indoors in loamy Haplic Luvisol soils (Werth and Kuzyakov 2008). Inputs of easily degraded C can significantly alter microbial biomass and activity, and depending on exudate chemistry, microbial community composition (Shi _et al._ 2011, DeAngelis 2016). For example, saccharide inputs can cause positive priming, where substrate additions increase microbial degradation of SOM. A positive priming event may be caused by microbes increasing SOM decomposition to obtain N or P in response to C rich substrate inputs (Hamilton and Frank 2001, Phillips _et al._ 2011, Asmar _et al._ 1994). Such positive priming events can increase SOM loss by increasing mineralization (Dalenberg and Jager 1989, Hamer _et al._ 2004, Zimmerman _et al._ 2011). Alternatively, a negative priming event is when the rate of SOM decomposition decreases after additions of substrate. Although negative priming events have been observed (Masayna _et al._ 1985, Nicolardot 1986, Schmitt 1990, 1991) they are far less common than positive priming events (Kuzyakov _et al._ 2000). In more C and N rich soils positive priming effects are typically higher due to greater microbial activity, and the size of priming events typically increases
with the amount of added organic substrate (Hyvönen et al. 2007, Phillips et al. 2011, Mary et al. 1993, Asmar et al. 1994). This raises the question: how does soil microbial biomass and activity change with soil saccharide inputs? We hypothesize that soil microbial biomass and activity (e.g. respiration) closely follow soil saccharides, because the saccharide inputs from exudates fuel microbial growth. We predict that as microbial biomass increases with exudate inputs there should also be a corresponding drop in the production of C acquiring ecoenzymes (enzymes existing/functioning outside of cells (Sinsabaugh et al. 2009)), and an increase in N and P acquiring ecoenzymes due to increased C availability and nutrient demand (e.g. Rinkes et al. 2011). Due to influences on microbial activity, variations in soil saccharides and microbial biomass are also likely to influence concentrations of available nitrogen from ammonium (NH$_4^+$-N), nitrate (NO$_3^-$-N), and phosphorus from phosphate (PO$_4^{3-}$-P) throughout the growing season, which in turn may affect microbial investment in N and P acquisition.

To address the above questions and hypotheses, the seasonal dynamics of exudation from two plants with contrasting phenology were observed, alongside a set of trenched control plots where root exudate inputs were eliminated, in a temperate deciduous forest in NW Ohio, USA: Acer rubrum (red maple) trees and Lonicera maackii (honeysuckle) shrubs. The impacts of root exudates from these two plants on soil microbial activity and nutrient availability within the rhizosphere were also observed. Soil extracts and soil solution samples were collected, providing a comparison for estimating the size of the spatially inaccessible pool. Measurements of soil respiration, total reducing saccharides (TRS; a proxy for total dissolved monosaccharides and root exudates), microbial biomass, respiration and ecoenzyme activities, and available N and
P were determined in soils associated with both plant species and trenched controls from spring through fall (through leaf senescence) 2015.
Chapter 2

Methods

2.1 Species Description

Two woody temperate deciduous forest plant species with contrasting phenology were selected for comparison. The first study species was honeysuckle (*Lonicera maackii*) is an invasive understory shrub, and, where it occurs, is one of the earliest woody species to complete leaf-expansion and among the last to senesce (Hutchinson 1997). This helps makes honeysuckle a successful invasive species, outcompeting many other understory plants with relatively low shade tolerance (Hutchinson 1997). *L. maackii* roots are typically concentrated between the top 1 to 6 cm of the soil (U.S. Department of Agriculture 2015). *L. maackii* leaves senescence from late October through November (Trisel 1997, Arthur et al. 2012). The second plant species is red maple (*A. rubrum*), a canopy tree that begins leaf expansion almost a month later and senesces 1 - 4 month(s) earlier than honeysuckle but obtains more light during the summer because of its height. *A. rubrum* has a much larger range of senescence depending on locality and genetics, giving it a wide range of senescence dates anywhere from late July to September
(Anderson and Ryser 2015). *A. rubrum* has mostly horizontal roots concentrated in the top 25 cm of the soil (Lyford and Wilson 1964). Fine roots of *A. rubrum* are primarily in the top 8 cm of mineral soil, and below that there are primarily woody roots (Lyford and Wilson 1964). *A. rubrum* is one of the most abundant trees in the United States according to forest service surveys (Little and Service 1979). Little is known about the composition of either species’ root exudates. *L. maackii* root exudates have been studied, but this research was strictly focused on identifying potentially allelopathic compounds in an attempt to explain its success as an invasive species (Gorchov and Trisel 2003, Dorning and Cipollini 2006). As far as we can tell from our literature search, exudate composition has not otherwise been analyzed for either species.

### 2.2 Site Description

All sampling occurred at the Stranahan Arboretum (GPS coordinates: 41.694368, -83.667b931), a 47-acre preserve in northwest Ohio consisting of cultivated ornamental trees, rolling lawns, natural woods, ponds, wetlands and prairie. The soil within this area is a coarse-loamy, mixed, nonacid, mesic Aeris Haplaquepts, it receives annual precipitation of 89 cm a year, and annual air temperature of 9.1 °C, on average (Web Soil survey 2015). There is anywhere from 0 to 2 percent slope within the site (Web Soil survey 2015). *L. maackii* is found near the edge of the forested area, forming a thicket (Fig. 1). All plots are in the same soil type (Web Soil survey 2015), but due to the topography of the site the soils vary somewhat in their hydrology, with occasional flooding in low lying areas, typically during the spring. Sampling occurred across these elevation differences in all plot types. The forest in this area is comprised mainly of pin
oak (*Quercus palustris*), red maple (*Acer rubrum*), and white oak (*Quercus alba*) (Web Soil survey 2015). There are also many shrubs, such as *Rosa multiflora* and *L. maackii* concentrated mostly around the edges of the forest stand, and various small understory plants such as *Podophyllum peltatum* (May apple), *Actaea pachypoda* (White baneberry) and *Alliaria petiolata* (Garlic mustard).

![Image](image_url)

Figure 1: Satellite view of the Stranahan arboretum. Red dots indicate the red maple plots, purple shows the trenched control plot, and blue shows the location of honeysuckle plots.

### 2.3 Plot Layout

We sampled from three types of plots in this observational study: *L. maackii*, *A. rubrum*, and plant-free control. These plots include the area around the bases of individuals of each plant species. Honeysuckle samples were always collected roughly
0.3 m away from the base of the plant, and 0.45 m from the red maple trees, in order to optimize sampling around fine roots and avoid larger woody roots. Mean DBH of sampled *A. rubrum* trees was 13 cm, ranging from 10-17 cm, and mean *L. maackii* DBH was 4 cm ranging from 3.5-4.6 cm. All three plot types were sampled using both destructive (when the soil is collected) and non-destructive techniques. For the non-destructive sampling, 15 plots were located around each *A. rubrum, L. maackii*, or >2 m from the nearest vegetation within a trenched control plot (see below). One microlysimeter and respiration collar (described below) were placed into each plot, with the microlysimeter installed to a depth of 10 cm in the center of the respiration collar. Small understory vegetation and leaf litter were removed within a 0.3 m of each collar upon installation, and emergent seedlings were removed throughout the sampling period. The collar was inserted 5 cm into the soil within 45 cm of the nearest *A. rubrum*, or 30 cm the nearest *L. maackii*. Fifteen control plots were constructed by digging a 10-15 cm deep trench around a >1 m² area at least 2 m from the nearest vegetation, in clusters of three. These 45 sampling plots were created in late March 2015 (as soon as the ground was thawed enough to install the microlysimeters and respiration collars) and samples were collected until December 2015.

For the destructive sampling, seven additional respiration collars and microlysimeters were installed within the 15 existing plots of each type, creating 21 destructive sampling points within the 45 plots described above. Destructive sampling points were used for soil collection after microlysimeter sampling, and hence could only be sampled once each. After soil collection, the collars from those plots were then moved to one of the eight plots of the same type that were not sampled that day.
2.4 Soil Respiration

Soil respiration was measured with a Li-8100A Automated Soil CO\(_2\) Soil Flux System (LI-COR Biosciences, Lincoln, Nebraska, USA). Each plot had a 11.5 cm diameter and 9-12.8 cm high PVC soil collar inserted 5 cm into the ground (the 8100A accounts for variable aboveground height). Any plants or organisms found in the collars were removed upon installation, and disturbance effects were given a minimum of a week to subside. CO\(_2\) flux in the collars was measured for one minute and expressed as \(\mu\text{mol m}^{-2}\text{s}^{-1}\). The Li-8100A also recorded soil temperature at 10 cm and soil moisture at 5 cm, using a soil temperature thermistor probe and an ECH\(_2\)O volumetric water content, dielectric permittivity sensor (LI-COR Biosciences, Lincoln, Nebraska, USA).

2.5 Plant Metrics

To determine when leaf expansion was complete, the diameter of the largest three accessible leaves was measured with a ruler on each sampling date until leaf expansion was complete. To determine the onset of leaf senescence a relative index of leaf chlorophyll concentration was measured using a SPAD-502 chlorophyll meter (Konica Minolta Business Solutions, U.S.A., Inc.). The meter has two light emitting sources, one at 650 nm and the other 940 nm, and a photodiode detector measuring light transmission through the leaves (Markwell et al. 1995). The measurements from this unit are only comparable within a single plant species, but can be used to determine when chlorophyll is at its peak during the growing season, and when the chlorophyll concentration begins to drop just after senescence begins. On each sampling date ten leaves of similar orientation and size were measured on a given plant, and the average recorded (For 15
individuals). Both plant measurements began as soon as leaves were present, and were collected at the same time as soil pore water/soil harvest (see below) and respiration. Leaf diameter measurements stopped when leaves finished expanding, while relative chlorophyll measurements continued until leaf fall.

**2.6 Soil Pore Water Sampling**

To collect the soil pore water samples, microlysimeters, a.k.a. rhizon samplers, (Rhizon SMS 10 cm male luer, 2.5 mm diameter; Eijkelkamp Inc., Nijverheidsstraat Giesbeek, The Netherlands) were placed within each soil collar to a depth of 10 cm. They consist of a 2.5 mm diameter porous PVC tube, pore size 0.12-0.18 µm, with a metal wire running down the center for stability, and a tube coming off of the PVC ending in a luer fitting with a syringe needle. To collect pore water, a needle fitted into the luer fitting, located at the end of the lysimeter above ground, is inserted into a 6 mL vacuum tube plugged with a rubber septum (Greiner Vacuette No. 456089) and soil solution is sucked through the PVC and up into the evacuated tube. These vacuum tubes were left on the lysimeters for ~2 hours typically, though the sampling time was variable; if the soils were dry, up to 4 hours was allowed for sample collection. These samples were frozen (-20 °C) upon collection until analyses for total reducing saccharides and nutrients (see below).

**2.7 Soil Collection / Soil Processing**

Soil was collected using a soil corer (5 cm diameter) to a depth of 10 cm from around the lysimeters within the collars set aside for destructive harvest. Soil was only collected from a collar if it was wet enough to produce a soil pore water sample. Upon collection, soil samples were immediately returned to the lab and half of the sample (cut
length wise), 100-150 g, was set aside for root biomass measurements (see below). The remaining soil was hand homogenized to remove rocks, coarse roots, and other debris for 2.5 min. per sample. Up to 21 soil samples were collected during each destructive harvest.

The soil for root biomass determination was washed in a 0.25 mm sieve. After most of the soil was removed the roots were then picked out of the sieve using forceps and placed in metal dish and dried at 50 °C for 48 hours. Roots that were smaller than 3 mm in diameter were weighed.

Extractable nutrient pool sizes were determined using K₂SO₄ extraction (Weintraub et al. 2007). These were conducted by combining 5 g of soil with 25 mL of 0.5 M K₂SO₄ in a 50 mL plastic centrifuge tube and shaking for 1 hour on an orbital shaker. The sample was then filtered using a Whatman #1 paper filter (2 µm pore size) and a vacuum filtration system. Extractions were also performed as above on two days (August 11, and December 11), but with nanopure water, for comparing with the salt extracted samples and estimating adsorbed saccharide and nutrient concentrations.

Microbial biomass was determined using the chloroform fumigation extraction method (Brookes et al. 1985) as modified by (Scott-Denton et al. 2006): 2 mL ethanol-free chloroform was added to each 5 g soil sample at field moisture in a 250 mL Erlenmeyer flask, and then immediately stoppered. Twenty-four hours later the stoppers were removed and the chloroform was vented off for 30 minutes. Then, 25 mL of 0.5 M K₂SO₄ was added and the samples were extracted as above. All extracts were frozen (-20 °C) until analysis. Five g subsamples for moisture content measurements were dried at 65
°C for 48 hours. Soil moisture content is expressed as percent of dry mass (i.e. \((\text{wet-dry})/\text{dry}\)).

Table 1: soil pool table describing how each pool was calculated.

<table>
<thead>
<tr>
<th>Saccharide pools</th>
<th>Description</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile pool</td>
<td>Free floating, easily accessible to microbes and plants.</td>
<td>Saccharide concentration in soil pore water (Collected via lysimetry)</td>
</tr>
<tr>
<td>Adsorbed pool</td>
<td>Weakly bonded to the surface of mineral organo complexes in the soil.</td>
<td>Saccharide concentration in salt extractions minus saccharide concentrations in water extractions</td>
</tr>
<tr>
<td>Spatially inaccessible pool</td>
<td>Spatially separated in the soil matrix, enveloped by soil particles.</td>
<td>Saccharide concentration in salt extractions minus saccharide concentrations in pore water</td>
</tr>
</tbody>
</table>

2.8 Total Reducing Saccharide

Concentrations of total reducing saccharides (TRS) are used as a proxy for all dissolved monosaccharides, and TRS was measured in microlysimeter samples and soil extracts. Saccharide concentration was determined using a colorimetric microplate assay (Lever 1973, Fursova et al. 2012) and was used as a proxy measure of root exudates. Para-hydroxybenzoic acid hydrazide (PAHBAH) plus hydrochloric acid (HCl) (Reagent A) and sodium hydroxide (NaOH) plus calcium chloride (CaCl) and trisodium citrate (Na₃C₆H₅O₇) (Reagent B) were combined to form a TRS working reagent.
Samples (K₂SO₄ extract or soil porewater) were combined with the TRS working reagent into a 96-well polymerase chain reaction (PCR) microplate, which was placed in a heating block at 100 °C for 6 minutes, then quickly placed into an ice water bath for 5 minutes. The solution was then pipetted into a clear 96-well microplate and read at 410 nm on a Bio-Tek Synergy HT microplate reader (Bio-Tek INC., Winooski, VT, USA). Glucose was used as the standard, and TRS concentrations are reported in μmol glucose equivalents L⁻¹ for soil pore water or g dry soil⁻¹ for soil extractions.

2.9 Ammonium

NH₄⁺-N was quantified in microlysimeter samples and soil extracts using a colorimetric microplate assay (Rhine et al. 1998). Samples were pipetted in triplicate into a 96-well clear microplate along with citrate, 2-phenylphenol-nitroprusside reagent, and buffered hypochlorite reagents, plus nanopure water. Plates were incubated at 20 °C for 2 hours, and then read at 660 nm using a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). The three analytical replicates were averaged together. Soil pore water results are expressed as μmol NH₄⁺-N L⁻¹ and results from soil extractions are expressed as μg-N g dry soil⁻¹.

2.10 Total Free Primary Amines

Total free primary amines (TFPA) were quantified in microlysimeter samples and soil extracts using a fluorometric microplate assay (Jones et al. 2002, Darrouzet-Nardi et al. 2013). O-phthaldialdehyde, β-mercaptoethanol and borate buffer were combined with triplicates of sample in black 96-well microplates, incubated for 60 minutes and subsequently read at excitation/emission wavelengths of 360/460 nm on a Bio-Tek
Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). L-leucine was used as the standard, and concentrations are expressed as µmol leucine equivalents L⁻¹ or g dry soil⁻¹.

2.11 Nitrate

NO₃⁻N was quantified in microlysimeter samples and soil extracts using a colorimetric microplate assay (Doane and Horwáth 2003). Samples were pipetted in triplicate into a clear microplate and combined with vanadium chloride (VCl₃) solution (sulfanilamide, N-(1-naphtyl)-ethylenediamine dihydrochloride, and vanadium III chloride) in a 96-well microplate, and then incubated at 20 °C for 3 to 5 hours. The plates were then read using a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) at 540 nm. Soil pore water results are expressed as µmol NO₃⁻N L⁻¹ and results from soil extractions are expressed as µg-N g dry soil⁻¹.

2.12 Phosphate

PO₄³⁻-P concentrations were determined using a colorimetric microplate PO₄³⁻-P assay (D'Angelo et al. 2001). Samples were combined in triplicate with ammonium paramolybdate and malachite green in clear 96-well microplates. and then incubated at 20°C for 30 minutes and read at 630 nm in a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). Soil pore water results are expressed as µmol PO₄³⁻-P L⁻¹ and results from soil extractions are expressed as µg-P g dry soil⁻¹.

2.13 Microbial Biomass

The K₂SO₄ extracts from chloroform fumigated and unfumigated samples were analyzed for dissolved organic C (DOC) and total dissolved nitrogen (TDN) using a
Shimadzu total organic C (TOC-\text{V}_{\text{CPN}}) analyzer with a total nitrogen (TN) analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). DOC and TDN concentrations in the unfumigated K$_2$SO$_4$ extracts were subtracted from the fumigated extracts, yielding an estimate of extractable microbial biomass C and N. Three chloroform fumigated and extracted blanks were used to account for any possible C or N contamination. Extractable microbial biomass is expressed as $\mu\text{g (C or N) g dry soil}^{-1}$ and is not corrected for extraction efficiency, which has not been determined in these soils.

### 2.14 Ecoenzyme Activities

Microbial ecoenzyme assays were conducted, using colorimetric and fluorometric microplate assays derived from (Saiya-Cork \textit{et al.} 2002) and (German \textit{et al.} 2011), to determine if there are shifts in microbial activities in response to changes in exudation. $\beta$-1,4-glucosidase (BG), $\beta$-1,4-xylosidase (BX), leucine amino peptidase (LAP), $\beta$-1,4-N acetyl-glucosaminidase (NAG), acid phosphatase (PHOS), phenol oxidase (PHENOX), and peroxidase (PEROX) were all assayed in 1 g of soil set aside after soil sample homogenization. BG hydrolyzes cellobiose (a dimer of cellulose) into glucose, and BX hydrolyzes xylans (polysaccharides made from xylose, a major component of hemicellulose) into xylose, thus BG and BX are C acquiring ecoenzymes; LAP hydrolyzes various terminal amino acids from peptides, especially leucine; NAG hydrolyzes N-acetyl glucosamine originating from peptidoglycan and chitin derived oligomers; thus LAP and NAG are N (and C) acquiring ecoenzymes; PHOS hydrolyzes organic $\text{PO}_4^{3-}$ monoesters into phosphate and is a P (and C) acquiring ecoenzyme; PHENOX oxidizes phenolic compounds, and PEROX catalyzes oxidative reactions.
through the reduction of H₂O₂, and both degrade more persistent structures like lignin (Sinsabaugh et al. 2008, Sinsabaugh 2010, Rinkes et al. 2011, Bach et al. 2013).

Soil slurries were created by mixing 1 g of soil and adding 125 mL of modified universal buffer (MUB) (tris(hydroxymethyl) aminomethane (THAM), maleic acid, citric acid, boric acid, sodium hydroxide)) adjusted to the soil pH of 5.9, and then using a BioSpec Tissue Tearer (BioSpec Products, Bartlesville, OK) to homogenize the solution for one minute, in two 30-second intervals. The five hydrolytic ecoenzyme assays (BG, BX, LAP, NAG, PHOS) are fluorometric and performed in black 96-well microplates, and the oxidative ecoenzyme assays (PHENOX, PEROX) are colorimetric and performed in clear 96-well microplates. 200 µL of soil/buffer slurry was mixed with 50 µL of 200 µM substrate (BG: 4-methylumbelliferyl (MUF)-β-D-glucopyranoside; NAG: MUF-N-acetyl-β-D-glucosaminide; LAP: 7-amido-4-methylcoumarin (hydrochloride); PHOS: MUF phosphate; and L-3,4-dihydroxyphenylalanine for both PHENOX and PEROX). The other wells consist of: slurry and buffer (blanks); soil slurry and standard (BG, BX, NAG, PHOS use MUF, and LAP uses 7-amino-4-methylcoumarin (MC)) as quench standards for fluorometric assays; buffer and substrates, which serve as negative controls; and buffer and MUF standard, a reference standard for MUF fluorescence. PEROX plates also have 10 µL 0.3% hydrogen peroxide (H₂O₂) added to each well. All ecoenzyme assays were incubated at 14 °C (to represent a summer soil temperature, and analyze all ecoenzymes consistently throughout the study) in the dark for 2-4 hours and then read in a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA). The fluorometric plates were read at 365 nm excitation and 460 nm emission wavelengths.
The colorimetric plates are read at an absorbance of 460 nm. The activities are reported in nmol hr\(^{-1}\) g dry soil\(^{-1}\).

### 2.15 Statistical Analyses

All statistical analyses were conducted in the open source program R (R Core Team 2015). Analysis of variance (ANOVA) and Scheffe Post Hoc Tests was used to determine differences between plot types and different sampling techniques, i.e. soil pore water sampling or soil extraction. The seasonal dynamics of saccharides, our root exudation proxy in microlysimeter samples (dependent variable), were analyzed using a repeated measures two-way ANOVA, with time and plot type (control, *L. maackii*, *A. rubrum*) as the two factors, on samples from the same locations the whole season (non-destructive). All other dependent variables (saccharides, NH\(_4^+\)-N, NO\(_3^-\)N, TFPA, PO\(_4^{3-}\)-P, respiration) from these samples were analyzed the same way in an ANOVA together. The dependent variables from the destructive harvests (microbial biomass, saccharides, NH\(_4^+\)-N, NO\(_3^-\)-N, TFPA, PO\(_4^{3-}\)-P, ecoenzyme activities, respiration) were analyzed using a two-way ANOVA with time and plot type as factors. The spatially inaccessible pool of reducing saccharides was analyzed using one and three way ANOVAs with the dependent variables saccharide\(_{\text{pore water}}\) (mobile pool), and saccharide\(_{\text{extract-saccharide pore water}}\) (inaccessible pool) and time, plot type, collection method (i.e. water, compared to salt extracted and soil pore water) as factors. First a one-way ANOVA was used to compare extraction type, to determine if there was a difference between salt and water extracted samples. Then a three-way ANOVA was performed for saccharide concentrations using time, plot type and collection method (soil pore water or salt extraction), as factors. To
determine if soil temperature differed between *A. rubrum*, *L. maackii*, and control plots, a one-way ANOVA was performed using plot type as the factor. The relative chlorophyll measures had a smooth curve fit to time vs SPAD in R and inflection points were identified for *L. maackii* and *A. rubrum*. 
Chapter 3

Results

3.1 Temperature and Precipitation

Mean air temperature peaked at the study site in July and August. The maximum average air temperature was 26.6 °C (Fig. 2A). Air temperature data were obtained from a weather station 1.7 km north, station ID: KOHSYLVA18 (Weather Underground 2015). Air temperatures were between -10 and 0 °C until mid-April (Weather Underground 2015). Soil temperatures were similar between *L. maackii*, *A. rubrum*, and control plots. The highest mean soil temperature was just above 20 °C on August 8. Unfortunately, due to equipment issues, soil temperature measurements did not start until May 11 (Fig. 2C), thus we do not have soil temperature data from the start of sampling until then. March to June had a relatively higher frequency of precipitation, with the highest rainfall total on June 27 (Fig. 2B). Damage to vegetation, around but not in our plots, was caused by storms on April 25 (most plots were flooded) and May 26. Significant damage was considered to be when large plant branches or trunks were broken in or around our plots; for example, a group of five *L. maackii* was ripped out of
the ground and blown 10-20 m away on May 26. From July to December, rainfall intensity increased, while the frequency decreased, causing severe drying events, with soil moisture declining from 0.5 g water g\(^{-1}\) dry soil to as low as 0.05 g water g\(^{-1}\) dry soil (where severe mud cracking was observed around respiration collars).
Figure 2: A) Average daily air temperature (Station ID: KOHSYLVA18) (Weather Underground 2015) B) Daily precipitation (cm) (Station ID: KOHSYLVA18) (Weather Underground 2015) C) Average Soil temperature under plants and controls.
3.2 Leaf Expansion and Chlorophyll

Leaf expansion was estimated to be complete for *A. rubrum* on May 28 and for *L. maackii* on May 23 (Fig. 3B). The peak in leaf chlorophyll content was estimated for *A. rubrum* on July 3 and for *L. maackii* on July 23 (Fig. 3A). Relative chlorophyll content in both species followed a bell-shaped curve pattern over the growing season, increasing rapidly followed by a more gradual decline until an eventual drop off (due to *A. rubrum*’s relatively rapid color change the drop off is much more abrupt in this species, and measurements were below detection afterward).

3.3 Fine Root Mass

Fine root mass within soil cores collected from the *A. rubrum* plots was higher than *L. maackii* and control plot soil cores every day sampled. *A. rubrum* soil core fine root masses were significantly higher than both *L. maackii* and control soil core fine root masses (*p* ≤ 0.031). *L. maackii* soil cores also contained significantly more fine root mass than control plots (*p* = 0.0009). Overall, fine root masses in all three soils were significantly different (*p* ≤ 0.001), with no significant interaction between date and plot type. A peak in fine root biomass was observed in *L. maackii, A. rubrum,* and control soil cores on June 2; however, these measurements were highly variable and this may represent a spatial rather than a temporal difference (Fig. 3C).
Figure 3: Plant metrics: A) Leaf diameter measured on the largest leaves found using a ruler B) SPAD, a relative chlorophyll measurement. The lines on both graphs represent the inflection points of each given curve, indicating maximum leaf expansion, and peak chlorophyll content, respectively. C) Fine root (<3 mm) dry mass.
3.4 Total Reducing Saccharides

Pore water saccharide concentrations in *A. rubrum* and *L. maackii* soils were typically higher than controls until May, and were variable in April and May, with statistically insignificant peaks (Fig. 4B). Pore water saccharide concentrations in *A. rubrum* soils then converged with the controls from May to July, whereas concentrations in *L. maackii* soils increased between May and June, and soil pore water saccharides were significantly higher than both control and *A. rubrum* soils on May 18 ($p \leq 0.02$). After July, pore water saccharides were consistently low in the controls, while concentrations were higher and similar to one another in *A. rubrum* and *L. maackii* soils after July.

Overall, pore water saccharide concentrations did not differ significantly between the three soils ($p = 0.114$), but varied significantly by date ($p = 0.029$) (Table 2). When analyzed separately by date, *A. rubrum* had significantly higher pore water saccharide concentrations than the control plots on April 10 and 14 ($p \leq 0.01$). Pore water saccharides in *L. maackii* plots were significantly higher than control plots on April 14 and May 18 ($p \leq 0.018$). *L. maackii* plots only contained significantly higher concentrations of pore water saccharides than *A. rubrum* on May 18.

Table 2: Total reducing saccharides ANOVA results

<table>
<thead>
<tr>
<th>Total Reducing Saccharides</th>
<th>Plot type</th>
<th>Date</th>
<th>Plot *Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile TRS $p$ value</td>
<td>0.114</td>
<td>0.029</td>
<td>0.306</td>
</tr>
<tr>
<td>Mobile TRS F-ratio</td>
<td>2.18</td>
<td>1.73</td>
<td>1.11</td>
</tr>
<tr>
<td>Extractable TRS $p$ value</td>
<td>0.108</td>
<td>0.067</td>
<td>0.882</td>
</tr>
<tr>
<td>Extractable TRS F-ratio</td>
<td>2.27</td>
<td>1.90</td>
<td>0.59</td>
</tr>
<tr>
<td>Inaccessible pool of TRS $p$ value</td>
<td>0.176</td>
<td>0.013</td>
<td>0.868</td>
</tr>
<tr>
<td>Inaccessible pool of TRS F-ratio</td>
<td>1.76</td>
<td>2.47</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Figure 4: Total reducing saccharide concentrations in A) soil pore water (mobile pool) and B) Soil K₂SO₄ extractions. (•) denotes when both plants > control, (‡) when A. rubrum > control, and (*) when L. maackii > both A. rubrum and control (p ≥ 0.05, ANOVA).
To estimate adsorbed saccharide pool sizes, concentrations in water extractions were subtracted from those in salt extractions. Concentrations in the two types of extracts were not significantly different \( p \geq 0.48 \), and the differences centered around zero (Fig. 5A). The variation within these extractions was larger than the difference between them, thus there was no discernible difference between water and salt extractions.

To calculate the size of the spatially inaccessible saccharide pool, concentrations in soil pore water were subtracted from those in the extractions. Typically, concentrations in pore water are subtracted from those in the water extracted samples. For saccharide concentrations, the salt and water extractions did not differ and both yield similar results (Fig. 5A), thus we used concentrations from the salt extractions for these calculations because we have more data for them. Fig. 5B shows both salt extracted and pore water saccharide concentrations, the difference being the spatially inaccessible pool of reducing saccharides, shaded in green.

Spatially inaccessible saccharide concentrations were up to 10 times those in the mobile pool. Inaccessible saccharides were at their highest concentrations on the first sampling day in late March, and quickly dropped into the beginning of May. This was followed by a peak in inaccessible saccharides in August.
Figure 5: A) The difference in TRS concentrations between salt extracted and water extracted samples. B) The area shaded in pink represents the mobile pool of saccharides in pore water samples, and the green shaded area represents the spatially inaccessible pool of saccharides averaged across all soils (the extractable pool, black line, minus the mobile pool, blue line) (concentrations in the three plots types did not
3.5 Respiration

Respiration was similar in all three soils. The exception was between May and September, when respiration from both A. rubrum and L. maackii soils insignificantly increased, while respiration from the control plots insignificantly decreased (Fig. 6B).

Soil respiration in A. rubrum and L. maackii plots increased and decreased at the same times, including peaks at the same time in August. Soil respiration also peaked for all three soils in early May. These respiration dynamics resulted in a statistically significant interaction between plot type and date \((p \leq 0.0001)\) (Table 3). To separate the interacting effects of plot type and date on soil respiration, the data were then separately analyzed for plot type effects on each individual date. Respiration from L. maackii soils was significantly higher than the control soils on May 8, 11, 18, 21, Oct 29, and Nov 20 \((p \leq 0.04)\). Respiration from A. rubrum soils was never significantly higher than from L. maackii soils, but was significantly lower than L. maackii soils on Oct 29, Nov 17 and 20 \((p \leq 0.016)\). Respiration in A. rubrum soils never significantly differed from control soils.

3.6 Microbial Biomass

Microbial biomass in L. maackii soils was the highest on every sample date. L. maackii plots had significantly more soil microbial biomass (C and N), overall \((p \leq 0.0005)\) (Table 3) from April to December, but it was not significantly higher on any individual sampling day. After our first sampling date in March, microbial biomass C in all soils increased significantly until May 5, but faster in L. maackii soils, and significantly dropped after it peaked on May 13 (Fig. 6A).
Figure 6: A) Soil respiration measured in the field with a Li-COR 8100A soil respiration unit. B) Microbial biomass C measured via chloroform fumigation-extraction and total organic C analysis. (§) indicates L. maackii > control, (*) denotes L. maackii > both A. rubrum and control, and (T) indicates that all soils had a significant peak with respect to time ($p \geq 0.05$, ANOVA).
Table 3: Microbial biomass (C and N) and respiration ANOVA results

<table>
<thead>
<tr>
<th>Microbial biomass and soil respiration</th>
<th>Plot type</th>
<th>Date</th>
<th>Plot *Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration p value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Respiration F-ratio</td>
<td>4.58</td>
<td>6.88</td>
<td>3.99</td>
</tr>
<tr>
<td>Microbial biomass C p value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.50</td>
</tr>
<tr>
<td>Microbial biomass C F-ratio</td>
<td>17.0</td>
<td>40.6</td>
<td>0.97</td>
</tr>
<tr>
<td>Microbial biomass N p value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.54</td>
</tr>
<tr>
<td>Microbial biomass N F-ratio</td>
<td>20.7</td>
<td>53.9</td>
<td>0.93</td>
</tr>
</tbody>
</table>

3.7 Ecoenzymes

BG and PHOS had the highest activities, followed closely by NAG, while both LAP and BX activities were an order of magnitude lower (Figs. 7 and 8). BG, LAP, and NAG activities were similar between *L. maackii*, *A. rubrum*, and control soils (*p* ≥ 0.38). PHOS activities in *A. rubrum* soils were significantly higher than control soils over the whole study (*p* ≤ 0.033) (Table 4), although a post hoc test indicated no significant differences on individual dates. BX activities in *L. maackii* soils were significantly higher than in control soils on May 5, and control soils were significantly lower than in *L. maackii* and *A. rubrum* soils on June 2. All ecoenzyme activities varied significantly by date (*p* ≤ 0.014) (Table 4) due to a significant drop in all ecoenzyme activities in December (Fig. 7 and 8).
Figure 7: Ecoenzyme activities for N acquiring enzymes (A) LAP, B) NAG). Note the Y-axis scale difference, as LAP activities were lower. C) P acquiring enzyme activities (PHOS).
Figure 8: Ecoenzyme activities for C acquiring enzyme activities (BG, BX). (•) is when both plants > control, (§) indicates L. maackii > control ($p \geq 0.05$, ANOVA). Note the Y-axis scale is an order of magnitude lower for BX activities.
Table 4: Ecoenzyme ANOVA results

<table>
<thead>
<tr>
<th>Soil enzyme activities</th>
<th>Plot type</th>
<th>Date</th>
<th>Plot *Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG p value</td>
<td>0.977</td>
<td>0.0001</td>
<td>0.562</td>
</tr>
<tr>
<td>BG F-ratio</td>
<td>0.023</td>
<td>13.8</td>
<td>0.89</td>
</tr>
<tr>
<td>BX p value</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.141</td>
</tr>
<tr>
<td>BX F-ratio</td>
<td>9.14</td>
<td>15.7</td>
<td>1.51</td>
</tr>
<tr>
<td>LAP p value</td>
<td>0.675</td>
<td>0.015</td>
<td>0.117</td>
</tr>
<tr>
<td>LAP F-ratio</td>
<td>0.39</td>
<td>2.84</td>
<td>1.58</td>
</tr>
<tr>
<td>NAG p value</td>
<td>0.384</td>
<td>0.0001</td>
<td>0.082</td>
</tr>
<tr>
<td>NAG F-ratio</td>
<td>0.97</td>
<td>10.6</td>
<td>1.71</td>
</tr>
<tr>
<td>PHOS p value</td>
<td><strong>0.0233</strong></td>
<td>0.0001</td>
<td><strong>0.719</strong></td>
</tr>
<tr>
<td>PHOS F-ratio</td>
<td>3.95</td>
<td>9.12</td>
<td>0.73</td>
</tr>
</tbody>
</table>

### 3.8 Phosphate

Soil pore water in *L. maackii* soils contained higher PO$_4^{3-}$-P concentrations than the *A. rubrum* soils from May to July (Fig. 9B). Due to significant interaction effects between plot type and date (Table 5), the PO$_4^{3-}$-P concentrations were also analyzed separately for individual dates using plot type as the factor. Soil pore water PO$_4^{3-}$-P concentrations in *L. maackii* soils were significantly higher than in *A. rubrum* soils on April 24, 28, and May 21 ($p \leq 0.046$), and significantly higher than control soils on April 28, May 11, and 21 ($p \leq 0.013$) (Fig. 9B). Soil pore water PO$_4^{3-}$-P varied significantly for all soils over time ($p \leq 0.038$). Extractable PO$_4^{3-}$-P concentrations followed a similar pattern but with larger error and, thus, there were no significant differences between *L. maackii*, *A. rubrum*, and control extractable soil PO$_4^{3-}$-P concentrations (Fig. 9A) (Table 6).
Figure 9: PO$_4$$^{3-}$-P concentrations in A) soil pore water B) soil extractions. Significant differences are indicated by (*) when *L. maackii* > both *A. rubrum* and control, (§) when *L. maackii* > control, and (L) when *L. maackii* > *A. rubrum* ($p > 0.05$, ANOVA).
3.9 Nitrogen

Extractable NH$_4^+$-N concentrations decreased from April to June, and then increased again in August for both control and *L. maackii* soils (Fig. 10A). Extractable NH$_4^+$-N concentrations in *A. rubrum* soils followed a similar pattern, though increases and decreases appear to have been more abrupt. Extractable NH$_4^+$-N concentrations were significantly different between plot types (*p* = 0.035) and by date (*p* = 0.007) (Table 6). *A. rubrum* had, over the whole season, significantly more extractable NH$_4^+$-N. However, there were no significant differences between plot types on individual dates. Soil pore water NH$_4^+$-N concentrations increased from April to June whereas the extraction concentrations decreased (Fig. 10A and B). Soil pore water NH$_4^+$-N concentrations peaked for all samples on Nov. 20, and dropped by December 11 (Fig. 10B).

Extractable NO$_3^-$-N concentrations differed significantly between *L. maackii*, *A. rubrum*, and control soils as well as by date (*p* ≤ 0.003). *A. rubrum* soils had significantly less (*p* ≤ 0.003) NO$_3^-$-N from April to December, however, no individual dates differed significantly. All soils had significantly less extractable NO$_3^-$-N in December compared to April. Extractable NO$_3^-$-N concentrations increased in both control and *L. maackii* soils from April to May then decreased at the beginning of June, while the concentrations in *A. rubrum* soils started much lower in April and decreased steadily until December (Fig. 11A). Soil pore water NO$_3^-$-N concentrations differed significantly over time, but not between *L. maackii*, *A. rubrum* and control soils (Fig. 11B). Soil pore water NO$_3^-$-N dynamics were similar to PO$_4^{3-}$-P except that concentrations in *A. rubrum* soils increased
in August, and then all soil pore water sample NO$_3^-$-N concentrations decreased in December.

Extractable TFPA concentrations had similar patterns across all samples, starting high in April, decreasing to the lowest concentration on August 11, and then going back up slightly in December. The one exception is that *A. rubrum* extractable soil TFPA concentrations peaked on April 19, and *L. maackii* soils peaked immediately after on April 26. Overall, TFPA did not differ significantly between *L. maackii*, *A. rubrum*, and the controls, but varied significantly over time ($p \leq 0.0001$). TFPA was not measured in soil pore water due to sample volume restrictions on many sample days.

Figure 10: NH$_4^+$-N concentrations in A) soil extractions B) soil pore water.
Figure 11: NO$_3^-$-N concentrations in A) soil extractions B) soil pore water
Table 5: Soil pore water nutrient ANOVA results

<table>
<thead>
<tr>
<th>Pore water nutrient</th>
<th>Plot type</th>
<th>Date</th>
<th>Plot *Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺-N p value</td>
<td>0.271</td>
<td><strong>0.0001</strong></td>
<td>0.098</td>
</tr>
<tr>
<td>NH₄⁺-N F-ratio</td>
<td>1.31</td>
<td>5.1</td>
<td>1.3</td>
</tr>
<tr>
<td>NO₃⁻-N p value</td>
<td><strong>0.04</strong></td>
<td><strong>0.0001</strong></td>
<td>0.108</td>
</tr>
<tr>
<td>NO₃⁻-N F-ratio</td>
<td>3.24</td>
<td>5.08</td>
<td>1.31</td>
</tr>
<tr>
<td>PO₄³⁻-P p value</td>
<td>0.743</td>
<td><strong>0.0002</strong></td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td>PO₄³⁻-P F-ratio</td>
<td>0.30</td>
<td>2.48</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Table 6: Extraction nutrient ANOVA results

<table>
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Chapter 4

Discussion

The goals of this observational field study were to determine how soil saccharide concentrations relate to plant phenology, are distributed within the soil matrix, and influence microbial activity and soil N+P levels. We hypothesized that *L. maackii* and *A. rubrum*, two plants with differing phenology, would also vary in the seasonal dynamics of soil saccharide concentrations around their roots, because of the variability in leaf expansion timing and associated plant sugar production and exudation. It is widely believed that photosynthate moves through plants rapidly (Rattray et al. 1995, 2001, Hogberg et al. 2001), suggesting that the timing of leaf expansion and decline in chlorophyll concentrations should be good predictors for seasonal soil saccharide patterns. We predicted higher soil saccharide concentrations in association with *A. rubrum* when the leaf canopy was expanded, from late May to mid-October. *L. maackii* is an understory species that leafs expand early in April and senesces late; for example, some individuals kept their leaves until late November during our study. Thus, we predicted early and late growing season saccharide peaks in *L. maackii* soils, with lower concentrations while it was shaded by the forest canopy. Surprisingly however, we did not observe an overall difference between plant species in seasonal saccharide patterns in
rhizosphere soil pore water. Pore water saccharides in L. maackii soils were only significantly higher than in A. rubrum soils on May 18, just before leaf expansion for L. maackii was complete. Root free soil tended to have insignificantly lower concentrations; rooted soils had insignificantly higher soil solution saccharide concentrations on 16 out of 19 sampling dates. Similarly, Weintraub et al. (2007) used tree girdling to reduce P. contorta exudation in a coniferous subalpine forest with sandy inceptisol soil, and observed significant increases in soil DOC and microbial biomass C and N only around non-girdled trees during April, indicating these increases were due to root exudation. These DOC dynamics match our early season pore water saccharide concentrations well, as we observed reductions in saccharides from March to May and June 18 to July 23, and consistently low concentrations (< 50 µmol L\(^{-1}\) soil solution) through December within our trenched plots, suggesting that this difference, though not significant, was likely due to exudation.

We observed an insignificant increase in soil pore water saccharide concentrations with both species up to three weeks before peak chlorophyll concentrations, diverging from control soils as they decreased in saccharide concentrations after June. Soil pore water saccharide concentrations increased around A. rubrum on June 18 and around L. maackii on July 14, and we estimate chlorophyll content peaked in A. rubrum on July 3 and in L. maackii on July 23. Thus, soil pore water saccharides peaked around A. rubrum and L. maackii approximately 15 and 9 days before the peak in chlorophyll.
Root exudation has been estimated to range from 1-40% of fixed C. (Kuzyakov and Domanski 2000, Warembourg and Estelrich 2000, Hütsch et al. 2002). This suggests the lack of difference in soil saccharides between the two plant species in our study may simply be due to differences in exudation rates. Although *A. rubrum* obtains more light and produces more photosynthate when its leaves are out, it is possible that *L. maackii* exudes a larger proportion of recent photosynthate. A lower exudation rate combined with higher root mass in *A. rubrum*, which had the highest root mass on every sample date, and has a larger overall root system than *L. maackii*, could account for the lack of difference in soil saccharides between the plant species.

Soil saccharides varied little in relation to plant phenology in this study, suggesting they are less tightly connected than anticipated. Another possible explanation for the lack of variation, besides differences in exudation rates and root densities, could be that microbes are taking up exuded saccharides before we can measure them. Microbial uptake of monosaccharides can happen twice as fast or faster than sorption (Fischer et al. 2010). If microbes can take up the saccharides before we can measure them, then saccharide concentrations within the mobile pool should be consistently lower than in extractions. However, the differences we observed in soil pore water saccharides between *L. maackii* and *A. rubrum* and control soils suggest that microbes did not take up exuded saccharides faster than we could measure them. Aquatic systems typically have saccharide concentrations in the nanomolar range, indicating that microbial communities draw down saccharides to sub-micromolar concentrations when these substrates are accessible, whereas soils typically have concentrations on the order of tens of micromoles.
(Hobbie and Hobbie 2014). This, accompanied by the facts that soil microbes appear to live in a starved state, i.e. they activate quickly with added C substrate, and that densities of microbes are much higher in soil, suggests that most soil saccharides are inaccessible to soil microbes (Hobbie and Hobbie 2014; Darrouzet-Nardi and Weintraub 2014). This also suggests that our soil pore water measurements of > 50 µM may include some inaccessible saccharides captured by tension lysimetry.

Furthermore, plants can also take up exuded saccharides. For example, it has been shown using isotopic labeling that hydroponically grown Z. mays roots can take-up up to 50% of added glucose (Jones and Darrah 1992), indicating that plants can potentially be a significant source of uptake. Plants are a mixture of photosynthetic and heterotrophic cells and tissues and can utilize saccharides as their heterotrophic C source, and studies have shown various transporters for uptake of different monosaccharides (Büttner and Sauer 2000, Sherson et al. 2000, Sherson et al. 2003). Uptake of monosaccharides also happens passively through the active uptake zone on the root (driven by evapotranspiration), which can also be a net source of exudation in the evening when transpiration stops (Cardon and Gage 2006).

Another factor controlling soil saccharide concentrations is their fate and availability within the soil matrix. We hypothesized that the adsorbed pool of reducing saccharides would be negligible due to their lack of charge. The lack of difference in saccharide concentrations between the water and salt extractions supports this hypothesis.

We also hypothesized that a measurable spatially inaccessible pool of saccharides exists in these soils, because there is evidence for a spatially inaccessible pool of soil free
primary amines (Darrouzet-Nardi and Weintraub 2014), due to soil matrix structure rendering pockets of soil solution inaccessible. We predicted that the spatially inaccessible pool would be consistently larger than the more accessible mobile pool, which we also predicted would be more variable. Studies using Z. mays have shown an uptake rate of <1-10% of $^{14}$C-glucose (added “directly” to the mobile pool) within silty clay loam soils (Kuzyakov and Jones 2006, Biernath et al. 2008). The variability in plant uptake suggests that accessibility within the soil matrix is one of the primary controls on the fate and availability of soil saccharides, as plants can potentially take up anywhere from 1-50% of added sugar depending on the matrix it is growing in (Biernath et al. 2008, Jones and Darrah 1992).

We predicted the existence of a spatially inaccessible pool of saccharides based on the finding by Darrouzet-Nardi and Weintraub (2014) that NO$_3^-$-N was roughly ten times more concentrated in the inaccessible pool as the mobile pool (~110 vs. ~8.6 µmol N L$^{-1}$ soil solution), as saccharides also do not adsorb to cation exchange sites. However, contrary to our prediction, the spatially inaccessible pool varied more than the mobile pool. As was the case for the mobile pool of saccharides, the mobile N pool in this study also remained relatively low compared to the inaccessible pool. Just as Darrouzet-Nardi and Weintraub (2014) concluded that it will be necessary to consider spatially inaccessible N in our models of N cycling, our finding that the pool of spatially inaccessible saccharides was about 10 times larger than the mobile pool indicates that it is necessary to separately consider these pools to predict the fate and availability of soil saccharides.
Other studies have found similar differences between soil pore water concentrations and extractable saccharide concentrations. A review concluded that extractable mono- and di-saccharide concentrations are normally within 10-50 mM across several soils in incubation and non-incubation experiments (Ryan et al. 2001), whereas saccharide concentrations within soil pore water have been reported from 1-1000 µM (Jones 1998, Strobel 2001), mostly in Z. mays grown under sterile conditions in hydroponics (Jones and Darrah 1994, 1995, 1996). While we observed a ten-fold difference between soil pore water saccharide concentrations and extractable saccharides, this difference was relatively low compared to the above estimates. Taken together, these results suggest that spatially inaccessible saccharide concentrations can be anywhere from 10-500 times larger than the soil pore water concentrations.

We observed a relatively large decline in spatially inaccessible saccharide concentrations from the end of March to April 19. Spatially inaccessible saccharide concentrations were highest on the first day of sampling in late March. Concentrations of inaccessible saccharides then dropped quickly during April (Fig. 5B).

We speculate that this was due to thawing soils releasing potentially inaccessible frozen pockets of saccharide-containing soil solution, with the biggest effect occurring in March and diminishing rapidly into early April, but unfortunately we were not able to start soil temperature measurements until May 11, after the final spring thaw. Evidence for freeze and thaw cycles comes from air temperatures ranging from -10 to 0 °C, along with our occasional observation of ice on top of and inside soil cores until April 6. Interestingly, this sharp decline in spatially inaccessible saccharide concentrations was
not accompanied by an increase in soil pore water saccharides, supporting the idea that saccharides, once mobile in the soil pore water, are quickly assimilated by microbes (Hobbie and Hobbie 2014). Oztas (2003) determined the effect of freeze-thaw events on aggregate stability in four different soils, a Pasinler clay loam, Tuxcu sandy loam (both Typic Xerorthents), Karasu clay (Typic Cryaquent), and a Nenehatun clay (Xerollic Camborthid), containing various soil moistures and aggregate sizes. Freeze-thaw events reduced aggregate stability anywhere from 28.6-51.7%, depending on soil type and soil moisture at the time of freezing (Oztas and Fayetorbay 2003). A reduction in aggregate stability has the potential to alter the distribution of dissolved solutes, and we speculate that this mechanism was also at least partially responsible for the decrease in spatially inaccessible saccharides we observed between March and April. The large shifts in spatially inaccessible saccharides we observed and the decrease in spatially inaccessible N over the growing season observed by Darrouzet-Nardi and Weintraub (2014) suggest that the spatially inaccessible pools of DOC and nutrients vary more throughout the season than we had predicted, and may be impacted by freeze thaw events in this system throughout the late fall to early spring.

We also observed sharp increases in spatially inaccessible saccharide concentrations on May 5 and June 2, and attribute them to disturbance effects. Both sharp increases in spatially inaccessible saccharides corresponded with storms that were severe enough to damage the vegetation (i.e. when large plant branches or trunks were broken, for example a 20 m tree breaking at 10 m, or completely pulled out of the ground, in or around any plots). Damage to vegetation, around but not in our plots, was caused by
storms on April 25 and May 26, and increases in the inaccessible pool were observed on May 5 (there was not an increase immediately after this storm on April 26, presumably due to the dilution effect of added water), and on June 2 (plots had time to dry between May 26, storm date, and June 2). We speculate that the disturbance of these storms contributed to the increase in saccharide concentrations. Storms can cause significant DOC runoff into streams, increasing DOC up to 400% during storm events in several small watersheds in central Ontario, Canada, (Hinton et al. 1997). Such increases in stream water DOC have been found to originate from near-surface soil water within forested catchments in New York, USA (Inamdar et al. 2004). We observed increases in surface soil pore water saccharide concentrations after storm events, some of which may have become incorporated into the spatially inaccessible pool as soils dried out after rain events. Although the first storm was on April 25, we speculate that the delayed increase in spatially inaccessible saccharides was due to flooding causing a dilution effect (hence concentrations of saccharides were not elevated on April 26 due to a dilution effect). As soil water evaporates saccharides may be assimilated by microbes or plants, remain in the soil pore water, or become spatially inaccessible by becoming hydrologically disconnected (Darrouzet-Nardi and Weintraub 2014), or possibly precipitating out of solution with severe drying.

Drying and rewetting cycles can change soil properties in ways that may affect the distribution of dissolved solutes (Utomoto and Dexter 1982), and may also serve as a mechanism to move saccharides into and out of the spatially inaccessible pool. Because of the paucity of studies to date, no direct evidence exists, but we speculate that these
disturbances may affect the distribution of dissolved solutes through physical disruption of soil aggregates (Utomo and Dexter 1982). Utomo and Dexter (1982) found that natural wetting and drying events, in tilled and non tilled agricultural fine silt loam soils (Urrbrae, Strathalbyn and Mortlock), can increase the proportion of water stable aggregates briefly, however, under continued wetting and drying events (in the laboratory and field) concluded that this proportion of stable aggregates would decrease steadily. This is relevant because water stable aggregates are a potential reservoir for spatially inaccessible saccharides. Rajaram (1999) concluded that although the proportion of water soil aggregates in an agricultural clay loam soil goes down, there is an increase in the soil aggregate size with increasing drying severity. Furthermore, in a laboratory incubation, after four wetting and drying cycles in a silt loam soil (Aridic Paleustoll), microaggregate formation was reduced (Denef et al. 2001). Hence wetting and drying events may influence the spatially inaccessible pool of saccharides by altering the proportion and size of water stable aggregates.

Another factor likely impacting the spatially inaccessible pool is the presence of earthworms, as they are capable of impacting soil hydrological connectivity by creating large preferential flow paths as they burrow (Smettem 1992; Schaik et al. 2014), and they can also alter the distribution of nutrients within the soil by concentrating nutrients within their casts (Bohlen et al. 2004, Le Bayon and Binet 2006). The casts produced by earthworms are typically more structurally stable (Le Bissonnais 1996) and contain more SOC than surrounding soil aggregates (Jouquet et al. 2008, Lipiec et al. 2015). Another study, using $^{13}$CO$_2$ plant labeling and tracing it from leaf and root litter to soil aggregates,
showed that root C is incorporated into macroaggregates faster with earthworms present, though much of it remained labile and was mineralized within three years (Yavitt et al. 2015). Although the earthworms have been shown to impact C in macroaggregates, it has been suggested that microaggregate formation within macroaggregates is where long term C stabilization takes place (Six et al. 2004). While no measurements were taken, high densities of exotic earthworms (dominated by *Lumbricus terrestris*, also containing *Aporrectodea rosea*, *Amyntas agrestis*, and other species (Herman 2010)) were observed in these soils, and their presence likely affects these soils greatly through burrowing and cast formation, and likely further impact the spatially inaccessible pool hypothesized to be through their impact on aggregate size and stability. Earthworms are typically active from April until November, when the soils are not frozen, based on soil temperature and moisture requirements of the species. The dominant species, *L. terrestris*, is active between 0-20 °C (Nordstr et al. 1975), whereas *A. agrestis* has an active range of 12-25
°C (Richardson et al. 2009). Thus, earthworms were likely active and impacting the soil environment from April until December, when sampling stopped.

In light of these results, we propose extending the conceptual model of soil saccharide dynamics to explicitly represent mobile and spatially inaccessible saccharide pools, but not an adsorbed pool (Fig. 12). We also speculate that movement into and out of the spatially inaccessible pool can be caused by freeze/thaw and drying/rewetting cycles, as well as any physical disturbance that can affect soil aggregate size and stability. We further predict there to be slow diffusion from the inaccessible to the mobile pools, but our results suggest that these fluxes may be minor compared with those resulting from disturbances, such as spring thaw and summer storms.

Figure 12: Proposed extension of the conceptual model of soil saccharides with an added spatially inaccessible saccharide pool. Blue boxes represent pools of soil saccharides, while lines indicate fluxes. The red bars represent potential controlling mechanisms on saccharide movement into and out of the spatially inaccessible pool.
When accessible, microbes are able to take up saccharides rapidly (reviewed in Hobbie and Hobbie 2014), thus, our measurements represent a nearly instantaneous snapshot of the balance between production and uptake. If uptake is as fast as saccharide production, then microbial biomass and activity may be better correlated with root exudation than saccharide concentrations. For example, a study using $^{14}$C pulse chase labeling in Z. mays grown in a loamy Haplic Luvisol estimated that 69-86% of root exudate C was mineralized to CO$_2$ one week after labeling (Werth and Kuzyakov 2008). Because microbial uptake and assimilation of monosaccharides is rapid, we hypothesized that microbial biomass and respiration would track reducing saccharide concentrations. For example, glucose uptake was shown to be on the order of seconds to minutes across several soil types with varying plant communities (a sandy clay loam from a grazed grassland, a loamy sand from arable soil, and a loamy sand from eucalyptus and boreal forests were incubated in the lab), and microbial activation upon addition of substrates was virtually instantaneous (taking less than 60 seconds to stimulate microbial respiration after glucose additions) (Jones and Murphy 2007). Likewise, we also hypothesized that soil respiration would correlate with microbial biomass, because respiration is a function of microbial biomass and activity. We predicted that microbial biomass and respiration in L. maackii soils would follow the same dynamics we predicted for soil saccharides, forming bimodal peaks before and after a single A. rubrum peak. Soil saccharide dynamics did not follow this predicted pattern, however, as we observed no variation between plant species in seasonal patterns. Furthermore, microbial biomass and soil respiration did not follow patterns of soil pore water saccharides as predicted.
We speculate that biomass and respiration did not follow patterns of soil pore water saccharide concentrations because saccharides were taken up so fast that the concentrations we observed do not fully reflect patterns of saccharide production from plants. Although pore water saccharides in *L. maackii* soils formed a bimodal distribution, *A. rubrum* also followed a similar pattern. *L. maackii* soils had consistently, if insignificantly, higher respiration rates, but not higher concentrations of microbial biomass, and consistently lower root density than *A. rubrum*. This could result from differences in exudation rates and/or composition leading to differences in microbial activity (Broeckling *et al.* 2008; Falchini *et al.* 2003; Fan *et al.* 2001). *L. maackii* soils contained significantly more soil pore water saccharides than both *A. rubrum* and control soils immediately before leaf expansion was complete on May 18. The first respiration peak in May, observed in all soils, is speculated to be from a combination of increased C from overwinter root damage associated with freeze-thaw (including release of frozen spatially inaccessible saccharides), annual root turnover (Gill and Jackson 2000), spring root exudation, leaf litter decomposition, and increasing temperatures. These substrate inputs were likely a relatively greater factor contributing to the early increases in soil respiration and microbial biomass on April 17 and April 19, respectively, as spring root and litter inputs, and the effects of freeze-thaw dwindle as the growing season proceeds (air temperatures were between 3-7 °C on these dates, and soils were above freezing, indicating minimal to no effect from early season thawing) (Wipf *et al.* 2015). Peak soil respiration in both *A. rubrum* and control plots corresponded with the peak in microbial biomass on May 13 and 18, however respiration in *L. maackii* soils peaked in August. Our results suggest the soil respiration peak for *L. maackii* was driven primarily by
microbial respiration of root exudates, as respiration increased in both *A. rubrum* and *L. maackii* soils, but declined in root free bulk soil after leaf expansion. Thus, the deviation in soil respiration and microbial biomass after leaf expansion between controls (decline) and rooted soils (increase) suggests that the peak in soil respiration for *L. maackii* on August 20 was fueled primarily by root exudates and possibly increased root respiration. Additionally, soil respiration in the control plots decreased relative to both *L. maackii* and *A. rubrum* from late March to late April and again between June and August, while soil respiration in both *L. maackii* and *A. rubrum* plots increased, indicating that the trenching successfully reduced substrate availability to microbes.

There was a pulse of respiration in November from *L. maackii* soils, which was significantly higher than in *A. rubrum* soils on October 28, November 17, and 20, and significantly higher than control soils on October 28 and November 20. This late season pulse of respiration correlates with normal senescence times for *L. maackii* (Trisel 1997, Arthur *et al.* 2012). Current research suggests that root respiration, across a variety of species, decreases as senescence occurs (Comas *et al.* 2000, Fu *et al.* 2002). Because microbial biomass and ecoenzyme measurements were not conducted between August 11 and December 11, we cannot verify this, but we speculate that the pulse of respiration in late October early November was from microbial respiration and/or a pulse of exudation and fine root turnover with senescence.

Little variation between species in microbial biomass and saccharides was observed over the course of the growing season in our study. However, other studies have demonstrated barley, rice, and wheat exude different compounds (Fan *et al.* 2001), and
variations in the availabilities of these substrates have been shown to drive microbial community shifts (Griffiths et al. 1998, Falchini et al. 2003, Broeckling et al. 2008).

Because of variability in exudate composition between species, microbial communities in *L. maackii* soils may differ from soil communities within both *A. rubrum* and control soils. Microbial community composition was not assessed in this study, but a next logical step in this research would be a comprehensive analysis of how microbial community shifts relate to plant phenology and root exudation rates and composition.

Soil nutrient concentrations around the two species and in the control plots were predicted to inversely correlate with saccharide concentrations, but did not. Soil pore water P and extractable N concentrations in *L. maackii* soils were significantly higher than in *A. rubrum* soils on several days before leaf expansion was complete. After leaf expansion, P concentrations were less variable, however soil pore water sample volumes were too low to measure in every plot type on each sample date. N availability was typically high relative to other temperate deciduous forest soils, peaking at 2064 μmol-N (NO$_3^-$ +NH$_4^+$) L$^{-1}$ soil solution within *L. maackii* soils, primarily as NO$_3^-$ until June 2. Although this is relatively high N availability, several studies have reported much higher dissolved inorganic N concentrations, such as 4460 μmol-N (NH$_4^+$+NO$_3^-$) L$^{-1}$ soil solution in a German beech forest (Zhong and Makeschin 2006), or 6160 μmol-N (NO$_3^-$ only) L$^{-1}$ soil solution in a southern California conifer forest (Michalski et al. 2004). While soils around *L. maackii* began with the highest extractable N availability in the spring, *A. rubrum* soil had higher extractable N availability after leaf expansion was complete. Spring NO$_3^-$-N concentrations also increased in control soils, peaking slightly
higher than *L. maackii* on May 5, before leaf expansion was complete. The decline in NO$_3^-$-N concentrations in *L. maackii* soils after leaf expansion was complete was abrupt, going from 1730 to 569 μmol NO$_3^-$-N L$^{-1}$ soil solution. We speculate that *L. maackii* increased N uptake during this time because we observed no abrupt change in NH$_4^+$-N concentrations, as likely would have occurred if nitrification rates decreased (Hall *et al.* 2008), and a decrease in microbial biomass C and N, suggesting that microbial N uptake was not increasing. Furthermore, the absence of this pattern in the other soils, which experienced the same temperatures, suggests the high NO$_3^-$-N in *L. maackii* soils before leaf expansion was complete was not caused by winter-spring freeze-thaw events or by increased runoff, but rather soil properties relating to the litter chemistry and seasonal dynamics of *L. maackii*. We attribute this to high *L. maackii* litter decomposition and nutrient mineralization rates (Blair and Stowasser 2009, Poulette and Arthur 2012, Trammell *et al.* 2012). There is widespread support in the literature for the conclusion that easily decomposed, nutrient rich *L. maackii* litter increases rates of decomposition and nutrient mineralization in invaded forests (*e.g.*: Blair and Stowasser 2009, Poulette and Arthur 2012, Trammell *et al.* 2012). By increasing decomposition and nutrient mineralization rates in forest soils, *L. maackii* can significantly affect soil nutrient cycling dynamics and concentrations, independent of exudation.

### 4.1 Conclusions

We predicted that pore water saccharide concentrations in *L. maackii* and *A. rubrum* soils would track leaf expansion and senescence times for these species with
contrasting phenology. However, the concentrations were similar, which may be due to differences in exudation rates, composition, and/or root densities between species. The only difference was an apparent pulse of exudation around *L. maackii* just before leaf expansion was complete. We speculate that this was associated with high nutrient demand in *L. maackii* during leaf expansion, which was also indicated by apparently high nitrate uptake at this time. We conclude that soil total reducing saccharide concentrations, and possibly root exudation rates, do not necessarily track plant phenological events, and leaf presence does not always indicate higher soil total reducing saccharides. It is also possible that saccharides derived only from plants correlate more strongly with phenological events, as our measurements include microbially derived sugars, as well. Furthermore, the soil solution we collected already had the opportunity to be filtered by the microbial community in the rhizosphere and our data suggest that the microlysimeters may also sample some spatially inaccessible dissolved organic matter and nutrients, and therefore these concentrations may not reflect gross exudation rates. This raises the question of whether soil total reducing saccharides are a good proxy for plant derived saccharides, and how quickly the microbial community turns-over plant saccharides.

Once in the soil, the fate of soil saccharides was also assessed. The concentration of the adsorbed saccharide pool was negligible, as predicted. Spatially inaccessible saccharide concentrations were up to ten times higher than in the soil pore water. Although other studies have not measured pore water saccharides and extractable saccharides together, individual measurements in the literature suggest that the spatially inaccessible pool could be anywhere from 10 to 500 times greater than a mobile pool, implying that our system had relatively low levels of spatially inaccessible saccharides,
but we caution that additional direct measurements are needed. One factor that may reduce spatially inaccessible carbon and nutrient pools at our research site is the high abundance of invasive exotic earthworms, which have a large impact by increasing hydrological connectivity.

We speculate that the primary driver of large movements between the spatially inaccessible pool and soil pore water are earthworm activity and disturbances. We attribute the early spring decline in spatially inaccessible saccharides we observed to freeze thaw dynamics, as the ground was still frozen at the time of our first soil collection and contained the highest extractable saccharide concentration of this study. After this, the major increases in inaccessible saccharides appeared to occur around storms large enough to cause tree damage, which were coupled with the initial dilution of pore water concentrations from increased soil moisture, followed by increases in the inaccessible pool with drying. We speculate that this movement into the inaccessible pool might be caused by storm-related impacts to soil aggregate size and stability resulting in redistribution of soil saccharides, and likely N and P, within the soil matrix. However, further direct quantification of mobile vs inaccessible saccharides will be needed to determine the controls on the size of the spatially inaccessible pools of soil dissolved organic matter and nutrients.

We did not observe variations in soil microbial biomass or nutrient dynamics in response to changes in soil saccharide availability. In general, the biggest difference between *L. maackii* and *A. rubrum* soils was higher N and P concentrations in *L. maackii* soils before leaf expansion was complete. The higher N and P concentrations are
attributed to high litter quality and decomposition rates associated with nutrient rich \textit{L. maackii} litter and soils.

Because the majority of soil saccharides occur in the spatially inaccessible pool, we suggest that this pool should be incorporated into models of soil C sequestration. Saccharides stimulate microbial production of biomass, and ultimately necromass, believed to be the source of stable SOC. In order to address this, it will likely be necessary to correlate weather dynamics and soil properties (\textit{e.g.} aggregate size and stability) to spatially inaccessible saccharide pool sizes and movement.
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