Starch Analysis of Betula populifolia and Populus spp. in an Abandoned Urban Brownfield

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Abstract

In order to cope with a variety of environmental stresses, trees use starch storage as a buffering system to compensate for energy needs. Starch allocation into different tissues throughout the year can determine the health of deciduous trees. Natural environmental stresses including seasonal changes, water and nitrogen availability and defoliation are known to have direct effects on starch storage. Anthropogenic stresses such as heavy metal and other toxic contaminants in soils have not been investigated as factors that alter the normal starch trends in the different tissues of trees. This study assesses how starch storage in different tissues in *B. populifolia* and *Populus spp.* roots, twigs and leaves may have been altered by heavy metal contamination in trees growing in an urban brownfield over the course of a year. A heavy metal polluted site was used where varying levels of toxic metals including Zn, Pb, As, Cu and Cr were previously identified. Starch concentrations between the two species was significantly different (*p*=0.03) with *Populus spp.* having almost twice as high a starch concentration as *B. populifolia* when all tissue data for the year are considered. The level of metal contamination in the soil had some significant interaction effects on starch concentration in all of the tissues sampled between the two investigated species. Root and twig tissues of *Populus spp.* showed an increase in starch content with increasing metal load while *B. populifolia* showed no significant change in starch concentration at the different metal load sites. The difference in starch between the two species across the metal load sites indicates different responses to metal stress of the two species, which may in turn account for variations in species dominance where there is more or less metal pollution in the soil.
Starch Analysis of *Betula populifolia* and *Populus* spp. in an Abandoned Urban Brownfield

by

Ariel Valverde

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In Partial Fulfillment of the Requirements For the Degree of

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Department of Biology

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Starch Analysis of *Betula populifolia* and *Populus* spp. in an Abandoned Urban Brownfield

A Thesis

Submitted in partial fulfillment of the requirements for the degree of Master of Science by Ariel Valverde

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Montclair State University
Montclair, NJ
2017
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I would also like to thank my loving supportive family, Cesar, Lily, Jason and my parents.
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Figure 5: Starch data by tissue for Populus spp. for each collection date as a time series for each tissue.
Introduction

Nonstructural carbohydrates (NSC) which include starch and soluble sugars are the molecular components of a tree’s buffering system (Sala et al. 2012, Sauter and Cleve 1994). However, the primary photosynthate used by trees to cope with natural forms of stress is starch (McLaughlin et al. 1980). Starch levels respond directly with the degree of stress on trees (Vanderklein and Reich 1999) and are therefore an excellent measure of stress that can be used to determine which species can acclimate to a given stress better. Starch levels have also been used as an indicator of stress level in response to global climate changes including increases in atmospheric CO₂ (Taylor et al. 2003) and drought stress (McDowell 2011, Wang et al. 1995).

Both needle and broadleaf trees demonstrate a consistent pattern in seasonal shifts in starch storage and translocation (Regier et al. 2010, Landhäusser and Lieffers 2003, Vanderklein and Reich 1999, Loescher et al. 1990, Kimura et al. 1968, Webb and Kilpatrick 1993, Wight 1933). During the summer high photosynthate production leads to an accumulation of NSC in twig and root tissues (Wight 1933). During the late summer into the fall, starch levels in twigs decrease and accumulate in the fine roots where they are stored through winter with some use for maintenance (Loescher et al. 1990, Wight 1933). The highest starch levels are recorded directly before budbreak for both roots and twigs as the trees prepare for spring growth and reproduction (Loescher et al. 1990, Wight 1933). Once starch is mobilized after bud break, starch levels decrease about a third in twigs and roots (Wight 1933). After spring growth, starches begin to accumulate in the early summer renewing the cycle (Loescher et al. 1990, Wight 1933).
The mean trends of starch concentrations show fluctuations in response to different sources of stress on trees (Ostonen et al. 2007). Discrete stress exposure has the potential to interrupt productivity and kill trees, but with sufficient starch reserves trees have a better chance for survival (Sala et al. 2011). Excessive stress on trees during an entire growth season results in changes in starch reserves the following year (Vanderklein and Reich 1999). Additionally, short periods of stress, such as droughts, are detrimental to starch reserves (Wang et al. 1995).

Starch concentrations in trees subjected to seasonal herbivory stress have been used as indicators of stress and survivability (Vanderklein and Reich 1999, Dunn et al. 1987). Wood boring parasites in *Quercus* spp. have been shown to be correlated with trees that had low winter root starch storage, in contrast to trees that had higher starch storage indicating a response to herbivory in the previous year (Dunn et al. 1987). Defoliation stress also resulted in decreased starch reserves the following season with higher levels (75%) of defoliation resulting in lower starch reserves (Vanderklein and Reich 1999, Wiley et al. 2013).

Trees that are exposed to long-term stress, generally lasting the life of the tree, specific to one site have acclimation mechanisms. Bryant et al. (1983) found an association in pioneer species that when they are exposed to regular disturbances they will have higher C (carbon) storage. *Salix viminalis* also demonstrated higher starch storage when grown as an early successional species (Bollmart et al. 1999). Another ongoing stress trees must respond to is low soil N (nitrogen) availability, in this situation starch accumulation increases, likely due to the tree’s nutrient starved growth limits (McDonald et al. 1986).
Despite starch being used as an indicator of natural forms of stress, starch budgets have not widely been used to assess direct anthropogenic influences. How industrial waste pollution influences natural ecosystems is of great interest as more terrestrial area is utilized by humans (Grimm et al. 2008). Metal contaminated sludge, other industrial wastes and abandoned mines have been used to test ecosystem responses to heavy metal contamination on above- and belowground biomass (Pulford and Watson 2003). Due to the long-life history of trees relative to other species, polluted soils select for highly tolerant genotypes, such as species adapted to maintain large starch budgets, including pioneer genera like *Salix*, *Populus*, and *Betula* (Pulford and Watson 2003, Turner and Ross 1994).

No prior research appears to have been reported on how heavy metals (above ambient metal levels) in soils affect starch budgets in trees. Photosynthesis in an urban brownfield was assessed by Renninger et al. (2013), but no significant difference in photosynthetic capacity between the metal loads in *Populus spp.* was identified. Starch budgets in soil pollutant stressed trees are expected to be a strong indicator of pollution level, because soil pollution induces stress on many tree species and trends in decreased biomass at urban brownfields have already been found (Chapin et al. 1987, Chapin et al. 1990, Dahle et al. 2014).

In order to address the gap in research on urban brownfields affecting tree starch budgets I studied *Betula populifolia* Marsh. (grey birch) along with *Populus deltoides* Bartr. ex Marsh. (eastern cottonwood) and *Populus tremuloides* Michx. (quaking aspen) hybrids in a portion of Liberty State Park (LSP) which is contaminated with a variety of heavy metals. LSP is an industrial waste site in Jersey City, New Jersey with soil contaminated from construction fill along with metals that leached into it. The unique
hardwood assemblage delayed successional growth and soil contamination variations within the site make LSP an especially suitable location for determining soil pollutant effects on trees. I attempted to identify how soil pollution would influence starch budgets in two pioneer species, *Betula populifolia* and natural hybrids of *Populus tremuloides* and *Populus deltoides*. The species used are dominant at the site with very low abundance of late successional species (Gallagher et al. 2011). This study uses data collected over an entire year to compare starch budgets in twigs, roots and leaves of *B. populifolia* and *Populus spp.* surviving at sites with different levels of soil pollution. These species are of special interest due to being pioneer hardwoods and bioremediators (Bradshaw et al. 2000).

Several effects will be tested in this study based on starch content of the different tissues of trees. The first effect investigated will be the difference in starch content between *Populus spp.* and *B. populifolia*. The next effect to be tested will include differences between the species and how the annual trends of *Populus spp.* and *B. populifolia* were similar or different throughout the entire year. Seasonal trends will be assessed using specific tissue types of the trees to further differentiate energy budgeting differences between the species. Finally, the starch content of trees in the different metal loads, based on site, will be addressed. This method of accounting for known differences among the samples will help determine how much of sample starch differences are due to species, season, tissue and the actual soil pollutants.

It is expected that starch productivity and thus storage would be reduced in soils that have abnormally high metal concentrations in agreement with the reduced growth found in trees at polluted sites. It is also expected that the two species will share similar starch storage trends due to them being co-dominant at the site and that seasonal variations
will follow the trends seen in other tree species according to growing season starch accumulation and winter depletion (Sala et al. 2011).
Methods

Site

Liberty State Park (LSP), Jersey City, New Jersey (40°42'14” N, 74°03’14” W) is a 102 ha abandoned rail yard with a portion of the land recovered by the State of New Jersey for use as a public park (Gallagher et al. 2008). Initially LSP was a combination of marshland and mud flats along the Hudson River dominated by marsh grasses (US Army Corps of Engineers 2004). During the late 1800's the site was filled by the Central Railroad of New Jersey (CRRNJ) to be used as a rail yard (US Army Corps of Engineers 2004). The area was abandoned by CRRNJ in 1967 and purchased in 1970 by the state of New Jersey (Gallagher et al. 2008). Of the entire site, approximately 77 ha of LSP has varying levels of soil contamination and is considered a hazardous waste site. After approximately 30 years of abandonment with only vegetative growth, hardwood species began to quickly spread with a 23% increase in 8 years (Gallagher et al. 2008). A mixture of wetland grasses, early successional hardwoods and some invasive flora including Polygonum cuspidatum Siebold and Zucc. and Phragmites australis Cav. are present at the site. The unique composition of the soils at LSP consisting of primarily construction debris from New York lead the USDA to label it the Ladyliberty Series (National Cooperative Soil Survey 2012). Metals including: As (4.25 to 978µg g⁻¹), Cr (9.68 to 209µg g⁻¹), Cu (23.9 to 1870µg g⁻¹), Pb, V and Zn (24.8 to 6502µg g⁻¹) are all recorded on site at levels above the New Jersey soil ambient concentrations (Quian et al. 2012, Gallagher et al. 2008, Saunders 2002). The contaminated area is currently used for a variety of scientific investigations, but is otherwise undisturbed by humans.
This study used four of the sites at LSP previously mapped for metal contamination level (Figure 1). The sites were selected based on Gallagher et al. (2008) total metal load (TML) level with two sites (labelled 1416 and 14) being selected as high metal load sites and two sites (labelled 48 and 41) selected as low metal load sites. Betula populifolia Marsh. (grey birch) along with Populus deltoides Bartr. ex Marsh. and Populus tremuloides Michx. hybrids were the dominant hardwoods (51.2%) of the area with minimal presence of other trees (Gallagher et al. 2008). Site 1416 and 48 had a mixture of Populus spp. and B. populifolia, site 41 only had Populus spp., and site 14 only had B. populifolia.

Sample Collection

This project was initiated February 2014 and continued until March 2015 to account for annual variation in starch content in trees in different tissues (McLaughlin et al. 1980). At sites 48 and 1416 five B. populifolia and five Populus spp. trees were selected monthly for sampling. Because sites 14 and 41 were primarily homogenous, five B. populifolia were selected at site 14 and five Populus spp. were selected at site 41 monthly (Table 1). Any trees with morphological indication of poor health, such as cankers, shelf fungi, or crown die-back were excluded (c.f. Terho et al. 2007) to minimize other stressors affecting the results.

At each site, five trees of each species were randomly selected for sampling in order to minimize effects from digging and trimming to individual trees (Table 1). Root, stem and leaf (when available) samples were collected at each visit between 11am and 3pm (Barbaroux et al. 2003). Sites were always sampled in the same sequence so that tissue samples were always collected at relatively the same time of day. Root samples were collected at the smallest diameter possible (>5mm) in order to find the highest
starch content (Regier et al. 2010). Branches tended to be in the lower third of the crown due to limitations to access the upper branches. Generally, branches were in full exposure to the sun when sampled. Samples were kept in a cooler with ice and returned to the lab to be cleaned and dried for at least 48 hours at 50°C in a forced-air oven. After dehydration, the samples were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and run through a 0.4mm mesh. Samples were stored in closed vials until starch analysis could occur.

**Starch Analysis**

The starch analysis protocol was adapted from Haissig and Dickson (1979), whereby 50.0±0.4mg of ground dry sample tissue was vortexed with 10 mL of heated 80% ethanol. Samples were then placed in a centrifuge for 5 minutes at 3000 rpm. The supernatant was subsequently poured off the top of the samples removing compounds other than starch and structural carbohydrates. This process was repeated until the supernatant ran clear (approximately 4-7 times). Samples were stored in an oven at 50°C for at least 24 hours.

The next day the sample tissues were emoved from the oven and 0.2mL of 95% ethanol was added to each sample tube. Additionally, four mL of NaF buffer was added to each sample tube. Samples were covered and boiled for 15 minutes then immediately cooled to room temperature and 1 mL of 37.5U/mL amylglucosidase enzyme (reconstituted powder with NaF buffer Sigma Aldrich, St. Louis, MO) was added to each tube to digest starches; then tubes were vortexed, covered, and incubated at 50°C in an oven for 24 hours.

On the third day, tubes were removed from the oven, vortexed and then centrifuged at 3000 rpm for 12 minutes. 0.1mL of sample was pipetted into designated new tubes with
0.4mL of NaF buffer. Further, 0.5mL of glucose standards (GS) ranging from 0-180 mg/L were pipetted into corresponding centrifuge tubes. All tubes received 5.0mL of peroxidase glucose oxidase (PGO) enzyme solution (Sigma Aldrich, St. Louis, MO). Subsequently, the tubes were vortexed and placed in a water bath at 38°C for 30 minutes. At the end of this time, 2 mL of each sample and standard was placed into new cuvettes and read on a spectrophotometer at 450nm within 30 minutes of being incubated. Glucose standard results were regressed against their glucose concentrations to determine a standard calibration curve which was used to determine glucose concentrations of the samples. These glucose values were then converted to starch concentrations taking sample mass and dilutions into account and assuming a 1:1 ratio of starch to glucose.

**Statistical Analysis**

A generalized linear mixed regression model in Matlab version R2015b (fitglme procedure, The Mathworks Inc., Natick, MA) was used to assess the impacts of plant part, soil metal load, and time of year on starch concentration in the sampled trees. This method allows us to account for repeated measurements at the sites for the entire year as a fixed effect (time) with respect to seasonal changes (Blackwell et al., 2006) with the sites (14, 1416, 41 and 48) as grouping (nesting) variables and the metal loading (high or low) grouped within site. Since species were expected to have variations in results birch and poplar were analyzed for interactions with metal loads (high or low) that were included as potential explaining variables (fixed effects) in the model. Interaction between time and the plant parts were also fixed effects because different plant parts will change seasonally. The groups (sites) are evaluated in their tendencies (increase, decrease or no change) that by elimination of other variables can only be due to the metal loading. Significance was based on a $P$-value
of $<0.05$. This statistical analysis was chosen as it is not based on the assumptions inherent in the analysis of variance because there are unequal quantities of data so the design was not balanced (Blackwell et al., 2006, Bolker et al., 2009). Graphs were created using JMP® 11 (SAS Inc., Cary NC).

**Results**

**Effects of Soil Metal Load**

There was no overall effect of soil metal load on starch concentration. However, there was a significant interaction between soil metal load and species indicating that the species responded differently to soil metal load. This may be partially the result of trees at sites with similar metal loading showing very different trajectories in starch concentration for different plant tissues. For example, starch twig starch concentration responded quite differently in poplars at site 48 compared to site 41.

**Effects of Species**

Entire year data, including all tissues, reveal a significant starch concentration difference between species through the mixed effects model ($p<0.03$, Figure 2). This is primarily the result of the poplar species having much higher starch concentration in the high metal sites compared to the birch species (Figure 3). Because there was a significant effect of species and there was a significant interaction effect between species and soil metal load, due to this factor, species were represented separately for the other effects in Figures 3-5.

The primary starch storage tissue (twigs, roots or leaves) used by the trees was also different between the species when entire year and all sites’ data were considered (Figure 3). Despite the different species’ annual trends fine roots had the overall highest starch
levels for both species at 15.6±1.3mg/g above twigs and leaves were 10.3± 1.5 mg/g below
twigs (p<0.02, Figure 3).

Effects of Season

Starch accumulation in general occurred earlier in the growing season for Populus
spp. than for B. populifolia, with significant differences in July (P=0.0081), August
(P=0.017) and September (P=0.028). Populus spp. also tended to mobilize starch for spring
in March, whereas B. populifolia mobilized starches in late April (Figures 4 and 5). Root
starch mobilization was highly dependent on site and species where Populus spp. had the
highest starch accumulations prior to May budbreak (Figures 4 and 5). The effect of metal
concentration further exemplified species differences over the year in starch accumulation.
Leaves of both B. populifolia and Populus spp. were not significantly different at the
different sites (Figure 3).

Discussion

Effects of Species

Starch budgets between the two species were significantly different with Populus
spp. storing more starch than B. populifolia (Figure 2). Both tree species used fine roots
more than leaf or twig tissues for starch storage over the entire year as has been seen in
other tree species (Bollmark et al. 1999). Both species were within ranges of starch content
of similar species that were previously found by Essiamah and Eschrich (1985). They
sampled twigs of six different species (Acerpseudoplatanus, Betula pendula, Alnus
glutinosa, Fagus sylvatica, Quercus rober, and Fraxinus excelsior) and all starch contents
of these trees were between 5-70 mg/g for the study period (spring). This suggests that
despite the difference in starch between the species, the starch contents are not exceptionally low or high compared to the trees in our study growing on contaminated soils.

Sala et al. (2011) measured starch contents in ten genera including both deciduous and evergreen twig samples and found the highest content in *Quercus* at 160 mg/g in both spring and late summer which is well above the highest mean twig starch content found in either *B. populifolia* or *Populus spp.* at LSP (Figures 4 and 5). The two species clearly display differences in starch rationing, though these differences, when all data are included is not irregular compared to other deciduous trees (Sala et al. 2011).

The most significant difference of *Populus spp.* is seen in the summer, when starch accumulation occurs, and within the fine roots. The difference in *Populus spp.* begins slowly and increases with mean starch concentration doubling (24mg/g in August) in the summer and continues into October (37mg/g). Then mean starch concentration decreased in November (32mg/g) and returning to seasonal low means in December (18mg/g) (Figure 5). The trend of starch content was less consistent in *B. populifolia* where starch content increased prior to bud break and then again in the middle of the growing season, followed by another increase at the end of the growing season

*Effects of Seasons*

Both *B. populifolia* and *Populus spp.* maintained general trends of starch content spikes just prior to bud break and again after accumulation in late summer to early fall (Figures 4 and 5). However, regardless of site, twig starch accumulation peaked later in the season in *B. populifolia* (October) than in *Populus spp.* (August) (Figure 4 and 5). Trees that were growing in less polluted sites for both species had a dip in twig starch content in
September which is also typical of *Acer* and *Fagus* in general (Sala et al. 2011). This slight decrease in starch in September is not present at site 1416 with higher soil metal concentration (Table 3) possibly because of less growth or starch usage at this time, but no phenotypic differences of the leaves between trees were noticed. Root starch content in both species peaked in October, but *B. populifolia* had a less consistent decreasing range of root starch contents from October into the winter, while *Populus spp.* displayed a relatively consistent root starch decrease into winter. The unusual trend in *B. populifolia* through late fall into winter has not been previously observed in other species primarily because other studies do not record data past early fall (Sala et al. 2011, Essiamah and Eschrich 1985, Wight 1933). With more data, a significant difference throughout more of the year may have been found in *B. populifolia* starch content between site 48 and 1416 based on the graphical representation (Fig 5).

**Effects of Soil Metal Load**

Starch content variation was most significant within *Populus spp.* at the different metal load sites. Although this supports the expectation that there would be a difference in starch content due to metal contamination, the stress reaction to metals is contrary to natural stress reactions. Whereas decreased water availability or defoliation decrease starch availability (Schaberg et al. 2000, Vanderklein and Reich 1999), metal contamination increased starch production for *Populus spp.* (Figure 3). Increased starch storage in poplar twigs and roots at more polluted sites (1416 and 14) could be indications of pollutant exclusion or accumulation mechanisms creating higher energy costs (Dahmani-Muller et al. 2000).
Although metal contaminant levels for this experiment were based on a gradient system developed by Gallagher et al. (2008), it must be recognized that through natural methods including leaching, chelating and translocation the contamination levels change over time. The previously assessed metal content at LSP was re-evaluated at four sites (Hagmann et al. 2015) and sites that previously had high metal loads (e.g. site 14) were recorded with lower levels of contaminants (Table 2). The decrease in metals, seen most significantly at site 14, indicates that natural processes such as leaching, microbial chemical interactions and phyto-translocation (Gallagher et al. 2008) are actively altering the soil contaminants.

Among the sites that had considerable contamination level changes over time, site 14 changed the most, initially being especially high in metals and more recently being one of the least contaminated sites. The exception to this trend at site 14 is in Pb, which remained exceptionally high at 243.5±17.4 µg/g relative to sites 43 (adjacent to 41) and 48, which both were below 200µg/g (Table 2). However, although there was not a statistical significant difference in site 14 from the other B. populifolia sites, the annual mean starch concentration for all tissues was higher and potentially an significant effect could be detected if more data were available (Figure 3).

Other abiotic factors of the different sites make it difficult to isolate the effects of the metal pollutants. Since metal digestion and oxidation can alter factors such as pH and nutrient availability it is important to take variation in characteristics of the sites into consideration (Diaz-Ravina and Baath 1996). Site 48 had the highest pH of 6.1 while the other three sites had pH’s ranging from 5.2-5.4 (Hagmann et al. 2015, Gallagher et al. 2008). pH levels are known to impact metal translocation by plants along with high pH
leading to decreased N uptake in roots (Brunner et al. 2002, Zeng et al. 2011). However, starch tissue concentration have not directly been correlated with soil pH (Brunner et al. 2002).

Although soil water saturation varies across LSP, it is not expected that these differences had significant effects on starch storage, since other studies have found no difference in starch storage when elevation and water levels varied (Schaberg et al. 2000). Differences in soil type on starch storage have had inconsistent results. In one previous study (Schaberg et al. 2000), there was no difference in starch storage in trees growing in different soil types while in another study, more significant differences in starch content due to soil type than in either species and genera were seen (Brunner et al. 2002). More investigation into confounding variables that may influence starch such as organic matter at the different LSP locations would be necessary to elucidate some of the drivers of the differences seen (Zeng et al. 2011).

Prior research by Gallagher et al. (2008) indicates that in addition to having higher pollutant availability, site 1416 also has the highest total nitrogen (N) soil percentage (0.57%). Soil N at sites 14, 43 (adjacent to 41) and 48 ranged from 0.13%-0.39%; this range is relatively low in comparison to an undisturbed New Jersey deciduous hardwood forest soil of the same successional age as LSP (0.47%) (Hagmann et al. 2015). While poplars may be able to take advantage of the excess N available at site 1416, the *B. populifolia* may not be able to exclude the pollutants or tolerate them in a way that allows them to respond similarly. However, *Betula pendula* demonstrated increased starch production when growing in N depleted soils and if this trend is applicable, the reason why starch levels are not elevated in site 14 where N is the lowest, may be because of stress
from soil pollutants (McDonald et al. 1986). Another difference between the species may be metal accumulation in tissues. *P. tremuloides* and *P. deltoides* are identified as heavy metal hyper-accumulators, primarily in root tissues, but differences in accumulation in the species across sites has not been assessed (Table 2)( Qian et al. 2012, Gallagher et al. 2008).

While soil metal pollution is generally an indication of low enzymatic and microbial activities (Kuperman and Carreir 1997, Diaz-Ravina and Baath 1996) of the sites studied, 1416 had unpredictably higher phosphatase, cellobiohydrolyase and L-leucine-amino-peptidase than the other sites (Hagmann et al. 2015). Microbial activities are known to have a direct correlation on flora growth and vice versa (Wardle et al. 2004). Thus, the increased microbial activity, aboveground biomass and starch storage in relation to increased metal loads are complementary characteristics unique to LSP.

The findings in this research help explain the ability of pioneer hardwoods to dominate an urban brownfield after 47 years of abandonment. The variety of biotic and abiotic effects from pollutants at LSP grant a whole ecosystem approach to understanding how starch budgets are altered when there are heavy metals leached into the soil for over 100 years. Although it was expected that starch budgets would correlate with the soil metal pollution levels at LSP, it was not expected that starch levels would have a positive correlation. It is important to note that the cause of the positive relationship between starch levels and metal pollutant is not identified in this study and necessitates further research.


Tables and Figures

Table 1: Dominant trees sampled for starch and DBH at different sites over entire year.

<table>
<thead>
<tr>
<th>Site</th>
<th>Species Available</th>
<th>n Per Species Per Site</th>
<th>n Entire Year (some trees used repeatedly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Betula populifolia</td>
<td>46</td>
<td>134</td>
</tr>
<tr>
<td>48</td>
<td>Betula populifolia</td>
<td>19</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Populus spp.</td>
<td>9</td>
<td>130</td>
</tr>
<tr>
<td>41</td>
<td>Populus spp.</td>
<td>58</td>
<td>136</td>
</tr>
<tr>
<td>1416</td>
<td>Betula populifolia</td>
<td>11</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>Populus spp.</td>
<td>17</td>
<td>138</td>
</tr>
</tbody>
</table>

Table 2: Statistical analysis results including all data.

<table>
<thead>
<tr>
<th>Term</th>
<th>FStat</th>
<th>DF1</th>
<th>DF2</th>
<th>pValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.594</td>
<td>1</td>
<td>841</td>
<td>0.11</td>
</tr>
<tr>
<td>Tree Part</td>
<td>4.006</td>
<td>2</td>
<td>841</td>
<td>0.02</td>
</tr>
<tr>
<td>Species</td>
<td>4.598</td>
<td>1</td>
<td>841</td>
<td>0.03</td>
</tr>
<tr>
<td>Soil Metal Concentration</td>
<td>2.566</td>
<td>1</td>
<td>841</td>
<td>0.11</td>
</tr>
<tr>
<td>Date</td>
<td>2.541</td>
<td>1</td>
<td>841</td>
<td>0.11</td>
</tr>
<tr>
<td>Species X Soil Metal Concentration</td>
<td>4.114</td>
<td>1</td>
<td>841</td>
<td>0.04</td>
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<tr>
<td>Tree Tissue X Date</td>
<td>3.138</td>
<td>2</td>
<td>841</td>
<td>0.04</td>
</tr>
</tbody>
</table>
**Table 3**: Average contaminant levels of six metals based on most recent findings of Hagmann et al. (2015) and Qian et al. (2012). Sites are listed from lowest overall contaminant levels to highest. Figures in bold are at levels above the Generic Soil Remediation Standards (NJDEP 2004).

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>As (µg/g)</th>
<th>Cr(µg/g)</th>
<th>Cu(µg/g)</th>
<th>Pb(µg/g)</th>
<th>V(µg/g)</th>
<th>Zn(µg/g)</th>
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</thead>
<tbody>
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<td>14</td>
<td>soil</td>
<td>15.7±1.3</td>
<td>18.9±1.5</td>
<td>52.3±4.3</td>
<td>243.5±17.4</td>
<td>15.5±0.0</td>
<td>69.7±6.7</td>
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<td></td>
<td>B. <em>populifolia</em></td>
<td>87.4±41.3</td>
<td>37.8±33.4</td>
<td>103±37</td>
<td></td>
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<td>49.9±12.6</td>
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<td>(leaf)</td>
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<td>soil</td>
<td>9.3±0.2</td>
<td>23.0±0.6</td>
<td>66.8±2.0</td>
<td>178.0±1.5</td>
<td><em>31.4±0.4</em></td>
<td>97.8±4.6</td>
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<td>43</td>
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<td><em>16.4±1.4</em></td>
<td>20.7±3.1</td>
<td>67.7±2.7</td>
<td>209.5±5.5</td>
<td><em>26.4±0.4</em></td>
<td>80.7±0.5</td>
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<tr>
<td>1416</td>
<td>soil</td>
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<td>96.4±0.4</td>
<td>76.4±0.4</td>
<td><em>414.7±0.4</em></td>
<td><em>30.1±0.4</em></td>
<td>140.7±0.4</td>
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<tr>
<td></td>
<td>B. <em>populifolia</em></td>
<td>42.8±9.0</td>
<td>209±59.4</td>
<td>129±11</td>
<td></td>
<td></td>
<td>157±51</td>
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<td>(leaf)</td>
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</tbody>
</table>
Figure Captions

Figure 1: Scale of metal toxicity as of 2005 (Gallagher et al. 2008) based on Total Metal Load (scaling that accounts for variations in metal contents and their availability per site at above NJ ambient soil metal concentrations (Saunders 2002)).

Figure 2: Entire year starch data for B. populifolia and Populus spp. including all sites (*P<0.03). Standard error indicated. Y-axis represents mg/g of dry weight of tree tissue.

Figure 3: Tissue starch content including data from entire year for species per site (*P<0.02). Standard error indicated. Y-axis represents mg/g of dry weight of tree tissue.

Figure 4 (Betula Populifolia) and 5 (Populus spp.): Tissue starch content annual trend by site for the two species. Indicated are standard error of the mean. Y-axis represents mg/g of dry weight of tree tissue.
Figure 1:
Figure 2:
Figure 3:
Figure 4:

Figure 5: