# Tundra Plant Trait Collection Protocol

## Why?

- To improve the coverage of plant trait data from across the tundra biome
- Allow for the calculation of species, site and inter-annual variation in key plant traits
- Allow for improved analysis of plant trait data in the ITEX and ShrubHub syntheses

## How?

- Will take 1-2 days to implement
- Raw data/samples will be submitted to coordinators at the end of the summer for further analysis, collation and error checking
- Processed measurements will be returned to collectors and will be made available to the ITEX and ShrubHub networks
- Data will be submitted to the TRY database (www.try-db.org)
- If your data contributes to a larger synthesis project, you will have the opportunity to participate in that project

## One-day Trait Protocol

- 3 trait measurements
    1. Canopy height
    2. Leaf traits (e.g., SLA, leaf dry matter content)
    3. Seed mass
- Plus 2 optional measurements
    1. Leaf dry matter content (LDMC)
    2. Wood density
- For each of 10-30 individuals per species
- Across a range of habitat types, if possible
- The number of species sampled can be decided by the data collector

## Equipment need

Low-tech method:

- Ruler or other measuring instrument
- Pen or pencil
- Clear piece of plastic or glass
- Flat surface
- Digital camera
- Coin envelopes
- A warm and dry - or at least dry - place to store samples

High-tech method:

- Ruler or other measuring instrument
- Pen or pencil
- Leaf area meter or flatbed scanner
- Software, such as ImageJ, for measuring leaf area
- A scale sensitive to 0.001 g
- A warm and dry - or at least dry - place to dry and store samples

If you have questions, you can reach us at any time:

Anne Bjorkman, UBC/iDiv (annebj@gmail.com)

Janet Prevéy, SLF-WSL (janet.prevey@gmail.com)

Isla Myers-Smith, U Edinburgh (isla.myers-smith@ed.ac.uk) – limited contact: July – August

**Detailed Protocol**

**Which ones and how many?**

**Species:**

- As many different species as you have time for! With special emphasis on *shrubs* and the *dominant species* at your site.
- Additionally there are ~100 species in the ITEX/ShrubHub dataset that are completely unrepresented in the TRY trait database (see list at end of protocol). If you have any of these "neglected" species at your site this would be another top priority for trait measurement.

**Selection of individuals:**

- Measure traits on *10-30 adult individuals*.
- If the species is very rare, then just measure as many as you can easily find.
- Measured individuals should be *independent* of one another (e.g., far enough apart to ensure that you aren't accidentally measuring different parts of the same individual, or genetic clones, etc.) - generally *at least a few meters apart*.
- Individuals should be *randomly or haphazardly chosen* for measurement, preferably in areas relatively undisturbed by humans (e.g. not part of an experimental treatment, unless that treatment is expressly noted).
- Note that all traits can be (but don't have to be) measured on the same individual

**Number of habitat types:**

- As many as you have time for. We would like to encompass as much trait variation as possible.
- If you must choose between sampling many individuals in one habitat versus few individuals in several different habitats, we would prioritize the latter.

**Materials:**

- We have included high and low-tech versions of most protocols.
- If you do not have the time or capability to do the measurements in the field, you can send us the samples for processing (please see each trait measurement for details on what to send).
- Let us know if you would like us to provide you with materials (e.g., coin envelopes, etc.).

**Priority Traits to Measure:**

**1) Maximum Canopy Height**

Maximum canopy height will encompass two similar measurements:

a) Maximum vegetative height (unit = cm): Measure the vertical height from the ground to the tallest point on the plant. If the tallest point on the plant is a reproductive structure, measure only to the highest photosynthetic tissue. Do not pull drooping branches/leaves, etc. upright. Maximum canopy height is best measured toward the end of the growing season, when plants have reached their maximum vertical height.

b) Maximum overall height (unit = cm): as above, but including reproductive structures (flowers, etc.). This should be measured at the same time and on the same plant as maximum vegetative height.

**2) Specific Leaf Area (SLA)**

- Leaf area of a fresh leaf divided by its dry mass (unit = $mm^2/mg$). We are using the with-petiole method of measuring the specific leaf area.
- There are two different protocols for SLA, one "high-tech" and one "low-tech" depending on the tools you have available at your site. High-tech requires a leaf area meter or scanner and a scale, low-tech requires only a digital camera and a coin envelope.
- Choose young-mature leaves – fully expanded and hardened but without signs of damage or disease. Ideally, collect a branch or whole plant and remove the individual leaves only just before measurement to avoid drying out. Alternately, the leaves can be wrapped in a wet paper towel and placed in plastic bags until measurements are conducted. Measurements should be done within 24 hours of collecting the leaf. When possible, measure two leaves per individual, 5 – 10 for very small leaved plants like *Cassiope tetragona*.

**High-tech method**:

- Requires a leaf area meter or scanner (to measure leaf area), plus a scale (to measure dry mass)
- Cut the leaf, including petiole, from the stem for measuring leaf area.
- Use a leaf area meter (e.g., LiCor) to measure leaf area. Alternately, place the leaf flat on the bed of scanner with an object of known length (e.g., ruler, grid paper, etc.) to use as a scale and scan the leaf and scale object. You can then determine the area of the leaf using a software program such as ImageJ (free software; http://imagej.nih.gov/ij/) or send the scanned image to us and we will measure it. If you send us the scanned image, be sure to include information about the length of the object or size of the grid used as a scale in the image. One-centimeter grid paper can be downloaded online and printed (e.g., http://customgraph.com/SG/piart.php?art=654) but please be sure to double-check the size of the grid once it is printed, since the actual size of the printed grid might be altered by

your printer settings. You can also make your own scale, by hand, using a ruler to mark of the measurements. Measure the area in $mm^2$.

- Dry the leaf at 50°C (or however possible at your field site) for 72h and, as soon as it has been removed from the heat and cooled, weigh (unit = mg). Be sure to keep track of each individual's leaves (e.g., with unique identifiers) so that each leaf's dry mass can be paired with its leaf area measurement.
- Divide an individual leaf's leaf area measurement (unit = $mm^2$) by its dry mass (unit = mg). This gives you its SLA. If you send us the final SLA calculations, please also include each leaf's raw area measurement, as this is also a trait of interest.
- Note that if you have access to a scale, it would be relatively easy to also weigh each leaf before drying as well as after drying, thus providing leaf dry matter content (LDMC) – leaf dry mass divided by its water-saturated fresh mass. See the trait protocol reference (Cornelissen et al.) for more information.

**Low-tech method**:

- Requires a digital camera, a clear plastic or glass sheet, and a coin envelope
- Cut the leaf, including petiole, from the stem for measuring leaf area.
- Place the leaf on a white piece of paper on a clipboard, table, or other flat surface. Label the paper next to each leaf with a unique identifier that can later be used to match each leaf's photo with its dry mass.
- Place the clear sheet over the leaf (or leaves) to ensure that it lies flat. As above, be sure that there is an object that can be used for scale in your photo – either an object of known length (e.g., a ruler) or grid paper. Make rulers etc. do not overlap with the leaf, and that there are no reflections on the clear sheet, as this makes it more difficult to process area measurements. Take a photo through the clear sheet that includes the leaf and the scale object and send it to us for further analysis. Be sure to include information about the length of the scale object or the size of the grid.
- If you have a microgram balance to hand, you can take a fresh weight of each of the leaves.
- Place the leaf in a coin envelope, labeled with the same unique identifier used in the photograph, dry the leaf as much as possible (e.g. by placing the coin envelopes above a heat source for several days) and keep dry until you are able to send the coin envelopes to us. Please label all coin envelopes with the species name, site name and the unique individual leaf identifier, and place all coin envelopes in a paper bag with your name, the date, and a contact e-mail.
- You can scan (or photograph) more than one leaf at a time – just be sure to label each individual leaf in the photo so that it can later be matched up with its coin envelope.
- If you have access to a microgram balance after you have returned from the field, you can take a dry weight of each of the leaves once cooled to air temperature.

**3) Seed Mass**

- Oven-dry mass of a seed (unit = mg)
- The seeds should be sampled from a healthy adult (not diseased); the seeds should be mature
- Collect at least 5 seeds from an individual. If seeds are tiny, you may need to collect more in order to be able to weigh them (e.g. in batches of ten or even more).
- Remove any seed accessories (wings, comas, pappus, elaiosomes, fruit flesh) but do not remove the testa – in other words, measure only the seed, not the fruit.

- Dry the seeds at 50°C for at least 48h (or until dry in whatever conditions you have available in the field).
- Once dry, remove from oven and weigh immediately once cooled.
- If you do not have a scale available, place dried seeds in a coin envelope and send to us for measurement! Please write the species name, site name, and the number of seeds in the envelope on each envelope, and place all coin envelopes in a paper bag labeled with the date, your name, and a contact e-mail. Seeds from the same individual can be placed in the same envelope, but different individuals should be in separate envelopes.

**4) Optional: Wood Density**

- Equipment required: A saw, clippers or other instrument to cut a "cookie" (stem section) from the woody stem of shrub species (woody species only)
- Cut a ~3-cm-long (or longer) section of the main stem, at a height of approximately 1/3 the height of the plant of the largest stem (or a random/arbitrary stem if the largest stem can't be harvested or determined), remove any soil and loose bark. If you have a balance to hand you can take a fresh weight of the sample.
- If you can measure fresh volume in the field:

  1. Measure the volume using water displacement. If you have a precision balance place a small beaker of water on the balance and tare the balance. Then place the wood sample in the water and use a needle or tweezers to push the sample below the water level displacing the water. Record the weight in grams when the sample is under water (the measurement will fluctuate a bit as the sample moves around, so just estimate the median number). The weight in grams is equivalent to the volume in $cm^3$.
- Air-dry the sample and store in a paper bag. Please write the sample name, species name, site name on each envelope, and place all coin envelopes in a paper bag labeled with the date, your name, and a contact e-mail.
- Mail any samples collected to us at the end of the season for measurement, along with associated fresh mass and volume is measured.
- If you want to measure the density in the lab:

  1. Oven dry the samples (at 50°C) - take a dry weight once the samples are cooled.

  2. Measure the volume using water displacement. If you only have a volumetric flask available, submerse the stem in water-filled volumetric flask. Otherwise, if you have a precision balance place a small beaker of water on the balance and tare the balance. Then place the wood sample in the water and use a needle or tweezers to push the sample below the water level displacing the water. Record the weight in grams when the sample is under water (the measurement will fluctuate a bit as the sample moves around, so just estimate the median number). The weight in grams is equivalent to the volume in $cm^3$.

  3. To calculated the density divide the oven-dry mass of the stem section by the volume of the section (units: $mg/mm^3$)

  4. Soak the sample in water over night or for a couple of hours to take a water-saturated weight (which approximates the fresh sample weight).

For more information:

http://chave.ups-tlse.fr/chave/wood-density-protocol.pdf

**More information**

If you are considering sampling other traits or would like to read in greater depth about sampling protocols of the traits listed here (including exceptions and special instructions for "unusual" species), please see:

Cornelissen, JHC *et al*. 2003. **A handbook of protocols for standardised and easy measurement of plant functional traits worldwide**. Australian Journal of Botany. 51: 335-380.

PDF available online at: http://www.cedarcreek.umn.edu/biblio/fulltext/t1936.pdf

Please send data to:

Isla Myers-Smith, University of Edinburgh (isla.myers-smith@ed.ac.uk)

Anne Bjorkman, UBC/iDiv (annebj@gmail.com)

Janet Prevéy, SLF-WSL (janet.prevey@gmail.com)

And any samples collected (coin envelopes, stem sections in paper bags, etc.) to:

Isla Myers-Smith
School of GeoSciences
University of Edinburgh
113 Crew Building, The King's Buildings
West Mains Road
Edinburgh, Scotland, UK
EH9 3JN
phone: +44 (0) 131 650 7251
e-mail: isla.myers-smith@ed.ac.uk

Please e-mail us before sending samples so that we will know to expect them.

Please also fill out the attached metadata form with information about your site (location, lat./long. if possible, general description of site and habitat, etc.), along with any other information you think might be useful, including notes about how your sampling might have differed from the protocol, etc. This can be emailed to us or printed and included with the samples shipped.

**Thank you very much for your participation!**

**Species of greatest interest:**

(However - we could use measurements from any species observers are interested in!)

- *Alnus spp.*
- *Betula glandulosa*
- *Betula nana*
- *Carex aquatilis*
- *Carex bigelowii*
- *Carex flavocuspis*
- *Carex pyrenaica*
- *Cassiope tetragona*
- *Deschampsia spp.*
- *Dryas integrifolia*
- *Dryas octopetala*
- *Empetrum nigrum*
- *Eriophorum angustifolium*
- *Eriophorum vaginatum*
- *Geum rossii*
- *Juniperus nana*
- *Kobresia myosuroides*
- *Ledum palustre*
- *Potentilla spp.*
- *Salix arctica*
- *Salix herbacea*
- *Salix pulchra*
- *Salix richardsonii*
- *Vaccinium uliginosum*
- *Vaccinium vitis-idaea*