Patterns and Controls of Wood Stoichiometry:

A Globally Distributed Study Using Vertical Tree Observatories (DRAFT)

**Background and Rationale** — Ecosystem stoichiometry is a key driver of global biogeochemical cycling that will influence the ways ecosystems will respond to global change. For example, predicted increases in plant productivity (Hamilton et al. 2002; Norby et al. 2005; Zak et al. 2011) will likely persist under a range of tissue carbon:nitrogen:phosphorus (C:N:P) values, but limits to stoichiometric plasticity may govern future vegetation responses as nutrients become increasingly scarce. In addition, multiple lines of evidence suggest that ecosystem stoichiometry may be shifting globally due to interacting factors that include rising atmospheric CO2, N deposition, and climate change (McNeil et al. 2007; Fleischer et al. 2013; Du et al. 2019; Wang et al. 2021, Mason et al. 2022). Yet, while stoichiometric flexibility may be a key determinant of future C balance of terrestrial ecosystems, it remains uncertain whether plants exhibit sufficient flexibility to maintain growth despite anticipated imbalances in C, N and P availability from anthropogenically-altered biogeochemical cycling.

Despite patterns of stoichiometric flexibility in forest ecosystems globally, the effects of changing stoichiometry, especially over large temporal and spatial scales, remain largely unknown. More, the current understanding of stoichiometric flexibility we do have for trees has been informed largely from analyses of tree foliage, litter, and to a much lesser extent, roots (e.g., Dynarski et al. 2022). However, relatively little is known about the stoichiometry of wood and how it varies both among and between species over large spatial scales (but see Heineman et al. 2016), despite the fact that wood represents a large store of C and nutrients. Additional wood stoichiometric data would enhance our ability to predict how biogeochemical cycling will respond to changing resources and would improve model predictions of Earth’s C sink by establishing stoichiometric boundaries for productivity. Thus, we propose a globally distributed study of wood stoichiometry to explore the patterns and controls of wood stoichiometric flexibility in global forests.

**Selecting trees and establishing vertical observatories** — We propose that participants establish a set of semi-permanent *vertical tree observatories* in sites where wood (tree core) samples can be obtained now, but that also allow for return visits for additional sampling in the future. Sites may be located within an existing experiment, in areas with disturbance or management history, or co-located with other research. Site selection is left up to participants so long as relevant details are recorded in the datasheet below.

We suggest that when selecting observatory trees, participants consider either an intensive or extensive sampling regime, or a combination of the two. To contribute to our understanding of interspecific variation in wood stoichiometry over large spatial scales, we suggest North American participants consider *intensive* sampling from among the most common North American taxa (**Table 1**). Many of these genera are common in temperate and high latitude forests and we recommend that participants attempt to sample within these genera even if the trees at their sites are not one of the common species listed. Alternatively, for participants in geographical locations outside North America (e.g., tropical forests), or for North American participants who cannot access these common species or who are able to sample multiple species, we propose an *extensive* sampling regime in which participants sample as many different species as possible, irrespective of their abundance or distribution. Regardless of the species chosen, we urge that whenever possible, participants select tree species that occupy different plant functional types commonly used in modeling efforts (e.g., evergreen needleleaf, evergreen broadleaf, deciduous needleleaf, deciduous broadleaf) (e.g., Hanson et al. 2000). If there are multiple PFTs within a site, we recommend trying to sample across the PFTs as broadly as possible. **At a minimum, we would propose that participation in the data collection effort and database development include sampling, analyzing, and reporting wood C:N for at least one species sampled in triplicate (three individuals per species).** Mature individuals (≥ 20 cm diameter at breast height, DBH) should be marked (e.g., using tree tags) and precisely geolocated for future sampling (latitude/longitude in UTM format). Date, time, location, GPS coordinates, environmental data, including elevation, aspect, and tree DBH should be recorded on the data sheet provided. If available, other available site data may also be reported (e.g., mean annual temperature, mean annual precipitation, soil type, etc.). If trees are located within existing experimental treatments or site gradients participants should record relevant details in the data sheet as well.

**Tree core sampling and analysis —** Once the observatory trees have been located, identified, their precise locations documented, and other necessary environmental data have been recorded (see attached data sheet), use the tree corer to collect one wood core sample per individual (see attached protocol). Once you have collected your tree core samples, they should be kept cool (e.g., in a refrigerator) until you are able to prepare them for drying and analysis (see attached protocol).

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| --- | --- | --- |
| **Eastern North America** | | |
| **Common Genera** | **Common Species** | **PFT** |
| Acer | *A. rubrum, A. saccharum* | DB |
| Betula | *B. papyrifera* | DB |
| Carya | *C. ovata, C. tomentosa* | DB |
| Fagus | *F. grandifolia* | DB |
| Fraxinus | *F. pennsylvanica, F. americana* | DB |
| Larix | *L. laricina* | DN |
| Liquidambar | *L.* styraciflua | DB |
| Nyssa | *N. sylvatica* | DB |
| Picea | *P. rubens* | EN |
| Pinus | *P. palustrus, P. strobus, P. taeda, etc.* | EN |
| Populus | *P. deltoides, P. grandidentada* | DB |
| Prunus | *P. pensylvanica* | DB |
| Quercus | *Q. rubra, Q. alba, etc.* | DB |
| Ulmus | *U. americana* | DB |
| **Western North America** | | |
| **Common Genera** | **Common Species** | **PFT** |
| Abies | *A. lasiocarpa, A. balsamea, A. procera, etc.* | EN |
| Juniperus | *J. communis, J. scopulorum, J. osteosperma, J. californica, J. grandis* | EN |
| Larix | *L. occidentalis* | DN |
| Picea | *P. engelmannii, P. mariana, P. glauca,* | EN |
| Pinus | *P. ponderosa, P. contorta, P. edulis, P. monophyla, P. flexilis, etc.* | EN |
| Populus | *P. tremuloides, P. deltoides, P. trichocarpa* | DB |
| Psuedotsuga | *P. menziesii* | EN |

**Table 1.** Common genera and species in temperate and high latitude forest ecosystems. Definitions: PFT = Plant functional type; DB = Deciduous broadleaf; EN = Evergreen needleleaf; DN = Deciduous needleleaf. Data from Knott et al. (2019) and Stanke et al. (2021).

Back at the lab, samples should be prepared for analysis (see attached protocol). They should be dried, ground to a fine powder, and analyzed for C and N (percent), typically using dry combustion analysis on an elemental analyzer. Measuring the C:N of wood is challenging due to relatively high C concentrations but relatively low N concentrations. Consequently, measuring both C and N for a sample requires analysis of two subsamples of different sizes—a smaller subsample to measure C and a larger subsample to measure N. Further, several analytical techniques can improve N detection and accuracy of results (see attached protocol). We will ask participants to at least use a larger sample size to improve N detection although following all additional steps is encouraged (see protocol appendix 1, analytical steps A and B). Finally, to ensure comparability of results between labs, our goal is to send analytical labs a small sample of a wood C:N check standard with known C and N concentrations. Prior to sample analysis, participating analytical labs will be asked to prepare a standard curve using their typical standard materials (often NIST Apple Leaf, atropine, or acetanilide) and run the check standard alongside the standard curve to ensure accuracy of results. During the analysis of tree core samples, analysts will also be asked to run one of the shared wood check standards every 10 samples. The specific details are described in the sampling and analysis protocol document. Previously collected tree core samples and data will also be considered if consistent with the protocols described below.

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**Tree Core Sampling and Analysis Protocol (DRAFT)**

**Part 1. Field Sampling**

Before you depart for the field, please watch this video carefully and consider practicing on a nearby tree: [*How to Core a Tree*](https://www.youtube.com/watch?v=jPJUewNcvao). In the field select your species and identify at least two other nearby individuals of the same species. Once your observatory trees have been selected and identified, please collect the information indicated on the data sheet and proceed as follows.

1. Use a tree tag (or equivalent) to mark the tree. Place the tag on the north side of the tree at DBH with some of the nail remaining exposed to allow for future tree growth. This will allow you to return to the same individuals for potential future samples (e.g, leaves, litter, roots, soil, etc.). Remember to select individuals that are at least 20 cm diameter at breast height (DBH), but whose diameter is no more than twice the length of the increment borer you are using.

2. Measure the tree DBH by using a tape to measure the tree circumference at 1.3 m above the ground. If you do not have access to a DBH tape, use a string (or similar) to encircle the tree, mark the circumference endpoints, measure the length with a regular tape measure, and divide the circumference measurement by 3.14.

3. Assemble the increment borer and clean it with rag or paper towel lightly doused with 70% ethanol.

4. Core the tree as described in the attached video. Core samples should be taken at breast height (1.3 m) to a depth of half the DBH of the tree, with the goal of intersecting the tree center (pith).

5. Carefully remove the tree core from the coring bit with the extractor, noting that they tend to break and fall to pieces on the ground. Make note of any differences between the inner (heartwood) and outer (sapwood) layers on the data sheet. Use a fine tipped sharpie to mark the hearwood/sapwood boundary.

6. Gently coax the hopefully still intact core from the extractor into an empty straw, cover the ends with masking tape, and clearly label the sample with the site, species, and replicate number (e.g., Lubrecht PICO R1; Lubrecht PICO R2, etc.). Also note on the straw which end of the core is from the inner part of the tree and which end is the outer part of the tree. If the core is not intact, try to separate the heartwood from the sapwood by hand in the straw or in separate straws.

7. Place all sealed straws into a ziplock bag and keep cool until further preparation and analysis (Part 2 below). To decrease confusion later, we suggest separately labeled bags for each species sampled.

**Part 2. Laboratory Analysis**

8. Upon return to the laboratory, samples should be gently removed from the straws and carefully separated into heartwood (dead wood) and sapwood (living wood) subsamples[[1]](#footnote-1) with a razor blade (Fig. 1). Taping cores to white paper can help to visually determine heartwood-sapwood boundary. However, if there is no discernable color difference between the inner and outer rings, assume the sapwood represents the outer 5 cm of the core (Heineman et al. 2022), excluding the usually much darker bark and/or phloem. The very dark outer-most layers can be discarded and should not be part of the analysis.

9. Once divided, heartwood and sapwood segments should be dried to a constant mass in a drying oven at 60°C for five days.

Chart, radar chart

Description automatically generated

**Figure 1.** Cross section of a tree trunk. Generally, the heartwood is darker (and higher density) than the sapwood, although the color differences can be difficult to discern in some species. However, sapwood is the outer portion of the wood, but inside the bark and phloem.

10. When samples are completely dry, they should be ground to a powder using a wiley mill or coffee grinder. To do so, carefully break each tree core subsample into pieces that will fit into the coffee grinder compartment. Grind each tree core section for several minutes until it reaches a powder (as fine as possible, but a few small chunks may remain). Thoroughly clean the inside of the coffee grinder between samples using compressed air and/or a clean towel.

11. Wrap each tree core subsample in a 9 x 5 mm tin capsule for analysis. For each increment sample, wrap two separate subsample tins:

* one containing 15 +/- 0.05mg of sample for N analysis
* one containing 2 +/- 0.05 mg of sample for C analysis

To do this, first weigh the empty tin and tare the scale. Next, weigh out the sample into the tin. Record the weight of sample (without the weight of the tin), fold it ([folding demo, see step 3](https://www.isotopeecology.com/collection-prep)), and place it in a 96 well plate. Record the well number corresponding with each sample. Do the same for each check standard to be run along with the samples. Once standards, samples and checks are weighed, wrapped, and identified in a spreadsheet, combust them in an elemental analyzer.

12. Once samples have been analyzed for C and N, archive remaining dried and ground wood tissue in labeled plastic or glass scintillation vials for future analysis of P and other elements of interest determined through INCyTE activities.

**Part 3. Data Reporting**

We will create a Google Sheet where participants can enter their data, and a Google Drive folder where all other project-related documents can be archived.

**Appendix 1: Instrument parameters for combustion (for Costech 4010)**

Left Furnace = 1020°C, Right Furnace = 650°C, GC Column = 70°C. Standard CN combustion and reduction column chemicals (chromium oxide + silvered cobaltic oxide, reduced copper pellets), magnesium perchlorate in drying tube, Ascarite/magnesium perchlorate in CO2 scrubber). Oxygen loop = 20 mL, mV settng of 156, gain = High w/multiplier of 6.

Additional analytical information:

If sufficient analytical capacity, proceed with the following steps as well:

A) It is recommended to supply extra oxygen for complete combustion. To do so, increase the O2 loop from the usual 2.5 – 10 mL, up to 20 mL. Use research grade oxygen (e.g., Praxair 5.0, Airgas 6.0), <1% N2). UHP is not sufficient for 20 mL oxygen loop because of increased background N levels (~30-40 mV) in blanks. Use fresh combustion and reduction chemicals for low blanks. Too little O2 for combustion causes underestimation of %N and/or sample carryover. Excess O2 must be removed (by Cu reduction tube) or else an O2 peak occurs near N2 peak.

B) Remove excess CO2 following combustion to isolate a clear N peak for measurement. Use ascarite/magnesium perchlorate to scrub CO2. When the ascarite is exhausted, CO2 breakthrough occurs and overlaps with N2 peak.

INCyTE Vertical Observatory Tree Core Collection Datasheet

**Sample Collector Name: Email:**

**Site Name: Date:**

**Site Location (general description):**

**Species Common Name:**

**Species Latin Name:**

**Species Replicate Number (Circle one):** 1 2 3

**Tree tag Number** (if marked): **Tree DBH (cm):**

**Tree Core Sample ID** (marked on straw):

**Heartwood/Sapwood Transition Obvious?** Y N

**Vertical Observatory Tree Location UTMs**

**GPS datum** (e.g.,NAD 83; WGS 84) **Zone** (e.g., 12 T)

**Stated GPS accuracy** (e.g., ± 5 m):

**GPS Coordinates:** (Please report in UTM ) (e.g., 272516 m E, 5193728 m N)

**Easting (E):** **Northing (N):**

**Additional Site Information**

**Elevation (m):** **Aspect:**

**Mean Annual Temperature (if available):**

**Mean Annual Precipitation (if available):**

**Soil type[[2]](#footnote-2)** (if available) (e.g., soil order, soil series, etc.):

**Notes:**

**Materials**

The following list includes the materials needed to collect and analyze tree cores for C and N. The most significant items are the corers themselves (need at least one, but recommend you carry a backup during sampling events), and the instrument that measures CN. Before purchasing anything, we suggest you ask colleagues if you can borrow things; most are available in other labs at universities or research institutions. Likewise, most large research institutions have someone with an elemental analyzer that is usually happy to help run samples for a small fee. The rest of it should be easy to obtain.

**Equipment to establish observatories:**

GPS

Tree tags, flagging, or other marking equipment

DBH tape (or regular tape and a section of small diameter rope/cord)

Ability to identify one/multiple tree species ☺

**Tree core sampling equipment:**

[Haglöf 10” Complete Increment Borer,](https://www.forestry-suppliers.com/p/63332/13981/hagl%C3%B6f-2-thread-increment-borers?key=GS2&gclid=CjwKCAjw_MqgBhAGEiwAnYOAeuFceMpSGPLXy2jkVtfxyGTEtr_u-RJmiuWLsRZCBsuIrQWLs_sU2hoCXSsQAvD_BwE) 2-Thread, 0.169 (4.3mm) increment borer (or equivalent)

Paper art (or plastic?) straws

Tape (masking tape, or most any tape will do)

Ethanol (for cleaning cores between samples)

Paper towels (for cleaning and drying cores between samples)

Fine tip Sharpie (to label cores and bags)

Plastic golf tees (to eject stubborn cores from the core bit)

**CN analysis:**

Razor blades (single-edged or something to divide cores with)

Aluminum weighing vessels, paper coin envelopes, or other heat resistant equivalent

Drying oven

CN Elemental Analyzer

[Tin capsules for weighing and holding wood samples (5 × 9 mm)](https://costechanalytical.com/shop/capsules-for-solid-and-liquid-samples/tin-capsules-for-solid-samples-5x9-mm/)

Coffee grinder

High-precision balance (± .001 mg)

Tweezers

96-well plate with lid or equivalent to organize, store, and transport wrapped samples

1. For some species, it may be difficult to distinguish heartwood from sapwood when cores have begun drying. If the differences are subtle after sampling, we suggest you separate heartwood from sapwood in the field and immediately after sampling or marking the boundary with a sharpie just after sample collection. [↑](#footnote-ref-1)
2. Detailed soil information for sites in the conterminous U.S. is available via the [USDA Soil Web Survey](https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). [↑](#footnote-ref-2)