Patterns and Controls of Stoichiometric Flexibility:

A Globally Distributed Study Using Vertical Tree Observatories

**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, especially as nutrient availability changes. 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 some evidence of stoichiometric flexibility in terrestrial ecosystems globally, our current understanding of the patterns and controls of stoichiometry has been informed largely from analyses of tree foliage, litter, and to a much lesser extent, soil and roots (e.g., Dynarski et al. 2022, Zhang et al. 2020). By contrast, 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. In addition, we have limited knowledge about how stoichiometry of different ecosystem components is coupled across space and time (Fig. 1). Additional stoichiometric data would enhance our ability to predict how biogeochemical cycling will respond to changing resources and could improve model predictions of Earth’s C sink by establishing stoichiometric boundaries for productivity.

To address these shortcomings, we propose a globally distributed study to explore patterns and controls of stoichiometric flexibility in forests. We propose that participants establish a set of semi-permanent *vertical tree observatories* in sites where wood, foliar, and soil samples can be obtained now, but that also allow for return visits for additional sampling in the future. Our core objective is to build a database of global wood stoichiometry. However, we also strongly encourage participants to collect foliar and soil samples to allow for us to test relationships between tree foliage, wood, and soil stoichiometry.

**Selecting trees and establishing vertical observatories** — We suggest that vertical observatories be placed within an existing experiment, in areas with disturbance or management history, along environmental gradients, and/or co-located with ongoing research. Sites may also include primary, secondary, or urban forests, and trees may be isolated individuals or grouped in a patch. Site and tree selection is left up to participants so long as relevant details are recorded in the datasheet provided. Please do not sample trees that are infected with esmerald ash borer or other pest species as we would like to prevent transporting them during sample shipping. **When collecting, all samples (leaves, wood, and soil) should all be collected at the same time**. For temperate and high latitude sites, we suggest sampling new growth after leaves/needles are fully emerged but before before leaves begin to senesce (e.g., midsummer for non-tropical ecosystems).

When selecting observatory trees, we suggest that 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, participation in the data collection effort, database development, and resulting products requires participants to collect and process leaves, wood, and soil C:N for at least three different species sampled in triplicate (three individuals per species**; total of 9 sets of foliar, wood, and soil samples). Participants are welcome to sample more, but INCyTE can only offset the analytical costs of running samples from three species. Sampling and sample preparation protocols, data sheets, and other information are included as appendices. Analytical costs   
(for up to three species) will be covered by INCyTE and will be performed in a single analytical lab (TBD).

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**Observatory Establishment, Sampling, and Analysis**

**Part 1. Observatory Establishment & Tree Selection**

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).

Record the date, time, location, GPS coordinates, environmental data, including elevation, aspect, and tree DBH on the data sheet provided.

If available, other 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 record relevant details in the data sheet as well. Sample wood, foliage, and soil as described below.

**Part 2. Observatory 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. We recommend trees be at least 10 m apart from one another, if possible. Once your observatory trees have been selected and identified, please collect the information indicated on the data sheet and proceed as follows.

Use a tree tag (or equivalent) to mark the tree. Aluminum nails are best to avoid rust and infection. Place the tag on the uphill side of the tree if it is on a slope, or the north side on level ground, 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.

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.

**Wood Sampling**

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

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).

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. Take a picture of each core to upload to the INCyTE database after sampling is complete.

Gently coax the hopefully still intact core from the extractor into an empty paper 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.

Place all sealed straws into a ziplock bag and keep cool until samples can be returned to the lab (Part 2 below). To decrease confusion later, we suggest separately labeled bags for each species sampled.

**Foliar Samping**

Sample wood as described above.

Sample foliage using pruning pole, shotgun, slingshot, or other technique (Cornellisen et al. 2003). Collect fully expanded and mature leaves from adult plants. Try to avoid (or discard) obviously sick leaves (e.g., leaves that appear like they have been affected by pathogens or herbivores) and leaves that are covered with epiphylls. Depending on your sampling approach, it may be easiest to sample whole twig sections with the leaves still attached.

Whenever possible, collect leaf samples from the upper, middle, and lower canopy positions to form one bulk foliar sample per individual. If this is not possible, please clearly document where leaves were sampled and how they were obtained. Collect at least 15-20 total leaves per species (e.g., 5-7 per canopy position) for grinding and archiving.

Place collected samples in labeled paper bags and return to the laboratory as soon as possible. Refrigerate unit processed, but no more than 24 hours after collection.

**Soil Sampling**

Sample wood and foliage as described above.

If the soil contains an organic horizon, remove it first to expose the mineral soil.

Using a hand bulb corer or equivalent, collect surface (0-10 cm) mineral soil samples at 120° intervals 2 m from the tree trunk (i.e., *N=3* per tree) and combine to form one bulked sample per tree.

Place collected mineral soil samples inlabeled plastic bags. If possible, keep soil samples cool until they can be processed.

**Part 3. Observatory Sample Preparation & Analysis**

**Wood Samples**

In the laboratory, wood (tree core) 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.

Once divided, heartwood and sapwood segments should be dried to a constant mass in a drying oven at 60°C for five days. We recommend drying immediately upon return to the lab, but if this is not possible, please freeze the cores until you can proceed with additional processing.

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.

When samples are completely dry, they should be ground to a powder using a wiley mill, bead beater or coffee grinder. To do so, carefully break each tree core subsample into pieces that will fit into the 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 grinder between samples using compressed air and/or a clean towel.

If you are packaging your own samples, 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 +/- 0.5mg of sample for N analysis
* one containing 2 +/- 0.5 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, ship them to the Cornell Stable Isotope lab (see details below) for combustion in an elemental analyzer. If you are not packaging your own samples, please ship dried and ground samples to the Cornell Lab as described in the ‘Sample Analysis’ section below.

Once samples have been prepared for C and N analysis, 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.

**Foliar Samples**

In the laboratory, remove leaves from branches, cut all petioles from leaves including separating leaflets from petioles in the case of compound leaves, and dry processed leaves at 60° C for at least 72 hours.

Grind dry samples to 20-mesh using a Wiley Mill, coffee grinder, ball grinder, or equivalent (Cornellisen et al. 2003). Wrap 1.5-2 mg of finely ground sample as described above and archive the remaining material for future analysis, or split your material in half (if possible) and ship the subsamples to the Cornell Stable Isotope Lab as described below.

**Soil Samples**

At the laboratory, sieve fresh or air-dried soils to 2 mm and dry a subsample (1-2 heaping tablespoons) of sieved soil at 105° C for at least 72 hours.

Grind dried soil subsamples to 20-mesh using a mortar and pestle, ball grinder, or equivalent. Wrap 15-20 mg of finely ground sample as described above and archive the remaining material for future analysis. If not packaging your own samples, split your material in half (if possible) and ship the subsamples to the Cornell Stable Isotope Lab as described below.

**Sample Analysis**

INCyTE is working with the Cornell Stable Isotope Lab to analyze samples collected by participants. INCyTE will cover the analytical costs of stoichiometric observatory samples sent to the Cornell lab. This includes tree core, foliar, and soil samples. Currently, we anticipate being able to cover the cost of samples from 9 trees (3 replicates of 3 species, so 9 tree cores, 9 foliar samples, 9 soil samples) from each participant. If participants wish to collect more samples than that, that is great! We will consider paying for additional samples on a case-by-case basis, but only after pre-approval from members of the steering committee.

**After you collect and prepare your samples,** **please ship them to the Cornell lab at the address below. Unfortunately, we are unable to cover shipping costs but hope that doesn’t deter anyone from participating.**

Kim Sparks/Cornell Stable Isotope Lab  
Corson Hall E440  
526 Campus Road

Cornell University  
​Ithaca, NY 14853

The Cornell lab can accept ground sample materials or tinned samples if you have access to a microbalance and would prefer to package your own samples. Dried and ground (untinned) samples should be stored in scintillation vials or whirlpak/small ziplock bags and clearly labeled with “INCyTE”, your last name, and the date (e.g. INCyTE\_Hauser\_062023). Tinned samples should be shipped in a tightly sealed 96 well plate with a sample list noting the ID of each sample by plate row/column. When you ship your samples to Cornell, please submit a digital version of your sample list via email to Emma ([emma.hauser@umt.edu](mailto:emma.hauser@umt.edu)) in addition to including the printed list in your shipment. **Please make sure your name and “INCyTE” are written clearly on each sample label**so the lab can group all our samples together easily. Please note that the Cornell lab will not be able to return leftover samples so if possible, please archive small subsamples of your sample material for later.

**Part 4. Observatory Data Reporting**

Once data and samples are collected and samples are ready to ship, participants should email [emma.hauser@umt.edu](mailto:emma.hauser@umt.edu) to gain access to the INCyTE Stoichiometric Observatories google sheet and google folder. Participants should transfer the data they collected in the datasheet in this document into the appropriate columns of the google sheet. In the folder, participants are encouraged to upload photos of their tree cores taken before grinding and any additional information they think is useful for describing their sample submission.

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):

**Foliar Sample ID (marked on bag):**

**Soil Sample ID (marked on bag):**

**Sampling notes (if needed):**

**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). 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 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 Sharpies (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

**Foliage and soil sampling**

Pole pruner, shotgun, bigshot, or something to sample tree leaves

Hand clippers/scissors/razor blade

Paper lunch bags

Hand corer

Plastic bags

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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).

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)