TAPIOCA trait sampling protocol

Updated 3/7/2012

1. WHEN- species will be sampled when at least 30% of the population in the experiment are reproductive, but before green tissues start to senesce on the sampled plants.
2. WHO- we want healthy, mature individuals. Nothing sick looking or heavily herbivorized. Plants should be selected haphazardly from the trait plots as the first priority, then from intermediate density background plots (that aren’t influencing targets!) as a backup. Plants along plot edges and walkways can be used if others are not available, and if all else fails, we can go to individuals growing the grassland immediately surrounding the experiment. I don’t want to use plants from lower down in the reserve, as we’ve noticed clear (likely plastic) morphological differences in the lower populations. When selecting individuals for root excavation we’ll be somewhat pragmatic, preferring individuals that aren’t growing next to large rocks or that are too near other high-value plants for trait sampling.
3. HOW MANY- we’ll target 8 individuals per species for whole- plant sampling (including root excavation), and another 7 individuals for above ground tissue harvesting only. Proper maximum height estimation will require sampling another 40 or so individuals (non-destructively).
4. HOW: **Trait measurements:**
5. **Measure Height:** Length from base to farthest vertical structure, along the main axis of the plant. This does not include scandant spreading braches in Lotus, for example. Max height of a population is often calculated as the 95% quantile of the population.
6. **Measure Canopy area:** The length of two major axes of the outline of the canopy projected straight down to the soil. Stand over the plant, looking down on the canopy to identify the projected canopy area. Measure the length of the longest axis along the ground. Define the second axis as perpendicular to the major axis. The area described by this ellipse can be used as a trait itself and as Asoil in the calculation of the leaf area index LAI= Aleaf/ Asoil.
7. **Excavate plant:** Using the trenching shovel and/ or hand trowels, cut a soil plug (core) that is 10 cm in diameter and 10 cm deep., centered on the target plant. Goal is to get representative sample of root system. If we can get entire root system in that core, even better. Remove the ABG of non-target plants prior to excavation. If the dimensions of the plug differ (might need to go deeper for larger plants, for example), record them- soil volume extracted will allow us to calculate root standing biomass (mg root per vol. soil) and the area of the core will allow us to calculate root length density (m root per area of soil). Bag the soil core in a ziplock, place in cooler. Sample needs to be kept cool and moist prior to processing.
8. **Harvest above ground biomass:** clip plant at base of soil (or slightly below), wrap in moistened paper towels, and place into ziplock in cooler. Sample needs to be kept cool and moist prior to processing.
9. **Go to processing area** ASAP.
10. **Check hydration status of AGB:** Leaves should have been kept cool and moist for no more than 24h, ideally just a few. If time is more than a few hours since harvest, follow rehydration procedure in Cornelissen et al 2003 (place plant in test tube of DI water for 6 H in the dark)- ideally we’ll work fast and not need to do this step. Blot any excess moisture from the plant.
11. **Select three leaves for detailed analysis** Leaves should be photosyntheticly active, in good shape, and fully hardened (ie- not expanding). Idea here is to capture a range of any ontogenetic change in leaf morphology. L1 will be oldest, ideally close to the base, L3 will be a newer leaf towards the tips, and L2 will be intermediate.
12. **Measure length and width of target leaves.** Take widest point for width.
13. **Record fresh weight of target leaves** for leaf dry matter content. Use 4 decimal balance in lab.
14. **Scan each target leaf one at a time** on Cannon scanner. Place ruler at one end of image, set options to 600 dpi, black and white photo, and letter size area scan. Each target leaf needs it’s own scan, and the area of each image needs to be the same to facilitate automated analyses later. Give each image file a name that reflects species, plant number, and leaf number, eg LACA\_P1\_L2. This will be used for SLA of each leaf, and also added to total plant leaf area later.
15. **Scan remaining leaves of plant**- Remove remaining leaves from plant, place all together on scanner bed and scan as one image (or more if needed- OK to split large leaves into pieces so long as they do not overlap). This will be used for measuring the total leaf area, which will give us leaf area index (LAI= Aleaf/ Asoil) and related measures.
16. **Dry all AGB fractions** at 60 degrees C for 72 or longer if needed to achieve constant mass. There will be separate envelopes for target leaves 1, 2 and 3, one envelop for all remaining leaves AGB (stems and flowers, and extra leaves).
17. **Wash roots:** Over a fine sieve (the 0.5 mm ones in the lab- there should be 2 of them), gently wash roots to remove soil from root system. Pick any disarticulated fine roots out of sieve with forceps and include in final sample. Be sure to remove roots from neighboring plants, if any. For time being, preserve sampled roots in 70% ethanol in a jar for later analysis- when ready go to root staining step.
18. **Stain roots** in neutral red dye. Might not be needed, but likely will be to enhance contrast for scanning. Stain for 24h then rinse.
19. **Scan roots** and analyse in WinRhizo software. Remove fine roots (<= 2mm) from sample in jar. Spread out in water on scanner. Use default settings in WinRhizo with dpi set to 600. Measure total root length, average diameter of fine roots (defined as roots < 2mm), and anything else that might be easy to capture with the software!
20. **Dry roots** in foil weighboats in oven at 60 degrees C for 72 hours (or longer as needed to reach constant mass).
21. **Weigh AGB fractions and root samples.** Allow tissues to cool inside desiccator or ziplock filled with desiccant. Tissue will absorb moisture from the air when warm otherwise- mostly an issue for small samples. Record weight of each part separately (L1, L2, L3, remaining leaves, non leaf AGB and roots). Might need microbalance to weigh target leaves and roots of small plants.
22. **Check all calculations** that might require re**-**weighing of dried samples (ie- SLA, LAI, total AGB fraction). Once tissues are ground there is no going back…
23. **Bulk leaf samples for grinding** Combine approximately equal biomass of leaves from each individual of each species.
24. **Grind bulked samples** from leaves of each species in WiggleBug grinder in D’Antonio lab. Be sure everything is super clean- this is for isotopes.
25. **Weigh out samples** of ground leaf material into tin capsules- microbalance in D’Antonio lab.
26. **Send to Dawson lab** for leaf [N], [C], and N and C isotope ratios.
27. **Still might decide to do:** leaf [P], root [N] and [P] (need to find lab). If we do want to do this, we might want to harvest the ABG of any trait plants as they hit maturity to be sure we have enough material. Some of the standard labs I know of (like DANR at UC Davis) require a lot of plant material for P analysis.

**What all this will give us:**

**“Performance” traits (not really traits per se, but might be helpful):**

Individual plant height

Above ground biomass

**Functional Traits (N= 15 unless noted)**

Seed weight (average per species)

Seed volume

Maximum height (feature of population, not individual)

Canopy area

Leaf Area Index (area leaf per area soil covered by canopy)

Leaf length and width

Specific Leaf Area (weight fresh leaf area per dry leaf mass)

Leaf Dry Matter Content (dry weight/ wet weight)

Leaf [N] (one value per species)

Leaf C/N ratio (one value per species)

Leaf 13C (one value per species)- linked to water use efficiency

Leaf 15N (one value per species)- linked to nitrogen use efficiency

Specific root length (N=8)- length fresh root per unit root dry mass

Fine root diameter (N=8)- average diameter of roots < 2mm

Root diameter distribution (N=8)- not really a codified trait per se, but there is more information in this distribution that we could use, particularly about taproot size and volume, perhaps.

**Additional traits we could get:**

Leaf [P]

Root [N]

Root [P]

Root depth distribution via coring. Will try this, but I’m doubtful it will work well with these plants in this soil type that we have. Might be better to measure something on the washed root sample than this- but hard to know you are getting deepest fine roots without really making a big mess and digging a ton.

Leaf pH- not sure exactly what this will yield, but is an option

Leaf [Ca]- Oscar’s suggestion- perhaps could be done @ same place as leaf [P]

Root/ Shoot ratio- will be hard without exhaustive excavation of roots. Current protocol doesn’t require that we get everything. We will be getting a BGB estimate, but it’s not necessarily exhaustive.