ASSESSING THE FUNCTIONAL SIGNIFICANCE OF WOOD NUTRIENT RESORPTION ALONG A SOIL FERTILITY GRADIENT

BY

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THESIS

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ABSTRACT

Soil nutrient availability can strongly influence tree community composition in forest ecosystems. Various life strategy trade-offs in species such as changes in relative growth rate and leaf tissue turnover rate have been shown to alter tree survival along gradients of soil fertility. In addition to these traits, trees are also able to employ nutrient resorption strategies that allow for nutrients to be translocated from older tissue into more functional juvenile tissues. While nutrient translocation in leaves is well understood, radial wood nutrient resorption has received far less investigation. To investigate this, I examined pre-leaf flush wood nutrients of *Quercus rubra*, *Quercus alba*, *Acer saccharum*, and *Acer rubrum* trees in 10 sites across a nitrogen (N) and calcium (Ca) fertility gradient in a North American temperate forest in Manistee, Michigan. At each site individual trees were fertilized with N, or a combination of all macronutrients ("All") for >10 years, along with unfertilized ("Control") trees. I analyzed N, C, Ca, Mg, and K concentrations in these species and compared how sapwood, heartwood, and differences in sapwood and heartwood nutrient concentrations varied according to soil fertility and fertilization treatment for each species. Additionally, I re-sampled *Acer* species post-leaf flush and compared differences in wood nutrients pre- and post-leaf flush to investigate whether stem-wood can serve as a seasonal store of plant nutrients. I also investigated if the fraction of wood tissue comprised of ray parenchyma is related to wood nutrient re-translocation as ray parenchyma are hypothesized to be the main vector of radial wood nutrient resorption.

I predicted that sapwood and heartwood nutrient concentrations would be highest in fertilized trees and trees found at high fertility sites. Additionally, I expected that trees grown at low fertility levels and without nutrient addition would have larger differences in sapwood and heartwood nutrients, indicating greater nutrient resorption. For *Acer* species I predicted that sapwood nutrients, and differences in sapwood and heartwood nutrients, would be lower post-leaf flush indicating nutrient draw from sapwood in support of leaf flush. I did not find any evidence for differential translocation of wood nutrients in response to soil nutrient availability, but found consistent support that sapwood nutrients serve as a seasonal repository for N. Additionally, I observed no consistent relationship between investment in ray parenchyma and wood nutrients with ray parenchyma found to only be a significant predictor of sapwood K. These results highlight the dynamic nature of wood nutrient stores and indicate that wood nutrient translocation is a complex phenomenon that is influenced by factors beyond soil nutrient availability.

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INTRODUCTION

Soil nutrients are a major driver of species composition in forest systems across the world (John et al. 2007, Kobe et al. 2014). When combined with climate and biotic interactions, shifts in soil nutrient availability can often be accompanied with notable shifts in tree species composition (Chapin and Kedrowski 1983, Maxwell et al. 2018, Umaña et al. 2021). Due to this natural variation in nutrient limitation, plant strategies can vary in terms of nutrient uptake, use efficiency, and allocation in response to limiting resources (Lambers et al. 2008a, Lambers et al. 2008b). Species adapted to low fertility soils may invest resources to root systems and long lived tissues, while those adapted to high soil nutrients may invest more in growth rate and high rates of tissue turnover (Chapin 1987). One additional component of these strategies is the storage of essential elements within various plant tissues, such as roots, bark, or sapwood, for use during periods of low resource availability, for reproduction, or injury response (Chapin 1987, Bazzaz et al. 2000, Yuan et al. 2006, Yuan and Chen 2015, Heineman et al. 2016, Muhammad et al. 2020). Nutrients stored for later use are often reserved in mobile forms that can be more readily shifted between tissues (Millard 1988, Muhammad et al. 2020). This movement or, translocation, permits plants to re-allocate nutrients away from senescing tissues into younger more functional ones, or to maintain resources for use when growth conditions improve (Millard and Neilsen 1989, Millard and Grelet 2010, Muhammad et al. 2020).

For nitrogen and phosphorus, resource resorption from leaf tissue has consistently been observed to be a critical strategy for nutrient recycling. Additionally, there has also been mixed evidence that, at the leaf level, this process can be altered by external factors such as fertilization (Brant and Chen 2015, Yuan and Chen 2015). However, resorption strategies are not restricted solely to leaf tissue; trees have been found to be capable of nutrient translocation across all plant organs that regularly go through senescence (Cherbuy et al. 2001, Kotowska et al. 2020). While much work has been done on leaf canopies, relatively little work has been conducted on woody tissue translocation despite the role these tissues play as large and long-term storage pools of both plant nutrients and non-structural carbohydrates (NSCs) (Meerts 2002, Brant and Chen 2015, Plavcová and Jansen 2015, Morris et al. 2016, Kotowska et al. 2020). Furthermore, this pool of wood nutrients has previously been viewed as relatively immobile with the implication being that woody tissues primarily function as structural support and the nutrients within them

are simply bound in structures within wood tissues (Eckstein et al. 1999, Meerts 2002, Kotowska et al. 2020).

Previous studies, however, have shown that wood nutrients contribute more significantly to plant function than simply facilitating the vertical transport of nutrients between a tree's canopy and its roots (Attiwill 1980, Andrews et al. 1999, Taylor et al. 2002, Millard and Grelet 2010, Kotowska et al. 2020, Muhammad et al. 2020). Several analysis of wood nutrients in silvicultural stands have demonstrated that wood nutrient translocation does occur with shifts in translocation occurring across various stand ages (Colin-Belgrand et al. 1996, Laclau et al. 2001). Additionally, studies comparing sapwood and heartwood differences within trees in natural settings have also demonstrated that there are marked differences in nutrient concentrations between the two woody organs, providing evidence that radial wood nutrient translocation regularly occurs in nature (Andrews et al. 1999, Meerts 2002, Kotowska et al. 2020). Furthermore, large variation in these differences among measured elements indicate that plant-specific biological processes may be tied to certain nutrients or are driven by environmental variables such as soil fertility (Meerts 2002).

Specific wood anatomical traits have also been hypothesized to facilitate radial wood nutrient resorption. Metabolically active cells in tree xylem oriented radially across tree stems, known as ray parenchyma, have been shown to be directly tied to a plethora of critical plant functions ranging from NSC transport to secondary metabolite storage for plant pathogen resistance (Plavcová and Jansen 2015, Morris et al. 2016, Słupianek et al. 2021). Due to the wide array of functions, radial orientation, and symplastic connectivity of ray parenchyma in tree stems (Meerts 2002, Morris et al. 2016), it has also been postulated that these parenchyma cells may also determine a tree's ability to translocate nutrients from developing heartwood into sapwood as a way to ensure that functional woody tissue retains nutrient pools that can be drawn upon (Frey-Wyssling and Bosshard 1959, Wardell and Hart 1973, Smith and Shortle 1996, Andrews et al. 1999, Meerts 2002).

Despite this, there has been little inquiry into the mechanisms of this nutrient translocation process. A few studies have investigated whether higher nutrient concentrations in sapwood compared to heartwood is an explicit plant controlled phenomenon to recycle wood nutrients under varying environmental scenarios or simply a consequence of the plant lignification process for plant sapwood regulation (Andrews et al. 1999, Meerts 2002, Kotowska

et al. 2020). However, many previous direct studies of translocation have primarily been confined to destructive sampling across silvicultural stands of a single species (Attiwill 1980, Colin-Belgrand et al. 1996, Laclau et al. 2001, Meerts 2002, Sette et al. 2013).

While much of the previously conducted work in natural forest settings has assessed wood nutrient translocation using natural gradients of soil fertility to compare differences of sapwood and heartwood concentrations, these analyses often do not account for times of high and low nutrient demand and so, are unable to provide evidence for the functional significance of the sapwood/heartwood difference presented. Thus, investigating seasonal differences in wood nutrient concentration (i.e. pre- and post leaf flush) in deciduous tree species provides a valuable framework for assessing wood nutrient translocation.

I used a natural soil fertility gradient, coupled with a tree fertilization experiment to investigate how soil nutrient availability influences the magnitude of sapwood and heartwood nutrient concentrations, differences in the concentration of sapwood and heartwood, and whether seasonal fluxes in wood nutrient concentrations occur in response to leaf flush. I collected tree cores from four tree species (*Quercus rubra*, *Quercus alba*, *Acer rubrum*, and *Acer saccharum*) pre-leaf flush (April 2021) in 10 sites spanning a natural gradient of nitrogen (N) and calcium (Ca) availability in temperate deciduous forest in Manistee Michigan, USA. In each site, individual trees were fertilized with N, or a combination of all macro and micronutrients (All) for >10 years, or were unfertilized (Control) trees. Post-leaf flush cores were also collected in June 2021 for *Acer* species to assess seasonal wood nutrient fluxes.

April wood nutrient data were used to address two hypotheses: (H1) If sapwood nutrients are important reserves used for seasonal leaf flush, then sapwood nutrients (N, C, K, Mg, Ca) will be higher for trees in higher fertility soils, and in fertilized versus unfertilized trees. Additionally, the same trend will occur for heartwood if lower amounts of translocation out of heartwood is necessary for trees growing under increased nutrient availability. (H2) If nutrient resorption from developing heartwood is energetically costly, but advantageous for leaf flush under low nutrient availability, then differences in sapwood and heartwood (S-H) nutrient concentrations will increase with decreasing soil fertility and will be higher in control than fertilized trees as a consequence of increased nutrient translocation from heartwood.

Wood nutrient data collected in April and June for *Acer* species were also used to address two additional hypotheses. (H3) If sapwood serves as a mobilizable store of nutrients allocated to

support seasonal leaf flush then there will be declines in both sapwood nutrients and S-H differences with no detectable decreases in heartwood nutrients following leaf flush. (H4) If trees are able to adjust allocation to wood depending on nutrient availability, then there will be smaller declines in sapwood nutrients for fertilized than control trees and no treatment effects on heartwood nutrient differences between April and June.

In addition to wood nutrient fluxes, I explored whether the fraction of wood tissue comprising of ray parenchyma is predictive of wood nutrient concentrations and S-H differences. In a subset of cores collected in my April sampling, I stained and imaged radial sections of wood to test whether the ray parenchyma fraction of sapwood is related to sapwood nutrients and S–H differences for species grown on soils with different nutrient availabilities. (H5) If ray parenchyma primarily serves as stores for wood nutrients to be drawn upon under periods of high nutrient demand, I expected for ray parenchyma to strongly correlate with wood nutrient measurements. Conversely, I expected that, (H6) if ray parenchyma plays a more critical role in nutrient resorption than nutrient storage then species grown at low fertility soils and unfertilized trees would have a higher fraction of wood allocated to ray parenchyma tissue.

METHODOLOGY

STUDY SITE

Sampling was conducted in the spring of 2021 within the Huron-Manistee National Forest, Manistee, Michigan, USA (44° N 85°W). This site is managed by the U.S. Forest Service and contains 12 forest sites spanning a natural N and Ca fertility gradient ranging from 4.03-31.3 mg kg^{-1} for N and 70–1160 mg kg^{-1} for Ca (Kobe et al. 2014). The forest is characterized as a northern temperate forest and is estimated to be between 80 and 100 years old and receives 700 mm of rainfall a year. This forest contains ~ 17 tree species > 10 cm DBH, with most individuals in the genera *Acer* and *Quercus* (Baribault and Kobe 2011, Kobe et al. 2014). Additionally, each site has been part of a long-term fertilization experiment (>10 years) in which several species have received nutrient additions directed to single trees. Single tree fertilization has been annually applied at each site with treatments consisting of N only (1900 g of $(NH_4)_2SO_4$); Ca only (3000 g of $CaSO_4$); N + Ca (1900 g of $(NH_4)_2SO_4$ and 3000 g of $CaSO_4$); and All nutrients (2790 g of $CaSO_4$, 3325 g of NPK, and 700 g of "MicroMAX" micronutrient fertilizer). Trees selected for this experiment were either in co-dominant or dominant canopy classes (Kobe 2022 unpublished).

Within Manistee, four species were sampled for plant material both pre-leaf flush (April) and post-leaf flush (June): *Quercus rubra*, *Quercus alba*, *Acer saccharum*, and *Acer rubrum* across 10 sites spanning the N and Ca fertility gradient (Table 1). While all four species were sampled in April, *Quercus* species were excluded from the June sampling due to the increased risk of transmitting oak wilt disease during the spring. Additionally sites were grouped as Low, Intermediate, or High fertility based on the Ecological Land Type Phase index that was specifically created for the Huron-Manistee National forest with increasing values of this index denoting both increased N and Ca soil nutrients (USFS 1993, Burger and Kotar 2003, Kobe et al. 2014).

PLANT MATERIAL COLLECTION

Wood core collection occurred pre-leaf flush in April (8^{th} – 16^{th}) and post-leaf flush in June (7^{th} – 9^{th}). At each tree, one 9 – 17 cm long tree core was collected for wood nutrient analysis using a 4.3 mm diameter increment borer (Haglöf, Långsele, Sweden). At each site, a

minimum of three individuals from each treatment were sampled for each species when present, however, not every species was fully represented across treatments at each site. To mitigate this, extra control trees were selected within or adjacent to their respective sites where some species were partially present across treatments.

Wood cores were stored in labelled plastic straws and kept in a freezer at -20 ℃ within 1-2 weeks of initial collection and until further processing. Because not all cores had visually distinct sapwood and heartwood boundaries, wood was partitioned according to distance from the cambium: The 1st cm of each core (excluding bark and cambium) was considered "sapwood" whereas the 10th cm of each core was considered "heartwood". For cores that were slightly less than 10 cm long, the last cm of the core was used for the "heartwood" sample. All sapwood sections were also examined for remnants of cambium tissues and any excess cambial tissues were removed with a razor blade prior to nutrient analysis. For my April sampling, a third 1 cm section was taken from the 2nd cm of the tree cores of 9 individuals from each species for wood anatomical measurements. For these same trees, a separate 6 cm long core was also collected in the field and stored in ethanol for use in the event that the first core did not yield quality images for parenchyma analysis. When usable sections were not able to be obtained from either of the 2 cores collected for wood anatomical analysis, a separate individual of the same soil fertility was chosen for analysis. (Table 1). All tree cores were taken below breast height at approximately 0.65 m above ground level but above tree buttresses.

NUTRIENT ANALYSIS

The 1 cm sections extracted from cores that were collected in both April and June sampling (hereafter referred to as A and J samples) were ground and dried at 65°C for 72 hours prior to nutrient analysis. For N and C, 10 mg of wood material was analyzed using a Costech 4010 Elemental Analyzer (Costech Analytical, Valencia, CA). For Ca, Mg, and K, 15 mg of wood was combusted in a muffle furnace at 450°C for three hours and then dissolved in a 1 M HNO_3 solution and analyzed through inductively coupled plasma optical emission spectrometry (ICP-OES, Avio, 200, Perkin Elmer, Waltham, Massachusetts, USA).

WOOD ANATOMY

To estimate the fraction of wood tissue consisting of ray parenchyma, nine individuals (three individuals of each treatment) were selected from each species with sites chosen such that, where possible, a species was present at all three fertilization levels (All, N, and Control). When this was not possible, sections were taken from other sites under the same soil fertility level as the chosen site (Table 1). For each individual, 1-5 μm thick tangential sections of wood were obtained using a GSL1-microtome (Gärtner et al. 2014). Prior to sectioning, each freezer stored sample was boiled for at least 30 minutes in a 1 or 10% glycerin solution to facilitate sectioning whereas ethanol stored cores were not boiled. Following Kotowska et al. (2020), tangential sections were stained with both Safranin and Astra Blue dyes to provide contrast for analysis of ray parenchyma. Sections were stained in a 1% Safranin 50% ethanol solution for 2 minutes, washed with distilled water, and subsequently dyed in a 1% Astra blue 50% ethanol solution for 15-30 minutes. After staining, sections were systematically dehydrated in 50%, 75%, and 99% ethanol solutions for 2 minutes at each step and mounted in a Euparal medium. Prior to imaging, sections were dried at either 65 °C for 72 hours or at room temperature for at least one week.

Image acquisition was conducted using a NanoZoomer (Hamamatsu Photonics, Hamamatsu, Japan) at 40x magnification. Images were exported from NDPViewer software at 2.5x magnification at 400 – 600 DPI. Acquired images were then imported into ImageJ where the highest quality portions of the image was cropped and used for analysis. Where image acquisition of a sample was difficult to obtain due to thickness or evenness of a microtome section, a z-stack was generated for the sample and a single 2-D image was created by combining the stacks in ImageJ. Final images were then overlayed with a grid with ~ 84 μm spacing and, following other wood anatomical protocols (Ziemińska et al. 2015, Kotowska et al. 2020), a minimum of 300 grid points was overlayed in each measurement and final image size ranged from 5.3 – 14 mm^2 across all species. From these images, ray parenchyma fraction was measured as the number of points that fell on ray parenchyma tissue divided by total points overlaid on the image (Figure 2). Ray parenchyma were identified according to features specified under the "IAWA list of microscopic features for hardwood identification" guidelines and referenced against the Inside Wood data base (IAWA 1989, Wheeler 2011), and both uniseriate and multiseriate rays were included in parenchyma fraction measurements. Generally, ray parenchyma could be identified as cell tissue symplastically connected across wood sections

(Figure 2). Due to slight deformation of wood section vessels and fibers that occurred throughout the sectioning process, accurate differentiation between these wood elements and axial parenchyma was inconsistent. Thus, analysis of parenchyma fraction was restricted to ray parenchyma to avoid inaccurate estimates.

In addition to quantifying ray parenchyma fraction, Wood Specific Gravity (WSG) was also collected for each core included in wood anatomical analysis. WSG was measured via the water displacement method specified by Wheeler (2011) using 4 cm of remaining wood tissue from each sample.

STATISTICAL ANALYSIS

All statistical analyses were carried out in R version 4.02 (RStudio Team 2015) using the "lme4", "lmerTest", "effsize", and "emmeans" statistical packages. Prior to analysis, extreme outliers were removed. This removal was only conducted for K, with nine measurements removed for *A. rubrum* and one removed for *Q. rubra* that appeared out of the biological range of K concentrations for that respective cut and/or species. Sapwood nutrients, heartwood nutrients, and S-H differences from the April sampling were pooled across all species and treatments to observe how wood nutrients varied across species. Here wood nutrients were related to "Species" and "Treatment" (All, N, Control) as fixed effects along with their interaction term with "Fertility" (low, medium, high), and "Site" (sites where sampling occurred across the fertility gradient) nested as random factors. When no significant effect of the interaction term was detected, it was not included for the final model. Additionally, comparisons among species wood nutrient concentrations were assessed using a Tukey post-hoc test.

Nutrient availability effects on wood nutrients

April S-H differences for each element were compared through mixed effect models on a per-species basis. To compare differences across treatments each elemental measurement for sapwood was subtracted from its corresponding heartwood section. This difference was then used as a response variable where "Treatment" was the fixed effect and "Fertility" and "Site" were set as nested random factors except for *A. saccharum* which was only present at one fertility level and thus, only had "Site" as a random factor (Table 1). From this model, comparisons of treatment nutrient concentrations were conducted via a post-hoc Tukey analysis.

To assess the effect of soil fertility directly, an additional mixed effect model was also generated for unfertilized (control) trees from each species that spanned two fertility levels (*Q. alba*, *Q. rubra*, and *A. rubrum*) with "Site" as a random factor. Additionally, individuals from all treatments were also combined to assess the effect of fertility in a separate mixed model. Both treatment and fertility effect analysis were also carried out for April sapwood and heartwood samples independently to observe any effects that acted solely on inner or outer wood samples.

Seasonal nutrient flux in wood

To test if overall percent S-H differences, sapwood, or heartwood nutrient concentration were higher pre-leaf flush (April) than post-leaf flush (June), I conducted separate paired t-tests for samples collected in both time periods. This analysis was conducted on both *Acer* species independently for each analyte with individuals from all treatments pooled.

To assess treatment effects on April and June wood sample comparisons, a separate mixed effect model was created where paired measurements of sapwood and heartwood samples between sampling periods were subtracted (April – June) and the difference of these measurements were then modelled against the fixed effect "Treatment" along with the random effect of "Site". From this model, Cohen's d effect sizes were calculated for differences among treatments and p-values for comparisons of means were obtained via a post-hoc Tukey analysis. Including fertility as a fixed factor had no additional effect on model outputs for *A. rubrum* despite this species being located at two different fertility levels and was left out of final analysis for effects of treatment on seasonal differences in wood nutrients.

Wood ray parenchyma fraction

Differences in ray parenchyma fraction among species were assessed using a one-way ANOVA analysis with species as a fixed effect. To assess the effects of fertilization treatments on ray parenchyma, a mixed effect model was conducted where ray parenchyma fraction was a function of fertilization treatment and Wood Specific Gravity (WSG) with species as a random effect. Differences in means were calculated using Tukey post-hoc analysis for both the one-way ANOVA and mixed effect model. For analysis of ray parenchyma effects on sapwood nutrients and S-H differences, a separate mixed effect model was used where ray parenchyma, treatment,

and WSG were coded as fixed effects. This model was run independently for each analyte and separately for sapwood nutrients and S-H differences.

RESULTS

There was a significant effect of species on wood nutrients in mixed effect model analysis of sapwood, heartwood, and S-H differences for all analytes except for C. However, post-hoc analysis revealed that differences in mean wood nutrient concentrations among species were inconsistent across analytes with no obvious trends in differences among species sapwood, heartwood, or S-H differences. Furthermore, sapwood nutrient concentrations were generally higher than those of heartwood, although there were exceptions, notably for Ca and Mg in *Acer* species (Figure 3, Table 2). For these two analytes, mean S-H differences were significantly smaller in *Acer* species than for *Quercus* species when comparing species individually (Tukey test; $p < 0.001$).

Fertilization effects on wood nutrient concentrations

I found only very limited support for the prediction of significantly higher sapwood nutrient concentrations in fertilization treatments. For April sapwood, there were significant effects of fertilization treatments on N and Mg for *A. rubrum* ($F = 3.85$ and 6.42, $df = 26.06$ and 24.97, $p < 0.05$) and for Mg in *Q. rubra* ($F = 7.13$, $df = 41.69$, $p < 0.01$, Table 3). However, for *A. rubrum*, differences among individual treatments were not significantly different for N. For Mg, the All treatment was significantly higher than both N and control treatments (Tukey test; $p < 0.01$, and $p < 0.05$ respectively). For *Q. rubra*, sapwood in the N treatment had significantly higher Mg concentrations than control trees (Tukey test; $p < 0.01$) while sapwood nutrient concentrations in the All treatment were marginally higher than control trees (Tukey test; $p = 0.05$). For heartwood nutrient concentrations, there was a significant effect of treatment in *Q. alba* ($F = 6.44$, $df = 36.25$, $p < 0.01$, Table 3) where individuals in the All treatment had significantly higher Mg than individuals in the N treatment (Tukey test; $p < 0.01$).

For S-H, I predicted differences would be greater in control trees compared to fertilized trees however, there was only a significant effect of treatment in *Q. rubra* for Mg ($F = 7.57$, $df = 41.68$, $p < 0.01$, Table 3). Trees in the control treatment had significantly smaller S-H differences than trees in the All or N treatments (Tukey test; $p < 0.05$, and 0.01 respectively, Figure 4).

Soil fertility effects on wood nutrient concentrations

I found little support for my prediction that trees found at higher soil fertility levels have higher sapwood nutrient concentrations. In April, considering only the control trees, there was a significant fertility effect on sapwood Mg in *Q. alba* ($F = 6.56$, $df = 14$, $p < 0.05$, Table 4) however sapwood nutrient concentrations were higher in low fertility sites than in intermediate fertility sites. When considering all fertilization treatments combined, there was only a significant effect for N in *Q. rubra* ($F = 8.60$, $df = 48$, $p < 0.01$, Table 4) where individuals from intermediate fertility sites had significantly lower sapwood N concentrations than for those in the high fertility sites.

For heartwood, there was a marginally significant effect of Mg in *Q. alba* ($F = 4.61$, $df = 14$, $p = 0.049$, Table 5) when considering only control trees, with higher Mg concentrations in low than intermediate fertility soils. This result remained significant after combining trees from the three fertilization treatments ($F = 5.33$, $df = 38$, $p < 05$, Table 4).

I found no evidence of larger S-H differences in trees on lower fertility sites compared to higher fertility sites. There were no significant effects for any species or analytes when considering only unfertilized trees. When individuals from all fertilization treatments were combined, there was only a significant effect of fertility for N in *Q. rubra* ($F = 7.98$, $df = 48$, $p < 0.01$, Table 4) with trees in the high fertility sites having larger S-H differences than trees grown in intermediate fertility sites (Figure 5).

Seasonal nutrient flux in wood

I predicted that for *Acer* species, sapwood nutrient concentrations, as well as, differences in nutrient concentrations between sapwood and heartwood would be greater in April than June, reflecting a seasonal flux of nutrients out of sapwood associated with leaf flush. When comparing sapwood nutrients on a per-species basis, there was mixed support for this prediction. For *A. rubrum* sapwood there was a significant decrease in N (7.8%) ($t = 4.66$, $df = 28$, $p < 0.01$) but a significant increase in K (21.9%) between leaf flush periods ($t = -4.89$, $df = 27$ $p < 0.01$, Figure 6, Table 5). For *A. saccharum*, there was a significant decrease in both N (9.8%) ($t = 6.28$, $df = 29$, $p < 0.01$) and K (29.4 %) for sapwood between sampling periods ($t = 2.1$, $df = 29$, $p < 0.05$, Figure 6, Table 5). For heartwood samples, there was a significant seasonal increase in N (5.9%) and K (32.7%) concentrations for *A. rubrum* ($t = -2.79$ and -3.27, $df = 30$ and 27, $p <$

0.01) and a significant decrease in N (4.9%) for *A. saccharum* ($t = 2.36$, $df = 29$, $p < 0.05$, Figure 7, Table 5).

For differences between sapwood and heartwood (S-H), there was a significantly greater difference pre-leaf flush than post-leaf flush for N in *A. rubrum* ($t = 10.36$, $df = 28$, $p < 0.01$), with the opposite for K ($t = -3.09$, $df = 24$, $p < 0.01$). No significant differences were detected for any other analyte. For *A. saccharum*, S-H differences were significantly higher for Ca ($t = 2.49$, $df = 28$, $p < 0.05$) and N ($t = 6.48$, $df = 28$, $p < 0.01$) pre-leaf flush than post leaf flush while C differences were significantly greater post-leaf flush ($t = -2.76$, $df = 28$, $p < 0.05$, Figure 8, Table 5). No other analytes showed any significant changes between April and June.

Fertilization effects on seasonal differences of wood nutrients

I found no strong support for treatment effects on seasonal wood nutrient fluxes. For sapwood there was no significant differences among treatments for *A. saccharum* (Figure 9). *A. rubrum* only showed a significant effect of treatment for N and K ($F = 4.20$ and 4.86, $df = 26$ and 25, $p < 0.05$). For N the All treatment had significantly smaller A-J sapwood differences than the N treatment (Tukey test; $p < 0.05$), while for K, the All treatment had significantly larger A-J differences than the N treatment (Tukey test; $p < 0.05$, Figure 9). For heartwood, there were no significant treatment effects on heartwood nutrient differences between April and June, with the exception of Mg in *A. saccharum* ($F = 9.33$, $df = 27$, $p < 0.01$), where A-J differences were greater for trees in the N treatment compared to control and All trees (Tukey test; $p < 0.01$, Figure 10).

Wood ray parenchyma fraction

Ray parenchyma fraction varied significantly among species ($F = 9.14$, $df = 3$, $p < 0.01$) with low coefficients of variation found within all species (*A. saccharum* = 0.47, *A. rubrum* = 0.37, *Q. rubra* = 0.28, *Q. alba* = 0.17). *Quercus alba* had significantly more ray parenchyma than any other species (Tukey test; $p < 0.05$) while ray parenchyma fraction in *A. saccharum* did not differ from *A. rubrum* or *Q. rubra* (Figure 11, Table 6). There were no significant effects of fertilization treatment on ray parenchyma. Lastly, I found little support for the expectation that ray parenchyma was a significant predictor for sapwood nutrients or S-H differences with the only significant result occurring for K sapwood concentrations where wood nutrients were

positively associated with ray parenchyma fraction ($F = 7.5$, $df = 31$, $p = 0.01$), however, the effect of treatment and WSG were not significant.

DISCUSSION

Nutrient availability effects on wood nutrients

From my analysis of fertilization treatments and soil fertility effects, I found only very limited support that sapwood nutrient concentrations are sensitive to nutrient availability at the scale of variation present in the Huron-Manistee National Forest. Both *A. rubrum* and *Q. rubra* species had higher sapwood Mg concentrations in either N or All fertilizer treatments. However, fertilization effects were not consistent with changes in nutrient concentrations across sites that differed in soil fertility. *Quercus rubra* was the only species for which sapwood nutrient concentrations responded positively to soil fertility, with the effect limited to N.

Previous work has provided support that wood nutrients respond to increased nutrient availability. In a study of fertilization effects on wood nutrients in a silvicultural plantation of *Liriodendron tulipifera*, individuals had significantly higher stem-wood P and K after an initial liming application (McClenahen et al. 1989). Additionally, Heineman et al. (2016) showed that nutrients in the outer 5 cm of wood increase with soil nutrients within a tropical forest when observing community weighted means of trees at the forest plot scale, however, while this relationship was present for P, Ca, and Mg there was no significant relationship for N. As my study site is located in a temperate forest as opposed to a tropical forest, I would expect my results might differ from those of Heineman et al. (2016) due to relatively low bioavailable N in temperate forests (Rennenberg and Dannenmann 2015). The lack of consistent effects across species, fertilization treatments, and soil fertility levels may indicate either that species are unable to accumulate additional available nutrients in wood, or that uptake capacity is contingent on a combination of limiting resources.

Previous research within Huron-Manistee forest has found evidence for soil resources limiting productivity of leaf and wood tissue. Baribault et al. (2010) measured plant available soil nutrients in 13 sites within Huron-Manistee National Forest and related them to leaf and wood above ground productivity separately. Soil N was positively associated with leaf production, while Ca and Mg were both related to wood production especially for trees found in low fertility sites. Thus, as N is not the sole limiting nutrient within this system, uptake of nutrients across fertilization treatments and fertility sites may be influenced by among-nutrient interactions that may limit nutrient uptake and storage within sapwood (Heineman et al. 2016).

Analysis of heartwood nutrient concentrations also revealed results that conflicted with my hypothesis. In *Q. alba*, heartwood Mg concentrations were found to be highest in the All nutrient treatment yet were also higher in individuals grown under low soil fertility when compared to intermediate fertility individuals. Few studies have explicitly analyzed heartwood nutrient variation across soil nutrient gradients. However, one study that analyzed how wood nutrients in the conifer *Chamaecyparis thyoides* varied with soil fertility found that while sapwood concentrations of these analytes did not differ between two sites, heartwood Ca, Mg, and K nutrients were lower at the low fertility site, indicating greater wood nutrient translocation (Andrews et al. 1999). While my study failed to find support that heartwood nutrient concentrations are lower in trees on lower fertility sites, this discrepancy may be due to the fact that my study did not account for variability in heartwood nutrient concentrations according to distance from pith, as was the case in (Andrews et al. 1999). As wood nutrients have been observed to increase or decrease from pith to sapwood with occasional spikes in nutrient concentration within the sapwood/heartwood boundary (Okada et al. 1993, Taylor et al. 2002), not accounting for this variability may mask any true differences in heartwood nutrient concentrations among species sampled in my analysis.

Consistent with the treatment effects on sapwood and heartwood nutrient concentrations I found no evidence that additional nutrient translocation can provide nutrients for seasonal leaf flush for plants grown under low soil nutrient availability. I expected S-H differences to be higher in control trees than in fertilized trees and for differences to be higher in individuals at lower fertility levels, but found no support for either prediction. For a majority of the analytes assessed, there were no significant effects of fertilization treatment or soil fertility on S-H differences.

For the one analyte that was significantly different between fertilization treatments (Mg in *Q. rubra*), I found that these differences were contrary to my original hypothesis with lower Mg S-H differences in control trees than fertilized trees (Figure 10). Furthermore, when analyzing differing fertility levels the only significant finding (N for *Q. rubra*), indicated that trees growing at higher fertility levels had higher S-H differences. These results show that, while nutrient availability may increase sapwood nutrient concentrations, individuals do not appear to translocate more nutrients out of developing heartwood when growing under lower soil nutrient conditions.

Limited fertilization effects on sapwood nutrients and S-H differences in my study, may also reflect the experiment timescale. While trees have been fertilized for ~13 years the heartwood sampled in much of this study was deposited prior to the establishment of experimental treatments. The average increment in DBH for all trees in the sites sampled (All, N, and CTR) since the experiment began was less than 6 cm for all species (*A. rubrum* = 4.2 cm, *A. saccharum* = 3.5 cm, *Q. rubra* = 5.6 cm, *Q. alba* = 3 cm, Kobe 2022 unpublished). As heartwood sections were obtained from the 10th cm of each core to control for differences in sapwood width within and among species, much, if not all, of the heartwood sampled in this study was developed before fertilization began. Previous work has also shown that tree growth response to fertilization can vary with age, with responsiveness to fertilization higher in young Douglas-fir stands (20 years) compared to older stands (40 and 60 years) (Miller 1988).

Additionally, wood nutrient translocation can vary with stand age. Colin-Belgrand et al. (1996) found that N, P, and K translocation increased with stand age but decreased for Ca. Furthermore, Laclau et al. (2001) observed increased accumulation of N, P, Ca, and Mg in the inner rings of a Eucalyptus hybrid, indicating negative translocation, near the end of a stand's rotation, however this was speculated to be related to changes in soil nutrient availability. As this analysis was carried out in mature forest stands, it is possible that nutrient draw from senescing sapwood may be a critical resorption strategy for trees, but that this reliance may be less important than in juvenile trees. For example, wood nutrient translocation might be expected to be more prominent in younger individuals with shallower root systems under periods of high nutrient demand. Conversely, differences in plant reproduction strategies may also strongly influence nutrient draw from wood. For some species in temperate forests, mass reproduction can require large irregular and synchronous nutrient inputs into reproductive tissues (Cleavitt and Fahey 2017, Fernández-Martínez et al. 2019). In a recent study analyzing seed masting intensity of 219 species in relation to foliar N and P concentrations, Fernández-Martínez et al. (2019) found that species with low foliar N and P were more likely to have higher intensity masting events. Thus, wood nutrient translocation and storage might also vary in accordance with non-regular reproductive events, such as masting.

Nonetheless, the fact that the only significant effect of fertility showed high fertility trees having significantly higher S-H differences than individuals grown at intermediate fertility for *Q. rubra* provides support against my original hypothesis as I would expect for higher differences in

trees grown at lower fertility levels as a result of increased translocation out of heartwood into sapwood.

Another important point to consider is that for both *Acer* species, I saw no clear transition of sapwood to heartwood in the majority of tree cores sampled. Previous work visually assessing sapwood thickness of *A. saccharum* and *Q. rubra* across differing silviculture management strategies found that *A. saccharum* maintained sapwood thickness greater than 10 cm across all management treatments whereas average *Q. rubra* sapwood thickness was less than 3 cm across all treatments (Wiemann 2004). Thus, it is possible that for *Acer* species with large volumes of sapwood, small changes in nutrient pool sizes could potentially result in large fluxes of nutrients. Additionally, this large proportion of sapwood may indicate that *Acer* species have a far greater pool of nutrients to draw from than *Quercus* species included in this study. So, while some effects of nutrient availability on wood nutrient concentrations were observed in *Acer* species in my study, large sapwood volume may have made fertility and nutrient addition effects more difficult to detect.

Seasonal nutrient flux in wood

For my analyses of seasonal wood nutrient fluxes, my sampling was confined to *Acer* species due to increased susceptibility of *Quercus* species to pathogen infection post-leaf flush. I predicted significant seasonal decreases in all nutrients for sapwood and S-H differences in both *Acer* species. I found consistent support for this hypothesis in sapwood N for both *Acer* species with average *A. saccharum* N concentrations decreasing by 9.8% and *A. rubrum* N decreasing by 7.8%. For the only other analyte that was significant, K, concentrations decreased for *A. saccharum* but increased for *A. rubrum*.

Consistent with my results, previous studies assessing seasonal wood nutrient fluxes have found that woody stems can serve as N reserves for seasonal leaf flush, with seasonal draw from wood possibly varying according to nutrient uptake strategies and nutrient status (Chapin and Kedrowski 1983, Silla and Escudero 2003). However, these studies assessed seasonal nutrient fluxes in far younger trees. One study that did assess wood nutrient variability in mature *Pinus sylvestris* trees conversely found no significant changes in seasonal stem-wood nutrient fluxes (Helmisaari and Siltala 1989), indicating that stem-wood nutrient fluxes are not universal across species. Nonetheless, my findings suggest that even mature trees found in relatively fertile sites

experience seasonal wood nutrient draw of N indicating that seasonal draws may be tied more directly to species specific nutrient use strategies and not necessarily tree nutrient status. These results are also consistent with the paradigm that northern temperate hardwood forests growing on low fertility soils are primarily N limited (Mitchell and Chandler 1939, Magill et al. 2000, Finzi et al. 2007, Rennenberg and Dannenmann 2015) and may reflect the critical role that N has been shown to serve in leaf production within my study sites (Baribault et al. 2010).

For heartwood analysis I found mixed support for decreased heartwood nutrient concentrations between measurement periods with N decreasing between measurement periods for *A. saccharum* but increasing for *A. rubrum*, with the only other significant change being an increase in K for *A. rubrum*. These unexpected fluxes in inner wood samples further indicate that true heartwood was not sampled for the majority of *Acer* species. However, lower fluxes within these samples provide evidence for reduced capacity for translocation indicative of gradually lower parenchyma metabolic activity as distance from the cambium increases (Shain and Mackay 1973, Taylor et al. 2002).

Considering that seasonal effects were primarily observed for only N and K, these changes may be more pronounced compared to Ca and Mg due to their potential increased mobility. Nitrogen and K have been shown to accumulate in ray parenchyma symplasts (Merrill and Cowling 1966, Wardell and Hart 1973, Saka and Mimori 1994) whereas Mg and Ca are thought to be less mobile due to the incorporation of a large fraction of these nutrient pools in cell wall structures and the tree lignin matrix (Cutter and Guyette 1993, McLaughlin and Wimmer 1999, Meerts 2002, Marschner 2011)

As for the differences in the magnitude of fluxes between *Acer* species this may reflect divergence in nutrient use strategies. While *A. saccharum* and *A. rubrum* can be found at similar sites within my study, *A. saccharum* has seen precipitous declines across its North American range over the last half-decade (Payette et al. 1996, Houston 1999, Horsley et al. 2002, Duchesne et al. 2005, Long et al. 2009) whereas *A. rubrum* has become increasingly common (Abrams 1998, Fei and Steiner 2007, 2009, Hanberry 2013). Where *A. saccharum* decline has been attributed to a number of environmental disturbances, such as extreme climate events (i.e. drought) and insect herbivory (Houston 1999, Horsley et al. 2002, Long et al. 2009), increased *A. rubrum* dominance has been attributed to forest fire suppression (Alexander and Arthur 2010). While there are many potential drivers of the inverse population trends of these two species it has

been speculated that these differences may also be driven by more favorable nutrient use and acquisition strategies within *A. rubrum* (Abrams 1998, St Clair 2004, St Clair and Lynch 2005a).

A. saccharum specifically has been shown to be more sensitive to nutrient poor and acidic soils than *A. rubrum* (Bigelow and Canham 2002, St. Clair et al. 2008). This sensitivity has been thought to be a result of lower nutrient requirements for *A. rubrum* (Abrams 1998, St Clair and Lynch 2005b) and therefore smaller fluxes. However, *A. saccharum* decline has been more closely tied to base cation availability (Ca and Mg) with trees containing high foliar Ca and Mg concentrations maintaining positive growth rates compared to other individuals with low Ca and Mg concentrations (Long et al. 2009). Furthermore, base cation addition experiments have demonstrated that both photosynthetic rates and mycorrhizal infection are more sensitive to Ca and Mg addition in *A. saccharum* than *A. rubrum* with the latter species maintaining higher rates of fungal infection under native soil conditions (St Clair and Lynch 2005a).

Considering these studies, significant changes in Ca S-H differences between pre- and post-leaf flush in *A. saccharum* may also be indicative of seasonal Ca translocation that was not captured in the analysis of sapwood and heartwood samples. Furthermore, I also found that *A. saccharum* had significantly higher sapwood Ca and Mg concentrations pre-leaf flush than *A. rubrum* with no significant differences present for any other analyte (t-test, $p < 0.001$), possibly highlighting a greater need for these base cations in *A. saccharum* compared to *A. rubrum*. Thus, while fluxes may be similar across species, the magnitude and importance of wood nutrient storage may vary between congeneric species according to varying nutrient use strategies.

Wood ray parenchyma fraction

Wood parenchyma cells have been tied to a plethora of plant processes with no one specific primary role identified as a driver of parenchyma distribution within wood (Kotowska et al. 2020, Słupianek et al. 2021). While parenchyma have been indirectly shown to be capable of wood nutrient translocation and storage (Wardell and Hart 1973, Meerts 2002, Kotowska et al. 2020), the primary functional role that these cells have for tree nutrient use is not well understood. Thus, I predicted that wood nutrient concentration would increase with the fraction of wood tissue allocated to ray parenchyma. Conversely, I predicted that if ray parenchyma fraction is more related to wood nutrient translocation as opposed to nutrient storage for trees within my sites, that ray parenchyma fraction would be higher in control trees than in fertilized

trees. Similarly, I expected for ray parenchyma fraction to be highest in *Q. alba* and lowest in *A. saccharum* as these species are primarily distributed in the lowest and highest fertility sites respectively within this study.

I found that ray parenchyma fraction did not explain variation within sapwood nutrient concentrations or S-H differences with the only significant effect found for K sapwood concentrations. Similarly, I found no evidence that fertilization treatments influenced ray parenchyma fraction. When comparing ray parenchyma fraction across species I did observe that *Q. alba* maintained significantly greater ray parenchyma than other species, but differences among the other three species were less pronounced (Figure 9), providing weak support for the hypothesis that ray parenchyma functions primarily to translocate nutrients as opposed to storing them. Additionally, the fact that *Q. alba* also tended to have higher N concentrations than *A. saccharum* (Table 2) suggests that storage may still be a major function of ray parenchyma within this species and further analysis into the actual wood nutrient content between these two species is needed to validate the interpretation of my findings.

Prior analysis assessing the relationship between wood nutrients and parenchyma fraction have found significant positive relationships for N. An early study relating axial and ray parenchyma to N concentrations across angiosperms from North, Central, and South America found a strong correlation of ray parenchyma fraction with sapwood N (Merrill and Cowling 1966). Similarly, a more recent study of wood anatomical traits across 16 tree species in Australia found a significant relationship between ray and axial parenchyma fraction with stem-wood N, however, parenchyma tissue appeared to be relatively invariant within a species with no relationship found for stem-wood P or soil N and P (Kotowska et al. 2020). Additionally, this study also observed a stronger relationship between N and parenchyma when excluding ray parenchyma from analysis. Taking into consideration that axial parenchyma have also been shown to store N (Magel 2001, Carlquist 2007, Kotowska et al. 2020), discrepancies between my results and prior analysis may be due to the fact that axial parenchyma were not included in my analysis, indicating a more complex relationship between ray parenchyma and wood nutrient translocation beyond symplastic transport across stems. While few if any studies have related base cation nutrient concentrations to wood parenchyma, my positive findings for K seem to indicate that ray parenchyma may facilitate the transport of highly mobile nutrients. However, given that I found no relationship with N, this translocation may not solely be dependent on the

mobility of nutrients but may vary according to plant specific nutrient strategies not accounted for in this study.

Nonetheless, the low within-species variation in parenchyma fraction observed in my study appear to indicate that ray parenchyma fraction is not likely directly influenced by nutrient availability, a similar observation made by Kotowska et al. (2020). Furthermore, while my results do not show that ray parenchyma consistently influences wood nutrients, the wide array of functions that parenchyma are able to fulfill within tree species, as well as, the wide variability that exists across species, points towards a functional significance for wood parenchyma tissue that spans several aspects of overall plant fitness (Kotowska et al. 2020, Słupianek et al. 2021). Thus, understanding the direct effects that ray parenchyma have on wood nutrient resorption is difficult to investigate under natural environmental conditions. Moreover, ray parenchyma cells can show physiological/functional differences depending on whether they are adjacent to wood vessels (VACs) or are non-vessel associated, isolation, cells. Here a major difference is that VACs lack large vacuoles typically found in isolation cells (Czaninski 1977, Morris et al. 2018, Kotowska et al. 2020, Słupianek et al. 2021). So, the influence of ray parenchyma on wood nutrient resorption may also depend on the actual storage capabilities within and among ray parenchyma cell types (Kotowska et al. 2020).

Conclusion

I analyzed nutrient concentrations of tree cores collected before leaf flush to assess if tree species grown in different soil nutrient conditions accumulate or translocate different amounts of nutrients in their wood. Furthermore, I analyzed differences in wood nutrient concentrations pre-and post-leaf flush to analyze how wood nutrients may contribute to seasonal periods of high plant nutrient demand. I also sought to relate the fraction of wood tissue comprised of ray parenchyma to wood nutrient re-translocation, by analyzing how ray parenchyma abundance predicted sapwood nutrient concentrations and differences in nutrient concentrations between sapwood and heartwood. For my experimental design, my sampling was constrained to one year of seasonal leaf flush across fertilization treatments that occurred in trees late in their developmental life stages. Furthermore, I did not account for large variation in heartwood depth between species or variation in heartwood nutrients which may have masked true effects of soil nutrient availability on wood nutrient concentrations. Additionally, sampling

was only able to be conducted at one height for each species meaning that my study was not able to account for significant heterogeneity of wood nutrients that can occur along the tree stem (Helmisaari and Siltala 1989).

Despite this, I found consistent support that sapwood nutrients may serve as a seasonal repository for N, but did not find any evidence for differential translocation of wood nutrients in response to soil nutrient availability. Additionally, I observed no consistent relationship between investment in ray parenchyma and wood nutrients. These results highlight the dynamic nature of wood nutrient stores and indicate that wood nutrient translocation is a complex phenomenon that is not solely dictated by soil nutrient availability or parenchyma fraction. Furthermore, seasonal fluxes in S-H differences for sampled *Acer* species also show that accounting for periods of low and high nutrient demand within a study is important for testing hypothesis concerning radial variation in wood nutrients as apparent differences in nutrient concentrations between inner and outer wood can be significantly influenced by plant nutrient demand at the time of sampling. Future work seeking to investigate wood nutrient translocation should assess other factors that may covary with soil nutrient availability (i.e. plant specific nutrient use/acquisition strategies) to investigate the primary drivers of wood nutrient concentrations across environmental gradients.

FIGURES AND TABLES

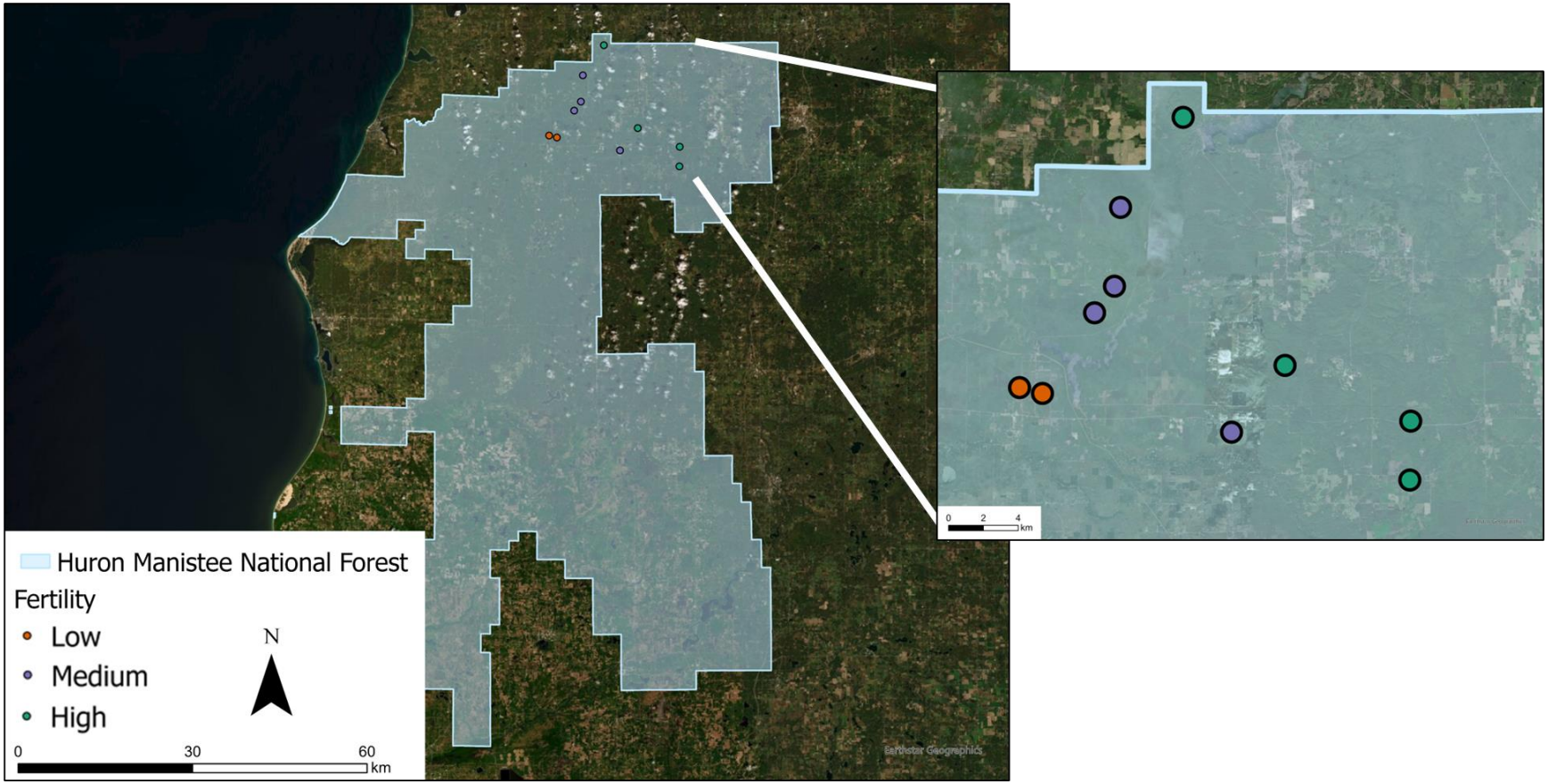

Figure 1. Map of the 10 sites sampled in Manistee, Michigan color coded by soil fertility level. Fertility levels were chosen based on the Ecological Land Type Phase index classification of each site that denotes relative soil fertility.

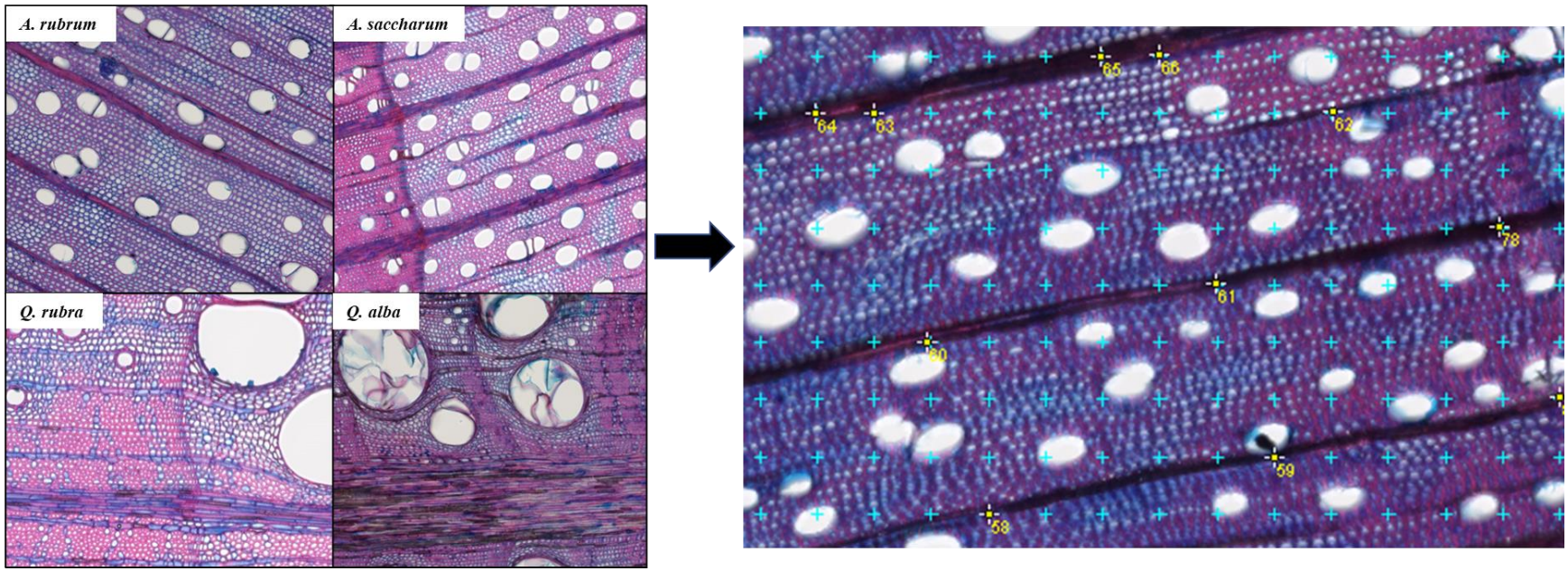

Figure 2. Transverse section for each species included in this study (left) and example of grid point method of ray parenchyma fraction estimation (right). Ray parenchyma identified as lines of symplastically connected cells that span across wood sections.

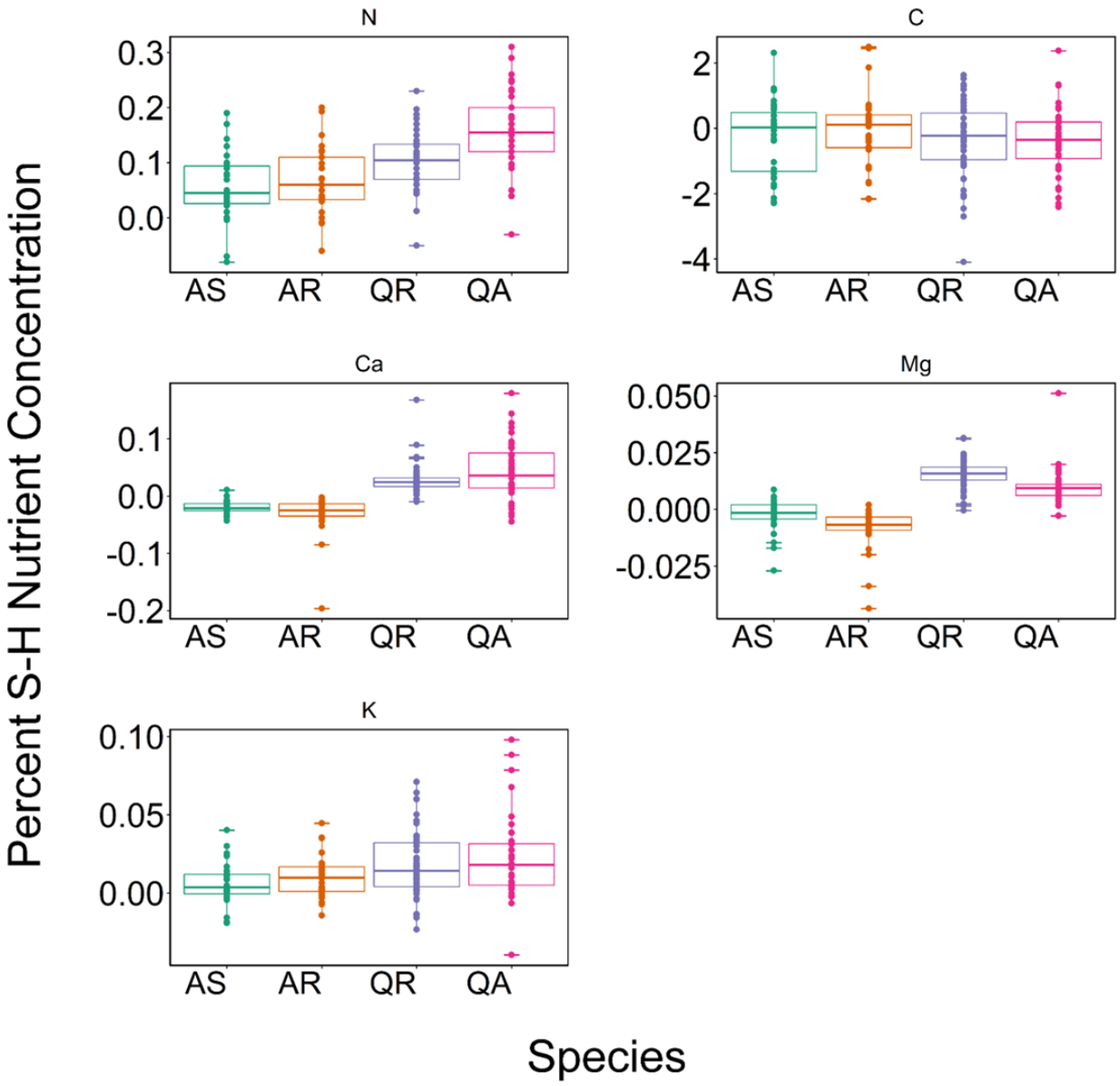

Figure 3. Boxplots of sapwood minus heartwood (S-H) nutrient concentrations for all species in April sampling. AS = *A. saccharum*, AR = *A. rubrum*, QR = *Q. rubra*, QA = *Q. alba*. Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively.

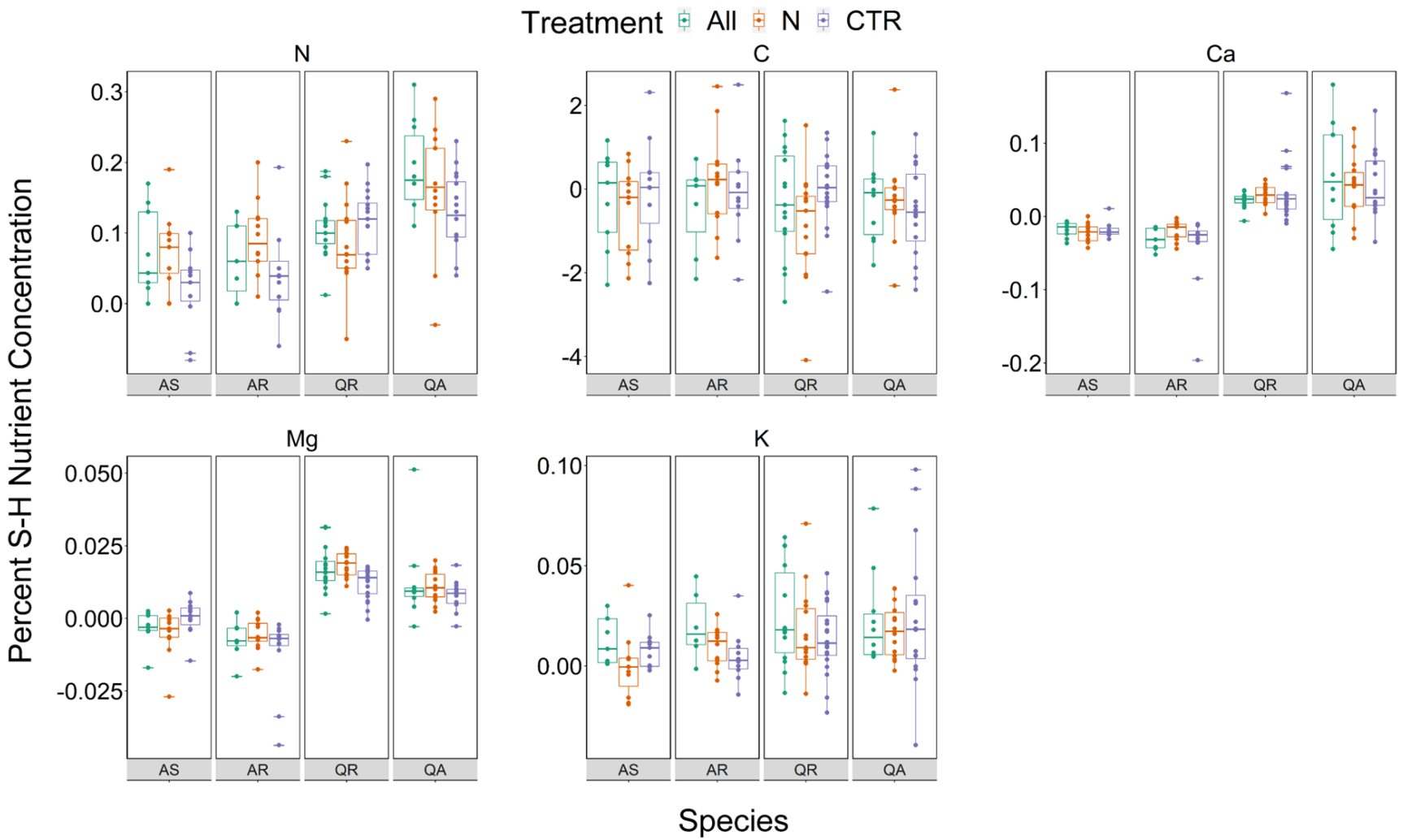

Figure 4. Boxplots of S-H nutrient differences for each treatment (All, N, and CTR), species, and analyte. AS = *A. saccharum*, AR = *A. rubrum*, QR = *Q. rubra*, QA = *Q. alba*. Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively.

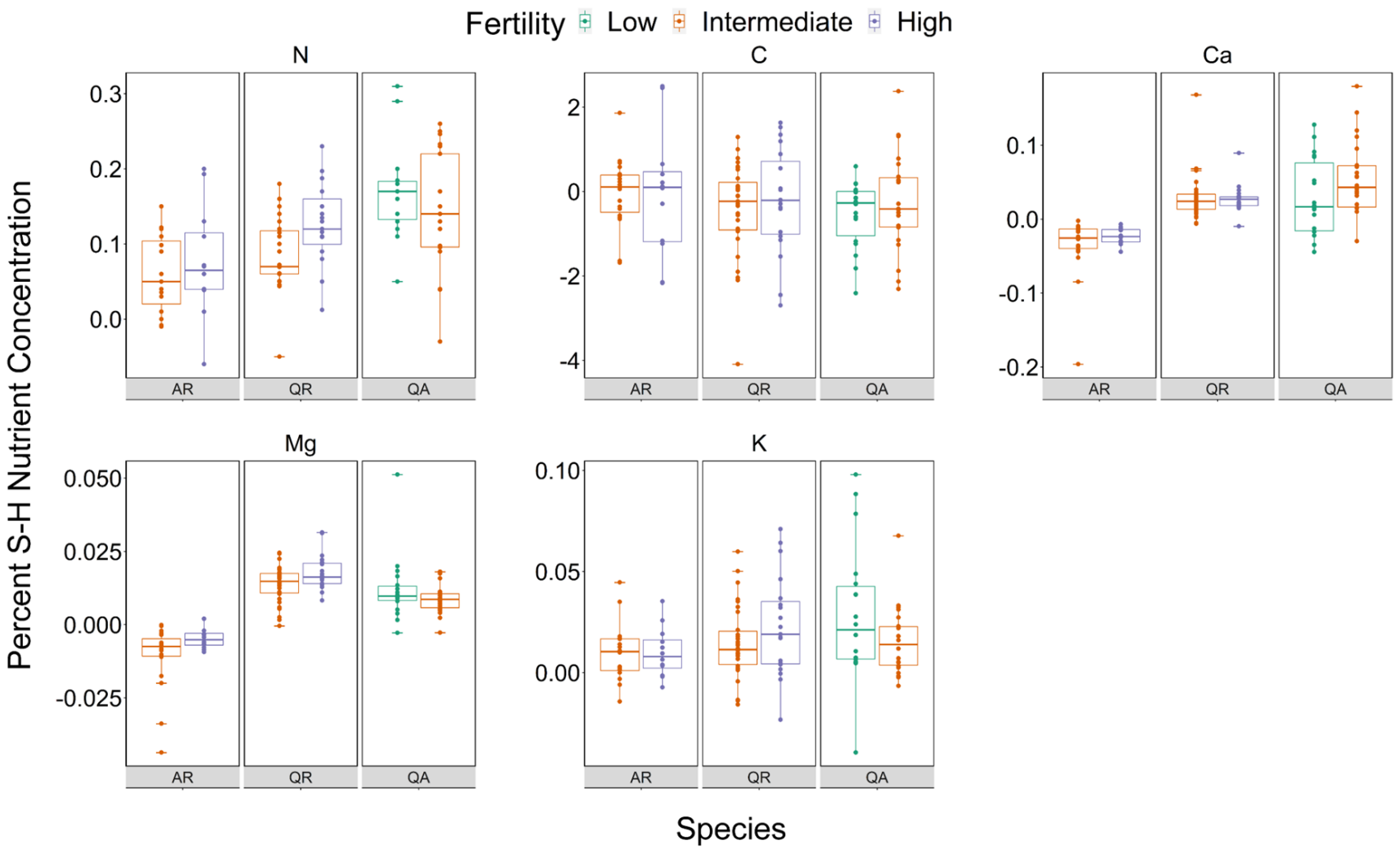

Figure 5. Boxplots of S-H nutrient differences for each fertility level, species, and analyte. AS = *A. saccharum*, AR = *A. rubrum*, QR = *Q. rubra*, QA = *Q. alba*. Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively and all treatments were pooled for each fertility level shown.

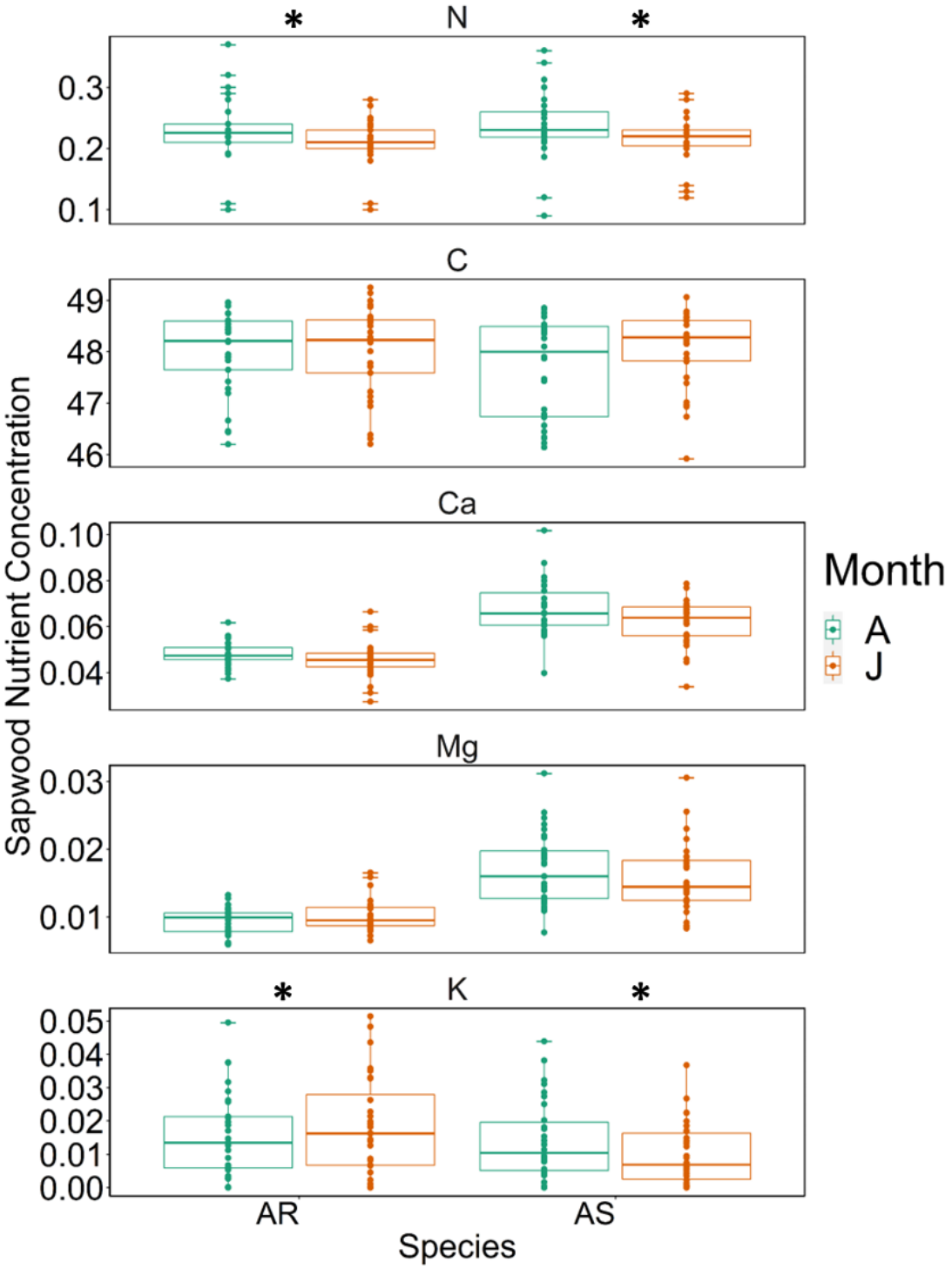

Figure 6. Sapwood percent nutrient boxplots of the 5 analytes measured (N, C, Ca, Mg, K) for *A. rubrum* (AR) and *A. saccharum* (AS) in April (A) and June (J). *A. rubrum* is shown on the left and *A. saccharum* is shown on the right. Green boxes denote April (pre-leaf flush) nutrient concentrations and orange boxes denote June (post-leaf flush) nutrient concentrations. Asterisks indicate statistcally significant differences between sampling periods ($p < 0.05$). Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively.

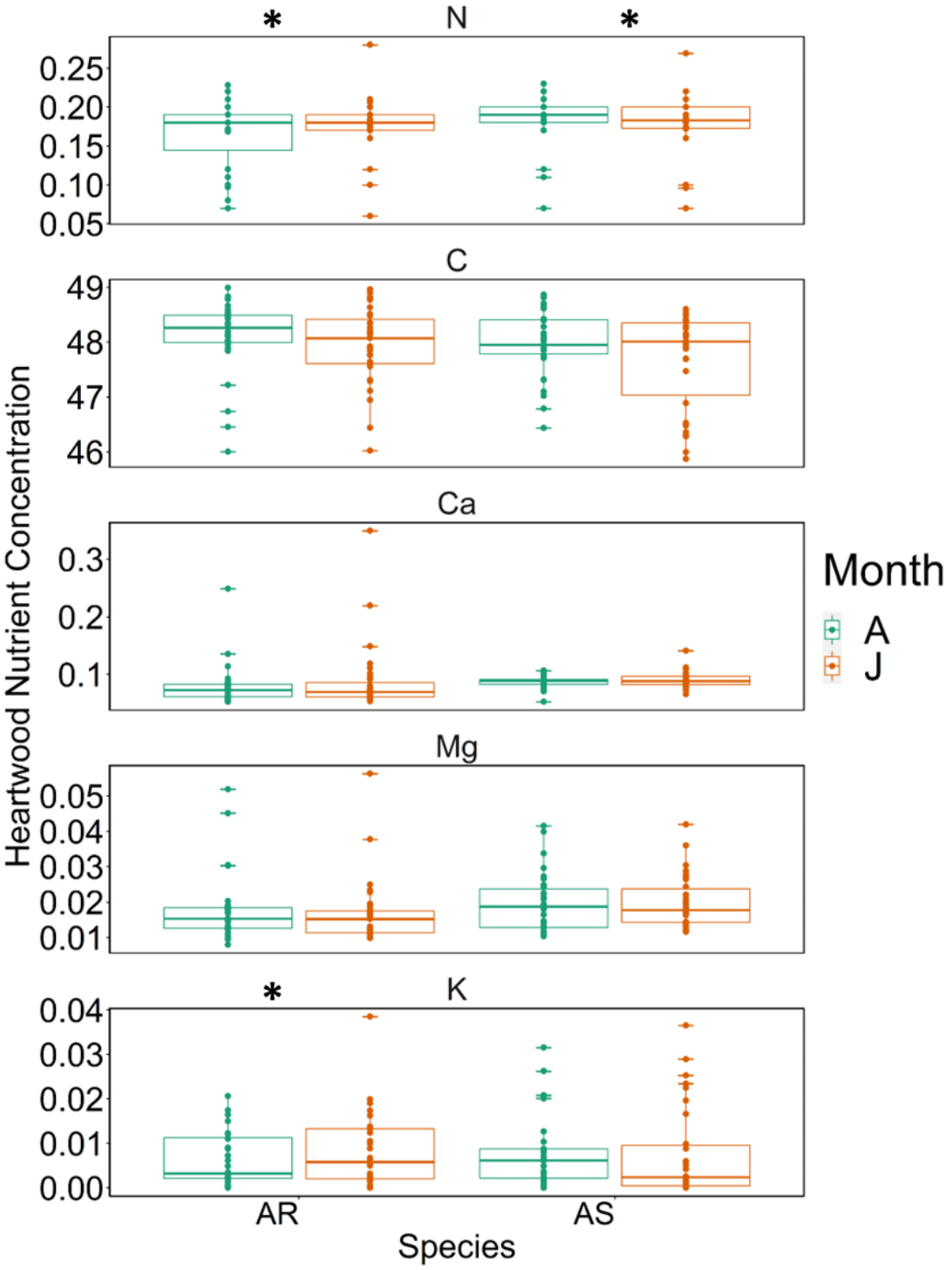

Figure 7. Heartwood percent nutrient boxplots of the 5 analytes measured (N, C, Ca, Mg, K) for *A. rubrum* (AR) and *A. saccharum* (AS) measured in April (A) and June (J). *A. rubrum* is shown on the left and *A. saccharum* is shown on the right. Green boxes denote April (pre-leaf flush) nutrient concentrations and orange boxes denote June (post-leaf flush) nutrient concentrations. Asterisks indicate statistcally significant differences between sampling periods ($p < 0.05$). Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively.

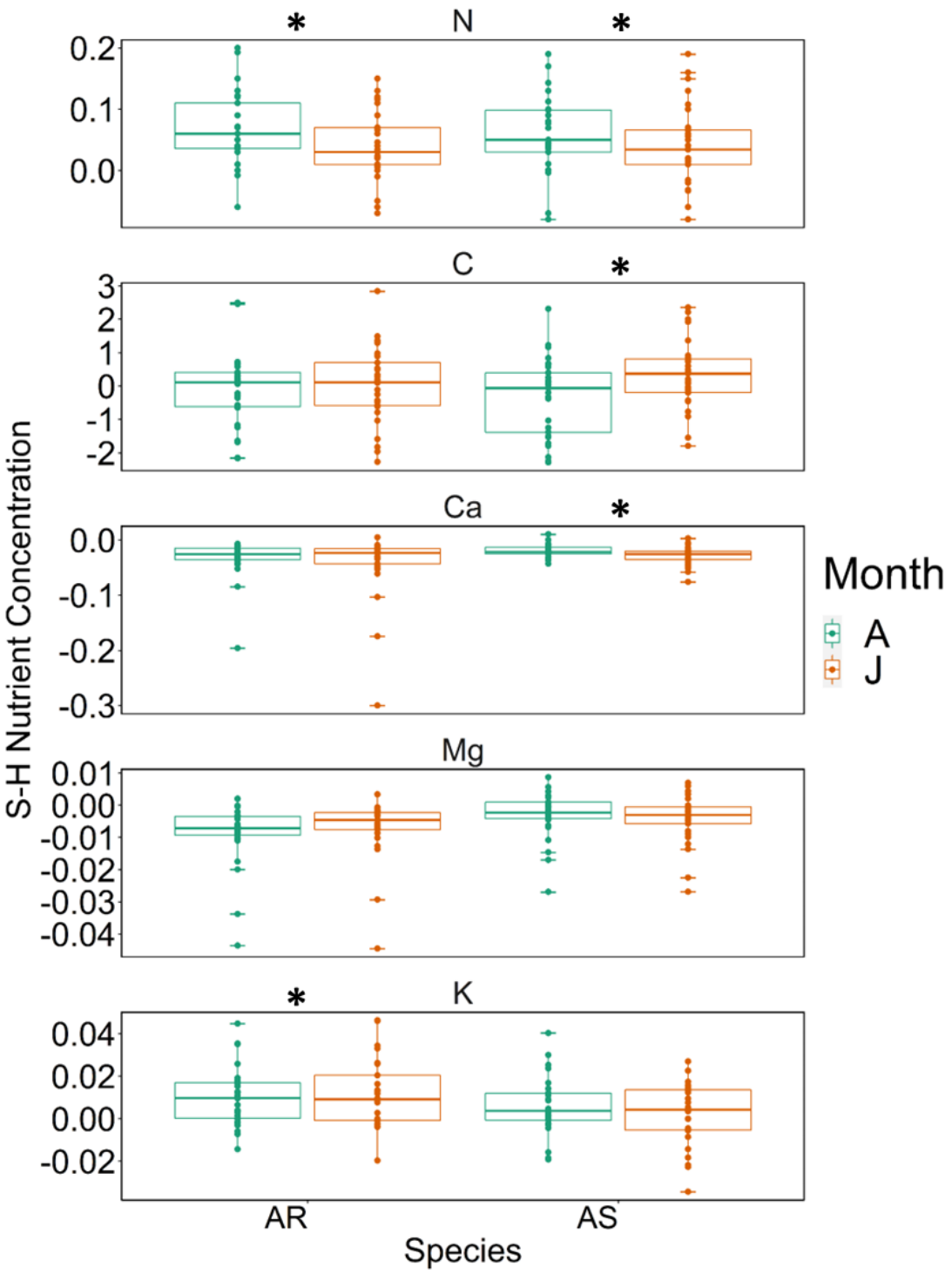

Figure 8. S-H percent nutrient boxplots of the 5 analytes measured (N, C, Ca, Mg, K) for *A. rubrum* (AR) and *A. saccharum* (AS) in April and June. *A. rubrum* is shown on the left and *A. saccharum* is shown on the right. Green boxes denote April (pre-leaf flush) nutrient concentrations and orange boxes denote June (post-leaf flush) nutrient concentrations. Asterisks indicate statistcally significant differences between sampling periods ($p < 0.05$). Box lines indicate the median with lower and upper edges of the box representing the 1st and 3rd quartile respectively.

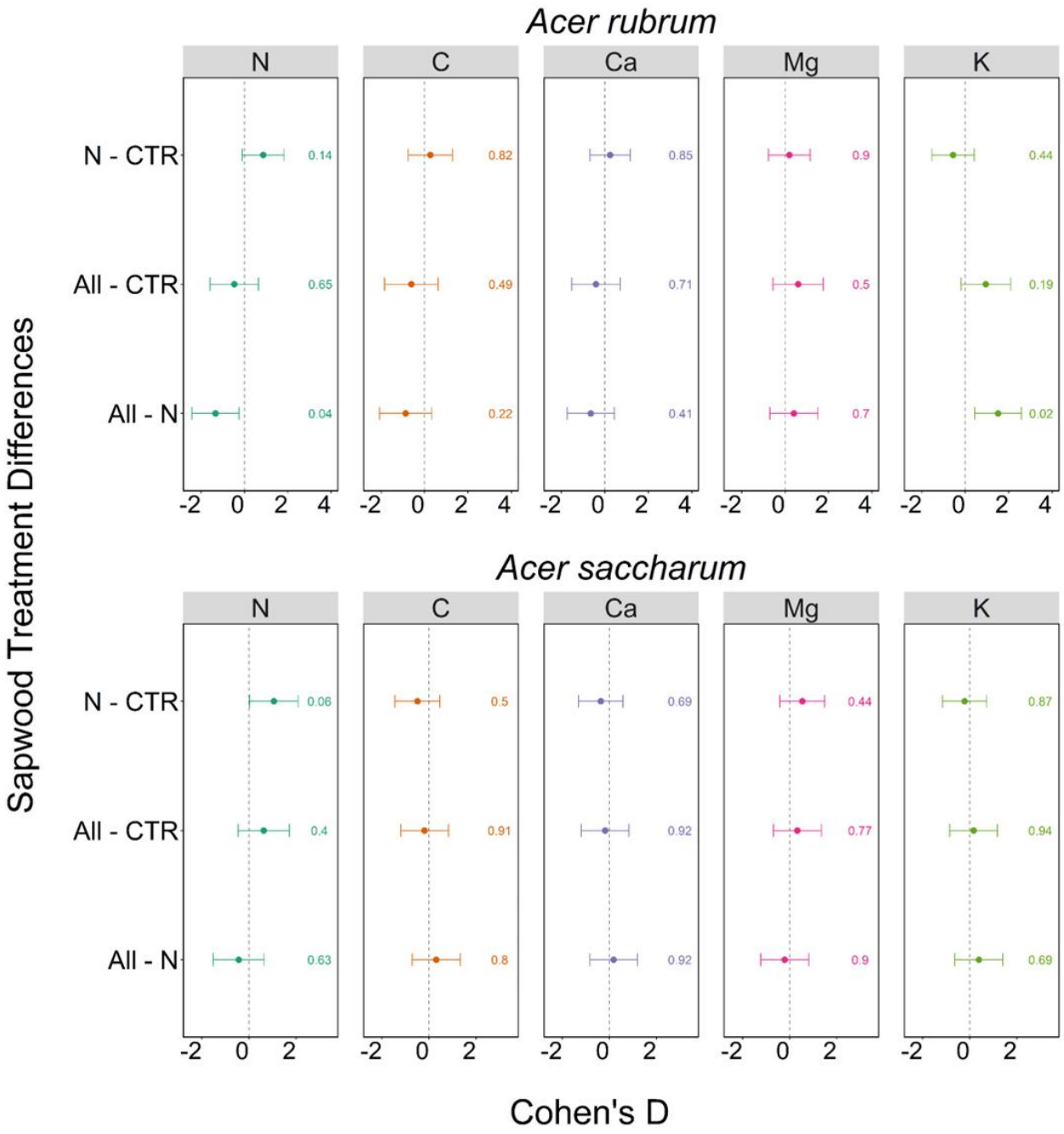

Figure 9. Cohen's D effect size plots for differences in pre- and post-leaf flush sapwood nutrient fluxes between treatments (All, N, CTR). Graphs are partitioned by analytes with p-values from a Tukey post-hoc test shown on the right hand side of each point. Positive values indicate higher nutrient concentrations in the first treatment than in the second treatment.

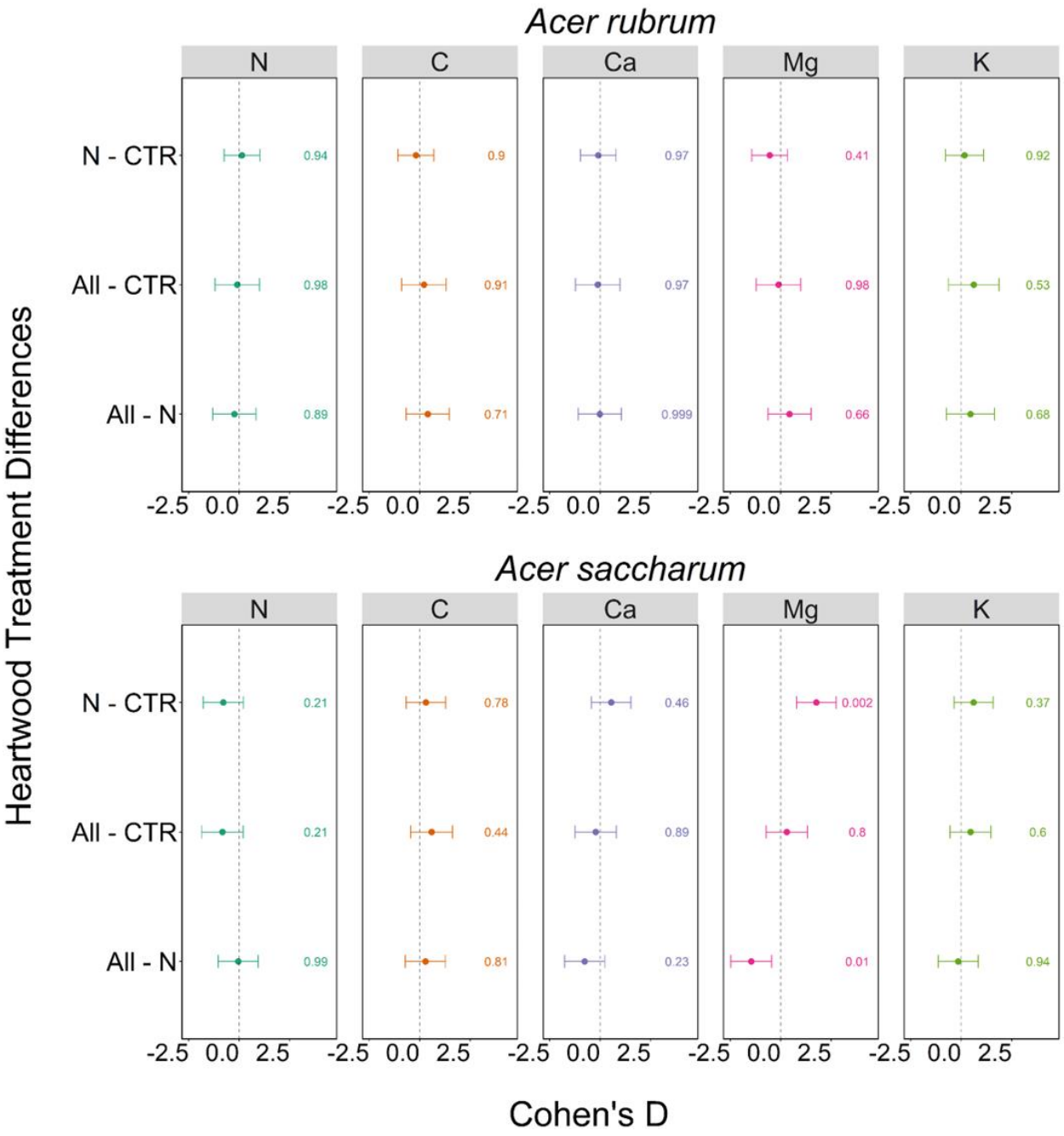

Figure 10. Cohen's D effect size plots for differences in pre- and post-leaf flush heartwood nutrient fluxes between treatments (All, N, CTR). Graphs are partitioned by analytes with p-values from a Tukey post-hoc test shown on the right hand side of each point. Positive values indicate higher nutrient concentrations in the first treatment than in the second treatment.

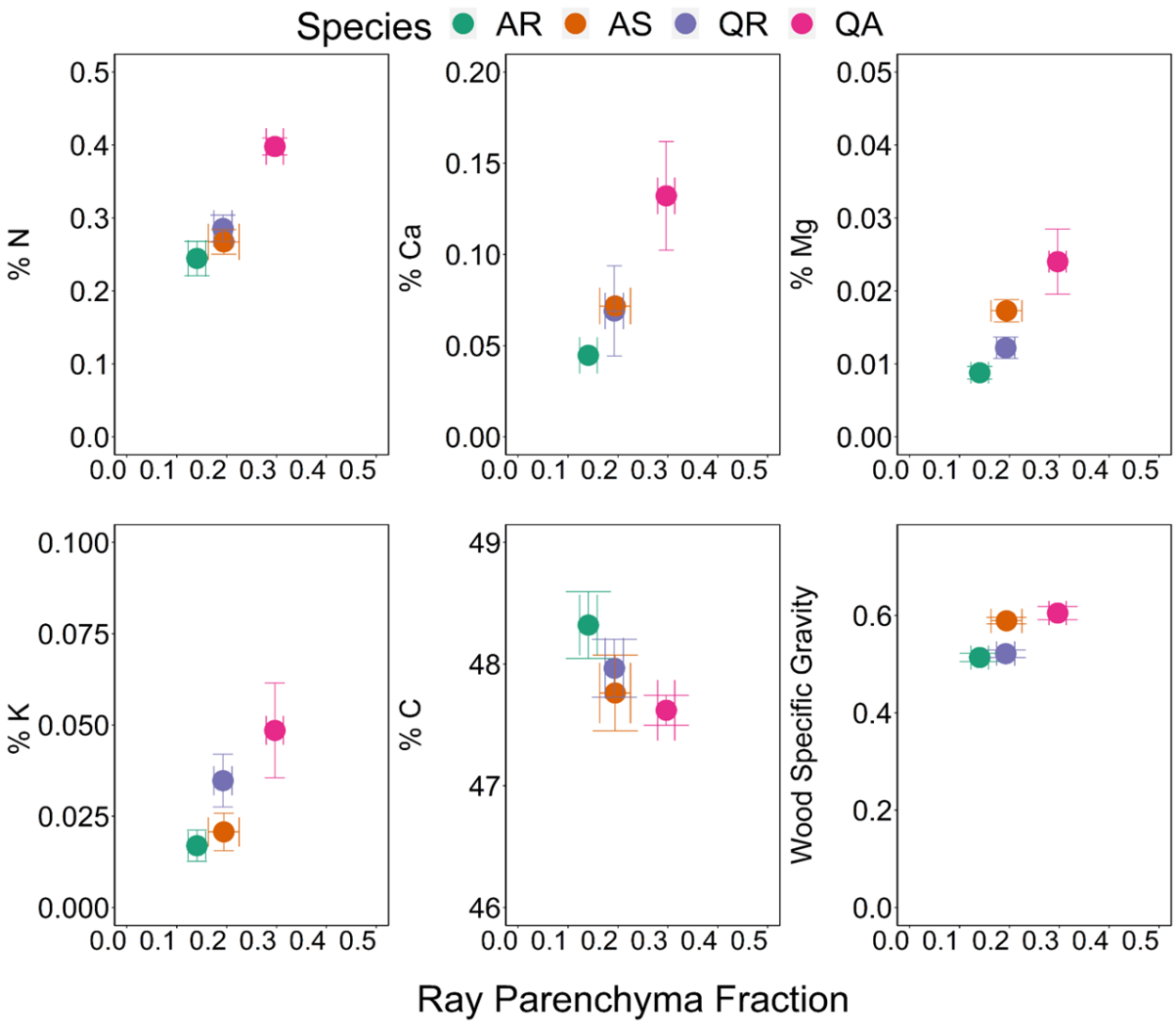

Figure 11. Average ray parenchyma fraction plotted against average percent nutrient concentrations of N, Ca, Mg, K, C, and wood specific gravity. AS = *A. saccharum*, AR = *A. rubrum*, QR = *Q. rubra*, QA = *Q. alba*. Error bars indicate standard error.

Table 1. Table of site fertility designation along with species found in each site. Numbers following the decimal point of each site denote Ecological Land Type Phase index classification and reflect increasing soil fertility from top to bottom. Sites marked in green indicate sites where wood anatomical sections were analyzed from and bold labelled species indicate the specific species that was sampled at each site.

Site	Species Sampled	Fertility Level
49.01	***Q. alba***	
59.12	*Q. alba*	**LOW**
8.21	*Quercus alba, Q. rubra, A. rubrum*	
39.21	*Quercus alba, Q. rubra, A. rubrum*	
16.35	*Quercus alba,* ***Q. rubra****, A. rubrum*	
60.35	*Quercus alba,* ***Q. rubra***	**INTERMEDIATE**
7.4	*Q. rubra, A. rubrum, A. saccharum*	
21.43	*Q. rubra,* ***A. rubrum****, A. saccharum*	
25.43	*Q. rubra, A. rubrum,* ***A. saccharum***	
6.45	*A.* ***saccharum***	**HIGH**

Table 2. Average percent nutrient concentrations of sapwood and heartwood samples of species collected both pre-leaf flush (April) and post-leaf flush (June). Sample size (*n*) and standard error (*SE*) are shown to the right of each element.

April Pre-Leaf Flush															
Species	**Sample**	**% N**	***SE***	***n***	**%C**	***SE***	***n***	**% Ca**	***SE***	***n***	**% Mg**	***SE***	***n***	**%K**	***SE***	***n***
Acer rubrum	S	0.231	±0.010	31	48.041	±0.140	31	0.049	±0.001	31	0.009	±0.000	31	0.016	±0.002	31
	H	0.164	±0.008	31	48.081	±0.124	31	0.080	±0.006	31	0.018	±0.002	31	0.006	±0.001	28
Acer saccharum	S	0.237	±0.009	31	47.704	±0.166	31	0.067	±0.002	31	0.017	±0.001	31	0.014	±0.002	31
	H	0.183	±0.006	31	47.977	±0.108	31	0.087	±0.002	31	0.020	±0.001	31	0.008	±0.001	31
Quercus rubra	S	0.317	±0.006	50	47.808	±0.128	50	0.059	±0.005	50	0.018	±0.001	50	0.031	±0.003	50
	H	0.216	±0.004	50	48.167	±0.106	50	0.030	±0.002	50	0.002	±0.0003	50	0.013	±0.001	49
Quercus alba	S	0.370	±0.009	40	47.748	±0.128	40	0.125	±0.010	40	0.016	±0.001	40	0.029	±0.004	40
	H	0.214	±0.007	40	48.129	±0.094	40	0.081	±0.006	40	0.006	±0.001	40	0.007	±0.001	40
June Post-Leaf Flush															
Species	**Sample**	**% N**	***SE***	***n***	**%C**	***SE***	***n***	**% Ca**	***SE***	***n***	**% Mg**	***SE***	***n***	**%K**	***SE***	***n***
Acer rubrum	S	0.213	±0.007	30	48.032	±0.155	30	0.045	±0.001	30	0.010	±0.0004	30	0.019	±0.003	29
	H	0.174	±0.007	32	47.956	±0.123	32	0.088	±0.010	32	0.017	±0.002	32	0.008	±0.002	29
Acer saccharum	S	0.212	±0.008	31	48.084	±0.131	31	0.062	±0.002	31	0.016	±0.001	31	0.010	±0.002	31
	H	0.174	±0.008	31	47.674	±0.153	31	0.090	±0.003	31	0.020	±0.001	31	0.008	±0.002	31

Table 3. Results of mixed effect models assessing for effects of fertilization treatments on April S-H percent nutrient differences, as well as, sapwood and heartwood percent nutrient concentrations of each analyte arranged by species. Degree's of freedom calculated using Satterthwaite's method. Values highlighted in bold indicate statistical significance ($p < 0.05$).

	S-H				Sapwood				Heartwood			
Species	***df***	**F-value**	**p-value**	**Nutrient**	***df***	**F-value**	**p-value**	**Nutrient**	***df***	**F-value**	**p-value**	**Nutrient**
Acer saccharum	28.00	2.914	0.071	N	26.20	3.035	0.065	N	28.00	0.166	0.848	N
	26.60	0.368	0.696	C	28.00	0.044	0.957	C	26.19	1.030	0.371	C
	28.00	0.475	0.627	Ca	25.90	0.406	0.671	Ca	28.00	0.031	0.970	Ca
	28.00	1.556	0.229	Mg	28.00	0.098	0.907	Mg	26.40	1.441	0.255	Mg
	28.00	2.274	0.122	K	28.00	1.575	0.225	K	28.00	2.937	0.069	K
Acer rubrum	28.00	2.377	0.111	N	26.06	3.846	**0.034**	N	26.35	1.605	0.220	N
	25.05	0.803	0.459	C	28.00	0.569	0.573	C	28.00	0.251	0.780	C
	27.14	1.363	0.273	Ca	25.95	0.027	0.974	Ca	27.08	1.325	0.283	Ca
	27.05	1.671	0.207	Mg	24.97	6.422	**0.006**	Mg	27.04	1.867	0.174	Mg
	24.36	2.773	0.080	K	28.00	1.788	0.186	K	25.00	0.082	0.922	K
Quercus rubra	46.02	1.383	0.261	N	46.02	1.088	0.345	N	47.00	0.612	0.547	N
	47.00	2.639	0.082	C	47.00	1.225	0.303	C	47.00	1.337	0.272	C
	42.94	0.648	0.528	Ca	42.94	1.201	0.311	Ca	42.20	1.440	0.248	Ca
	41.68	7.566	**0.002**	Mg	41.69	7.126	**0.002**	Mg	42.61	0.378	0.688	Mg
	39.23	1.208	0.310	K	40.64	3.106	0.056	K	45.11	2.231	0.119	K
Quercus alba	37.00	2.902	0.067	N	36.53	1.715	0.194	N	37.00	1.243	0.300	N
	36.60	0.378	0.688	C	36.59	0.346	0.710	C	37.00	0.731	0.488	C
	36.31	0.666	0.520	Ca	37.00	0.557	0.577	Ca	34.79	0.174	0.841	Ca
	35.02	1.017	0.372	Mg	34.42	2.925	0.067	Mg	36.25	6.440	**0.004**	Mg
	34.97	0.237	0.790	K	34.75	0.854	0.434	K	36.49	1.166	0.323	K

Table 4. Results of mixed effect models assessing for effects of fertility level on April S-H percent nutrient differences, as well as, sapwood and heartwood percent nutrient concentrations. Degree's of freedom calculated using Satterthwaite's method. Values highlighted in bold indicate statistical significance ($p < 0.05$).

	S-H Control				S-H Treatments Pooled			
Species	***df***	**F-value**	**p-value**	**Nutrient**	***df***	**F-value**	**p-value**	**Nutrient**
Acer rubrum	10.00	0.011	0.920	N	29.00	0.592	0.448	N
	3.80	0.485	0.527	C	2.33	0.441	0.566	C
	10.00	1.058	0.328	Ca	29.00	1.036	0.317	Ca
	10.00	2.002	0.187	Mg	29.00	3.700	0.064	Mg
	4.30	0.116	0.750	K	2.33	1e-04	0.994	K
Quercus rubra	18.00	4.154	0.057	N	48.00	7.978	**0.007**	N
	5.40	0.001	0.971	C	4.28	0.440	0.541	C
	5.26	0.089	0.776	Ca	5.20	0.021	0.890	Ca
	5.51	2.779	0.151	Mg	5.04	1.425	0.286	Mg
	18.00	0.473	0.500	K	3.80	1.669	0.269	K
Quercus alba	14.00	0.813	0.382	N	38.00	1.315	0.259	N
	3.86	0.539	0.505	C	38.00	0.850	0.362	C
	14.00	4.49E-06	0.998	Ca	38.00	2.642	0.112	Ca
	14.00	1.249	0.282	Mg	3.92	0.965	0.383	Mg
	4.04	0.858	0.406	K	4.00	1.612	0.273	K

	Sapwood Control				Sapwood Treatments Pooled			
Species	***df***	**F-value**	**p-value**	**Nutrient**	***df***	**F-value**	**p-value**	**Nutrient**
Acer rubrum	10.00	0.166	0.693	N	2.74	0.585	0.505	N
	4.01	0.881	0.401	C	1.44	0.240	0.689	C
	10.00	1.843	0.205	Ca	2.45	7.597	0.088	Ca
	2.73	0.097	0.777	Mg	1.97	0.425	0.582	Mg
	10.00	0.046	0.834	K	29.00	0.197	0.661	K
Quercus rubra	5.17	0.842	0.400	N	48.00	8.603	**0.005**	N
	18.00	3.342	0.084	C	4.73	0.011	0.922	C
	4.94	0.498	0.512	Ca	5.35	0.378	0.564	Ca
	5.27	1.505	0.272	Mg	5.06	1.223	0.319	Mg
	18.00	0.001	0.976	K	4.08	0.378	0.571	K
Quercus alba	14.00	3.862	0.070	N	38.00	2.238	0.143	N
	14.00	0.384	0.546	C	38.00	0.879	0.354	C
	2.48	0.005	0.951	Ca	38.00	0.324	0.573	Ca
	14.00	6.562	**0.023**	Mg	3.89	2.335	0.203	Mg
	3.93	2.166	0.216	K	3.33	2.660	0.192	K

Table 4 Cont.

	Heartwood Control				Heartwood Treatments Pooled			
Species	***df***	**F-value**	**p-value**	**Nutrient**	***df***	**F-value**	**p-value**	**Nutrient**
Acer rubrum	3.20	0.003	0.960	N	4.04	0.060	0.818	N
	10.00	0.031	0.865	C	29.00	0.013	0.911	C
	10.00	1.278	0.285	Ca	29.00	2.208	0.148	Ca
	10.00	2.320	0.159	Mg	2.92	4.176	0.136	Mg
	8.00	0.540	0.483	K	26.00	0.428	0.519	K
Quercus rubra	5.28	0.281	0.618	N	48.00	0.225	0.637	N
	6.42	2.419	0.168	C	48.00	0.888	0.351	C
	18.00	1.703	0.208	Ca	5.32	0.993	0.362	Ca
	6.58	0.648	0.449	Mg	5.62	0.258	0.631	Mg
	18.00	2.146	0.160	K	47.00	2.175	0.147	K
Quercus alba	14.00	0.638	0.438	N	38.00	0.002	0.965	N
	14.00	0.119	0.736	C	38.00	0.080	0.778	C
	2.86	0.024	0.887	Ca	3.84	0.976	0.381	Ca
	14.00	4.614	**0.0497**	Mg	38.00	5.325	**0.027**	Mg
	14.00	1.126	0.307	K	38.00	1.259	0.269	K

Table 5. Paired t-test results for pre- and post-leaf flush (A-J) analysis for S-H percent nutrient differences, as well as, sapwood and heartwood nutrient concentrations. Values highlighted in bold indicate statistical significance ($p < 0.05$).

S-H							
Species	***df***	**t-statistic**	**p-value**	**Nutrient**	**Mean of Differences**	**Lower Conf.**	**Upper Conf.**
Acer rubrum	28	10.356	**4.43E-11**	N	0.028	0.022	0.033
	28	-0.514	0.611	C	-0.148	-0.735	0.440
	28	0.820	0.419	Ca	0.010	-0.015	0.036
	28	-0.895	0.379	Mg	-0.002	-0.006	0.002
	24	-3.094	**0.005**	K	-0.003	-0.004	-0.001
Acer saccharum	28	6.481	**5.07E-07**	N	0.015	0.010	0.020
	28	-2.757	**0.010**	C	-0.680	-1.186	-0.175
	28	2.485	**0.019**	Ca	0.009	0.002	0.017
	28	0.573	0.571	Mg	0.001	-0.003	0.005
	28	0.988	0.331	K	0.004	-0.004	0.012

Sapwood							
	df	**t-statistic**	**p-value**	**Nutrient**	**Mean of Difference**	**Lower Conf.**	**Upper Conf.**
Acer rubrum	28	4.661	**0.0001**	N	0.018	0.010	0.026
	28	-0.014	0.989	C	-0.002	-0.358	0.353
	28	1.349	0.188	Ca	0.003	-0.001	0.006
	28	-1.533	0.136	Mg	-0.001	-0.002	0.0003
	27	-4.889	**0.00004**	K	-0.003	-0.005	-0.002
Acer saccharum	29	6.282	**7.38E-07**	N	0.023	0.016	0.031
	29	-1.620	0.116	C	-0.366	-0.827	0.096
	29	1.860	0.073	Ca	0.006	-0.001	0.012
	29	1.043	0.306	Mg	0.001	-0.001	0.004
	29	2.096	**0.045**	K	0.004	0.0001	0.008

Heartwood							
	df	**t-statistic**	**p-value**	**Nutrient**	**Mean Difference**	**Lower Conf.**	**Upper Conf.**
Acer rubrum	30	-2.788	**0.009**	N	-0.010	-0.017	-0.003

Table 5 Cont.						
30	0.685	0.499	C	0.138	-0.273	0.549
30	-0.774	0.445	Ca	-0.009	-0.032	0.014
30	0.624	0.537	Mg	0.001	-0.002	0.004
27	-3.272	**0.003**	K	-0.002	-0.003	-0.001
Acer saccharum 29	2.361	**0.025**	N	0.009	0.001	0.017
29	1.397	0.173	C	0.302	-0.140	0.745
29	-0.933	0.358	Ca	-0.003	-0.010	0.004
29	0.049	0.961	Mg	0.0001	-0.004	0.005
29	0.185	0.854	K	0.0003	-0.003	0.004

Table 6. Summary table denoting average ray parenchyma fraction, wood specific gravity, and DBH along with standard error (*SE*) and sample size (*n*) for each species.

Species	**Parenchyma Fraction**	***SE***	***n***	**Wood Specific Gravity**	***SE***	***n***	**DBH**	***SE***	***n***
Acer saccharum	0.19	0.03	9	0.59	0.01	9	39.49	1.84	9
Acer rubrum	0.14	0.02	9	0.51	0.01	9	39.46	2.48	9
Quercus rubra	0.19	0.02	9	0.52	0.01	9	55.63	6.84	8
Quercus alba	0.30	0.02	9	0.60	0.01	9	32.40	1.47	9

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