**Field gear:**

* **Camping stuff, bear canister, bearspray, usual camping stuff. Sunscreen, sunglasses, hat.**
* **First aid kit.**
* **Permits.**
* **GPS, camera, tablet (all three should be included in tablet).**
* **MultispeQ**
* **Charging cord for tablet, phone.**
* **Field notebook and pencils. Make sure there is enough space to write everything down in case tablet fails.**
* **Laptop to make more detailed notes via typing.**
* **freezer with battery**
* **Small soft sided cooler**
* **Soft sided cooler with blue ice**
* **Latex gloves. As long as one is careful I dont think these are needed. All manipulation will be via forceps. Would need to change gloves constantly for them to really do any good.**
* **clipboard**
* **Whirl packs**
  + **Large ones for soil**
  + **Med. sized ones for plants**
* **At least three sharpies, and three pencils.**
* **Scotch tape for affixing leaves to paper. At least four rolls.**
* **Drop retention tool (binder with protractor and level)**
* **Plastic pipette.**
* **Water bottle that is easy to pipette out of.**
* **Compass**
* **Clinometer**
* **Height gauge tool**
* **DBH tape**
* **Possibly a slingshot and band saw and string and object to tie string to to shoot over branches. Could just physically throw a heavy object (like a bolt) with a line affixed to it over higher branches**
  + **Ignoring this for now, if it becomes problem to get samples then add back to list.**
* **2 fifty meter tapes and stakes to hold them down (tent stakes work, bring enough stakes to tack them down well in areas that are windy.)**
* **Flagging**
* **Duct tape**
* **Tape measure.**
* **Flora of Wyoming, Astragalus book, other flora and field guides.**
* **Scissors**
* **Forceps (big ones)**
* **Napkin/towel to wipe off scissors.**
* **Ethanol to spray on scissors to clean them if they get grimy**
* **At least 4 lighters to flame sterilize equipment. One of which should be wind resistant.**
* **Binoculars to look at bears and moose!**
* **SOIL SAMPLING equipment:**
  + **corer**
  + **larger whirl packs,**
  + **soil thermometer (marked to have 1cm increments**
* **Densitometer**
* **Quadrat frame and a sample card (or just mark the frame) to provide insight into what 5 and 10% is.**
* **Penetrometer and the associated plexiglass deck. Perhaps could affix this to the droplet retention folder.**
* **Plant press, newspaper, index cards for plant labels.**
* **Binder with plastic sleeves for paper that will have leaves taped to it for SLA measurements.**
  + **white paper**
* **Very big freezer ziplocks, biggest I can find. To hold all samples from a location.**
* **Mid size/smallish ziplocks for all whirl packs from a taxon at a location**

**Sampling locations**

Choose mountainous areas with at least a few thousand feet of vertical gain (so far all locations go from at least ~7k–11k, with some locations going from 5–6 all the way up to 12–13k). Sites do not need to be on the same mountain, but they should be within the general region (e.g. the Snowy Range). All sites will be on west aspects, or mountain tops/plateaus where aspect is uniform, to minimize between site variation due to aspect. Sample the top (alpine), the subalpine meadow/forest boundary, and the subalpine forest (might skip this in favor of a site lower in the sagebrush), and sagebrush/prairie. Need to sample 6 mountains, 3 locations on each mountain, possibly 4 locations on each mountain. I suspect if I sample in a dense subalpine forest there wont be very many taxa to sample, so these sites could be gained at low cost. Sampling on a mountain could be random within a habitat type, but it may work out to where I pick locations based on plants present, similarly to the surroundings, and ease of access. I wanted to go with pseudorandom sites (mountain chosen, but site random), but the more I think about the less practical it seems as I need to target certain plant taxa and I am not sure if it will really add that much to pick sites in a truly random way for my questions. All transects are car accessible for the lower sites, and most the lower two sites are car accessible, only the high alpine is not.

**Sampling time-**

Peak biomass prior to wide-scale senescence across focal non-evergreen taxa. This is defined as 30-40% of non-evergreen taxa beginning to senesce. Ideally everything will have had true leaves a month, and the vast majority of taxa will be flowering or fruiting. The latter both to signal maturity, but also to aid identification. Will only sample plant taxa that are flowering or fruiting/ or post fruiting. No purely vegetative individuals will be sampled unless they don't reproduce each year.

-Sampling time will be confounded with altitude. TRY to sample higher altitude sites at lower latitudes at the same time as lower altitude sites at higher latitudes in a few cases. Also, save several low altitude sites for sampling later in the season, once the high altitude sites will be sampled. By sampling in terms of PHENOLOGY and not date some standardization will be applied. Will include sampling date and phenology and hopefully first flowering date as covariates (the latter to be gleaned from the interwebs and herbarium records etc.). I think this is a weakness of the study that I see no way to avoid.

**Species selection**

-the most abundant taxa in each functional group at each site must be sampled.

-ideally the taxa sampled should sum to >80% of the total biomass in the area (see citations in Perez-Harguindeguy et al. 2016)

-rare taxa should be sampled as well. These will be taxa that are unusual to the area, and/or are at very low abundance (<1% total biomass as estimated occularly. Basically there aren’t many individuals of these taxa present.)

-Need to sample across life histories and growth strategies.

-Need to sample across as many plant families as possible

If I sampled every taxon at only 3 locations, then I could sample 42 taxa. Here I will choose which taxa to sample.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Species** | **#of sites** | **type of site** | **Family** |
| Trees | *Pinus contorta* | 5 | high elevation subalpine forest and meadow | Pinaceae |
|  | *Pinus albicaulis* | 3 | high elevation subalpine forest and meadow | Pinaceae |
|  | *Pinus flexilis* | 3 | high elevation subalpine forest and meadow | Pinaceae |
|  | *Picea engelmanni* | 5 | high elevation subalpine forest and meadow | Pinaceae |
|  | *Abies lasiocarpa* | 5 | high elevation subalpine forest and meadow | Pinaceae |
|  | *Pseudotsuga menziesii* | 3 | mid elevation subalpine forest and meadow | Pinaceae |
|  | *Populus tremuloides* | 5 | subalpine forest and meadow | Salicaceae |
|  | *Juniperus communis* |  |  |  |
| Total | 7 species of trees expected. |  |  |  |
| Shrubs | *Alnus incana* | 3 | riparian areas mid elevation | Betulaceae |
|  | *Amelanchier alnifolia* | 3+ | low elevation subalpine forest | Roseaceae |
|  | *Artemisia tridentata* | 6+ |  | Asteraceae |
|  | *Mahonia repens* | 3+ | subalpine forest | Berberidaceae |
|  | *Physocarpus malvaceus (*ninebark) | 3+ |  | Roseacae |
|  | *Purshia tridentata* | 2+ | lower elevation meadows. May not sample down low enough to get this plant often | Roseaceae |
|  | *Ribes montigenum* | 3+ | subalpine forest | Grossulariaceae |
|  | *Salix arctica* | 4+ | alpine, subalpine | Salicaceae |
|  | *Vaccinium myrtulus* | 3+ |  | Ericaceae |
|  | *Arctostaphylos uva-ussi* | 5+ | subalpine forest | Ericaceae |
|  | *Acer glabrum* | 3+ | riparian areas in lower subalpine | Sapindaceae |
| Total | At least 10. |  | Will pick only one Roseaceae probably. |  |
| Forbs | *Frasera speciosa* |  |  | Gentiaceae |
|  | *Erythronium grandiflorum* |  |  | Liliaceae |
|  | *Lomatium spp.* | 4+ |  | Apiaceae |
|  | *Chaenactis alpina* |  |  | Asteraceae |
|  | *Polygonium bistortoides* |  |  | Polygonaceae |
| Graminoids |  |  |  |  |

This is not complete. See species sampled csv for more.

**Individual selection within a focal taxon**

-choose 10 reproductively mature, healthy looking individuals (Perez-Harguindeguy et al. 2016). I will avoid seedlings/saplings, and will only choose trees that are large enough to produce fruit.

-Individuals will be choosen randomly by plotting xy coords and choosing nearest individual. If individuals do not grow throughout site, such as forbs, grasses, and rare plants, then sample haphazardly for these taxa. Choose individuals that encompass the variation in obvious trait values present (e.g. floral color, leaf size), but avoid non-reproductive individuals.

-If taxon is clonal (e.g. aspen, willow) try to sample multiple clones if present. If only one clone present, then sample ramets as you would non-clonal organisms (e.g. randomly, haphazardly, depending upon abundance).

-try to choose plants that are growing in the “typical” way for that plant at that location. In other words, if 90% of the taxon is growing in the shade, don’t sample the ones in the sun. This is a bit different than normal, where traits are often measured on plants that are in the sun and growing in favorable conditions (Perez-Harguindeguy et al. 2016). It makes more sense to me to sample what is “typical” rather than the trait values of plants growing in optimum conditions.

NOTE: was originally going to sample only 5 individuals, but I think it makes more sense to sample 10 individuals per taxon and fewer taxa because it would be a real shame to not be able to look at intraspecific variation due to poor sampling.

**Leaf selection within a focal individual**

-Sample three leaves/leaflets from each focal taxon. Avoid obviously young or old leaves. Whenever possible make sure each leaf is from a different branch/stem and is facing a different cardinal direction. Do not pick leaves from the inside of a canopy, but instead choose leaves on the outside so long as they are not recently unfurled. I considered standardizing cardinal direction, but we know microbes will differ on either side of plant. Since we want to know about the totality of microbes on leaves, then we should sample all sides of plant, even though this will inject noise into the data. If we want to parse microbe habitat into microhabitat on a plant that will require a different study.

-if the plant is a cactus or has otherwise huge leaves that don’t make sense to collect in their entirety, cut at least a half inch x half inch chunk out of each leaf. Dont need to sterilize scissors between leaves for an individual because leaves will be pooled anyway.

-avoid leaves that have a lot of herbivory or are discolored.

-Measuring leaf traits. measure the nearest leaf to the leaf that is selected to be sequenced. Make sure the other leaf is similar in terms of cardinal direction, size, etc. Will collect all trait values from the exact same three leaves. *It might be possible to cut a piece off focal leaves of a standard dimension (e.g. a hole punch) then make all trait measurements on the same leaf as the one that got sequenced. EXCEPT this would not work on plants with very small leaves, such as most alpine plants. Need to think about this more. The way it would work is that the sample for sequencing would be collected, then everything else measured for the leaf. This might be worth doing when possible. The downside is that I would not be sequencing as much of the leafs microbiota. Also, I would have to wash epiphytes off the leaf, then I would have a wet bedraggled leaf that has been agitated to do measurements on...the microspeQ for instance wouldnt be happy. I don’t think I will be able to measure the exact same leaves….This is another weakness that I think may be unavoidable.*

**Covariates:**

Expected range or levels are shown for continuous and categorical variables respectively

**Site level measurements:**

**Sampling date:** (continuous) 0–365 from beginning of year to end of year.

**Location:** GPS measurement at center of plot.

**Aspect:** Continuous (0–365) Not accounting for declination. The direction of the prevailing slope. Measured with compass. Declination could be accounted for post hoc easily.

**Slope:** Continuous (0–30, I doubt I will sample steeper than that). The angle of the prevailing slope. Measured with clinometer.

**Four most abundant plant taxa:** Categorical. Don't see what I would do with this variable for this study, but it was measured for the sites from 2017 and will likely be measured into the future, so it could be a useful way to lump sites by vegetation similarity down the road.

**Atmospheric temperature:** Continuous. Should be measured by the multispeQ

**Barometric pressure:** Continuous. Should be measured by the multispeQ

**Cloud cover:** Categorical: sunny, partly cloudy, overcast. Won't collect if it is raining.

**Count number of living trees of greater than 8" dbh:** Continuous. This seems a bit redundant with canopy cover, but was measured in the past.

**Count number of dead, downed trees that are at least 12" at their widest:** Continuous.

**Pictures:** What good would sampling from the top of a mountain be without taking some good pics?! Also will get various nice pics of focal taxa for presentations, EOD, etc.

**Canopy cover:** Continuous (0–100). Densitometer measurements. 21 measurements for a plot. Do not keep track of the location of each measurement in 2d space.

**Daubenmire plots:** 5 plots. these will be collected with soil samples. See below. Will be used to measure plot **structural diversity,** and **% shrub, forb, graminoid** at the plot level.

**Tree canopy variation**– to be computed post hoc by taking the height measurements for the trees I sample and calculating the coefficient of variation, and maybe actual variation too...will just play with this post hoc.

**Metrics of plant diversity –** quadrat measurements will be taken at 4 locations throughout the plot that cooccur with soil samples. Will measure % cover of all taxa within the quadrats. If I dont know what a taxon is I will identify, or if time is very pressing (e.g. grizzly nearby, rampaging moose, or thunderstorm brewing) will just give it a number (e.g. unidentified forb #1 from sampling location "Medicine Bow mountaintop". Cover classes will be 0-5%, 5-10% and 10% increments after that. This should be enough to calculate veg diversity indices.

**Plant richness–** For richness, actually count the total number of plant taxa I find in a plot. Take pictures of all of them, and try to identify them if they are to be sampled. Will take voucher specimens of plants that I will sample.

*Data to be extracted from digital repositories or models:*

**Mean, max, min annual temp and precip:** Continuous. To be extracted from PRISM. Averaged over last 5, 10 years. (note: it might be interesting to do a sliding window analysis where we see how many years past is the best predictor of various microbes).

**Mean, max, min temp and precip by season:** Continuous. To be extracted from PRISM, see above.

**Variability in temp and precip annually (maybe by season too):** Continuous. To be extracted from PRISM, see above.

**NDVI:** Continuous. From landsat images.

**Palmer drought severity index:** Continuous. Averaged for last 5 and 10 years for each site

**Measurements made for every focal plant species:**

**Life history:** (categorical) **Levels (post hoc assignment, no need to do in field)**:

annual,

biennial,

monocarpic perennial (assuming this will be herbaceous, this category may not exist in sampled taxa. If woody monocarpic perennials exist that would be another level),

herbaceous perennial,

woody stemmed perennial.

**Growth form:** (categorical) after (Perez-Harguindeguy et al. 2016). Levels:

herbaceous: rosette;

herbaceous: elongated, leafbearing rhizomatous;

herbaceous: pulvinate;

herbaceous: many-stemmed herb;

graminoid (this might should be lumped with many-stemmed herb, but can do post hoc);

herbaceous: tussock;

Semi-woody: stem succulent; (note that I am not including bambusoid or palmoid here as we wont find any);

woody: prostrate subshrub;

woody: dwarfshrub (<0.5m tall);

woody:shrub(0.5-5m);

woody: tree;

woody: dwarfed tree (Krumholtz);

Epiphyte;

Lithophyte (growing out of a rock more or less, some ferns perhaps);

herbaceous vine;

woody vine;

parasite/saprophyte;

hemiparasite (e.g. Castilleja).

**Additional traits extracted from TRY (an online trait database):** For trees and shrubs and common, widespread plants, there will likely be extra traits measured at the species level. For instance, specific root area, wood density, relative growth rate, etc. These will of course vary by habitat, but I could either take mean trait values from plants measured in similar locales, OR just chose the “stable” traits that don’t vary much intraspecifically (see Perez-Harguindeguy et al. 2016 for references and a brief discussion on which traits are stable). LOOK for first flowering date, or date of leaf emergence, or leaf longevity as those traits would tell us how long a leaf had been exposed to inoculum.

**Drip tips:** categorical, yes or no.

**Species specific notes**: make note of odd things about the species that dictated minor changes in protocol. For instance, if species has big leaves (e.g. Heracleum) then all measurements other than SLA might be made on the same leaf without fear of contamination. Just want to keep track of which taxa I measure the same leafs that we sequence, and which taxa we measure similar but not identical leaves. Other notes of interest might be that they were totally covered in aphids or something.

**Measurements made for every individual**

**Phenological state:** (categorical) Levels: vegetative, flowering, fruiting, post-fruiting but not senescing, senescing. Notes: will try to only sample flowering and fruiting individuals for forbs and graminoids, but this may not be possible.

**Associated host**: for epiphyte and parasite, hemiparasite, saprophytes only. Record the host that the focal plant appears to be associated with. I suspect this will be important for Castillija and Orobanche, and maybe mistletoe).

**DBH (Trees only):** if multi-stemmed then measure the stems that you sampled from and include all measurements. Will likely average these. Measure 1.5m above ground (ish).

**Height of foliage:** Measure the height of the mass of foliage from ground. Do not include the odd branch sticking up or the inflorescence sticking up out of a rosette. The point here is to measure where the bulk of the leaves are and where the samples to be sequenced are coming from. For rosettes measure the height of the rosette. For herbaceous plants, will measure height at which foliage reaches, and ignore height of inflorescence as we won’t be sampling flowers/fruits. IF i measure epiphyte, measure its height above the ground, and the length of the longest stem to its point of attachment (these data will need to go in notes, as I doubt this will happen enough to warrant addition to the digital form).

**Height of samples:** Measure the height of all three samples and average. I know averaging loses data, but extracting each leaf individually would explode costs and is not practical.

**Proportion of plant above samples:** this is for trees mostly and will be calculated post hoc. It will be a continuous variable describing how much of the trees height is above where we sampled. The more tree above a sample, then the more inoculum will likely rain down on lower branches. Many, many studies have found higher microbial (or at leaf fungal) colonization in lower branches.

**Width and length of plant**–measure widest portion of plant and then the width perpendicular to that. These will be multiplied with height to get box measurements of plant size. Will be hard to measure for trees. I guess will just do my best.

**Branching architecture:** continuous. Not relevant for many plant taxa obviously. For branched plants, choose the three branches that leaves were sampled for sequencing, and measure the number of branching nodes before you get to a terminal branch that doesn’t have leaves. Measure the length of the branch to the tip of a leaf and then divide the number of branches by that length. This is called apical dominance index.

**Mean leaf angle**: measure three leaves that are about the same angle as those that are sampled for sequencing. This is a proxy for how long water would stay on a leaf I think.

**Droplet retention ability:** put a drop of water on a leaf and measure the angle the leaf has to be for the drop to first move. Would need to use a standard drop size (say 10ul) using a plastic pipette, that is light, cheap and could be reused. I could probably tape a leaf to a fixed, rigid surface (e.g. a hard plastic board affixed to a 3 ring binder, and raise this surface slowly until the drop moves. Could affix a protractor to the binder crease to measure the angle. Would always tape leaf with drip tip facing the crease in the binder, so that the leaf would be in a natural orientation. This sounds ridic. but I think this would be good to measure as leaf surface wetness is likely critical for foliar microbes.

**Leaf area:** See SLA below. Try to measure automatically. Would be cool if I could measure greenness or something too, but don’t put too much effort into figuring that out as such measurements will likely be redundant to what the microspeQ measures.

**Specific leaf area:** fresh leaf area divided by leaf dry mass. Measure leaf area in the field for three leaves by placing them on the binder, use the same leaves that we measured for everything else, which should have a square of known dimensions on it. Take a picture of the leaf and have a program compute leaf area. Save the image to the tablet in case there is a problem and imageJ used. Tape the leaf to a piece of paper that has labeling info (e.g. species, site, indiv, leaf) and put this in a binder with plastic over the leafs to protect them. Take them back to the lab and dry them either in an oven, or just let them sit there, then weigh each leaf once dry. Leave petioles on, this is important to remember bc some people cut the petioles off or measure them separately. This seems like a waste of time for my purposes.

**Force to punch/Leaf toughness**- use a penetrometer. Measure same three leaves measured for everything else. Use plexiglass deck. Likely will affix this to the folder for droplet retention measurement, so it is all in one spot. Just tie the clamp with the deck to the folder.

**C/N, %N, delta13C, and possibly N15 info –** will process the same three leaves as measured for other traits, ground, pooled, and aliquoted. Will get SIF, or the SIF facility at UNR to measure. This is 6.50 a sample and will likely require undergraduate help. Worst case scenario here is I only measure several individuals per taxon (say 3–5) if money gets too tight. Will do this last, so that I can gauge fund availability. These are critical traits though (especially %N) so I really would like to measure them. In a study of Astragalus lentiginosus I found that these traits were not predictive of overall microbial richness, but I didn’t do microbial taxon-specific analyses. Nobody has really looked at these traits as they relate to microbes as far as I know (Steve Kemble may have one paper that had some of these measurements, will have to check).

**MultispeQ measurements!! (super cool)–**For all of these measure the same leaves that we measured for everything else, except sequencing.

**Leaf angle of wilt - MultispeQ**

**Leaf cardinal direction - MultispeQ**

**Leaf temperature -MultispeQ**

**Chlorophyll amount - MultispeQ**

**Photosynthetically active radiation - MultispeQ**

**Leaf thickness**: measured by microspeQ. Measure middle of leaf while avoiding midrib or veins of course. Measure three leaves. Will measure along with all the other measurements conducted by microspeQ. May need to use dried pine needles and cut them and measure with calipers if the microspeQ doesn’t work on them.

**Soil measurements.**

1. in situ:
   1. temperature
2. fresh soil sample:
   1. Gravimetric moisture
   2. Microbial biomass C and N
   3. Mineral N (NO3, NH4)
   4. Dissolved organic C and N
3. air dried soil sample:
   1. pH
   2. EC
   3. total C, N, P
   4. Inorganic C
   5. Soil texture (particle size distribution)
      1. Phosphorus (available and total)
      2. CEC (cation exchange capacity)
      3. 13C, 15N
      4. GC-MS of Water Extractable Organic Matter (WEOM). Complements/ speciation of total C, N measurements
4. Frozen soil sample (-20C).
   1. DNA extraction
   2. enzyme analysis

**PROTOCOL**

NOTE: all data collection will be via an Android form, which has already been made.

Labeling of samples will be of the form: “Mountain\_site\_taxon\_indiv” where mountain will range from 1–6, site from 1–4, taxon from 1–25 followed by species name (e.g. 2AsLe, or 13PiCo), and indiv from 1–5.

Detailed protocol.

Note. Every step could be a stopping location. However it will be important to either leave the tape measures in place via staking them down, or, make sure to get the transect tapes in approximately the same location the next day. A few m either direction should not matter that much in my mind. Bring flagging to facilitate reinstalling the tapes. Will try to get sampling very early in day to avoid storms.

1. Choose sampling location. Sampling location needs to have a number of taxa that are focal and be on a West aspect. Focal taxa will include those that are common at a given elevation across the state (e.g. P. contorta at mid-elevation). Do not choose weird (e.g. strange soil, atypical plants), extremely steep or treacherous, or highly disturbed sites. Pick a location that has one major slope (e.g. dont pick a steep sided creek valley with both north and south aspects). Sites will be chosen by walking up a mountain and finding a spot with a lot of good plants. This will not be random at all, but will ensure we can address the questions of how is intra- interspecific variation in microbes associated with plant growth history, traits, and phylogeny. Try to avoid edges or sites that include drastically different habitat because then site level measurements wont be as useful (e.g. if canopy cover in half the plot might be 100% but the other half near 0%, then densitometer measurements may not be super useful, and soil type will probably change alot) The point is to pick typical locations with typical plants. This will involve judgement calls, but that is ok given that the focus here is on capturing plant trait variation.
2. Lay out 50m transect tapes in a big + such that the plant taxa I want to sample are in the plot. Make the axes of the plot align with cardinal directions.
3. Take a GPS coordinate at the center of the plot. Take pictures in the 4 cardinal directions.
4. Date should be auto-recorded by form.
5. Measure slope with a clinometer
6. Measure aspect with compass
7. Measure air temperature This will be done with multispec
8. Measure cloudcover. Categorical: sunny, partly cloudy, overcast These terms are vague, but I doubt this will be a useful covariate anyway.
9. Generate a random coordinate for soil sampling, and veg. composition measurement. Navigate to this point. The following steps in green are for each plot. Repeat these steps until finished. 5 Daubenmire plots and soil measurements will be taken at each site.
10. Toss a pencil at the random point and use this as the NW corner of the Daubenmire plot. If the plot is in rocky, or otherwise unsuitable ground, then shift the plot so that the pencil is in the SW corner, in worst case just pick a new location.
11. Make Daubenmire measurements. These are to match previously collected data. This involves visually estimating cover class for bare soil/rock, litter, lichen/moss, graminoid, forb, shrub, tree cover. Cover classes are: 0 (0%) 1 (0.1–5%), 2 (5–25%), 3 (25–50%), 4 (50–75%), 5 (75–95%), and 6 (>95%). These data may be used to generate structural diversity estimates for a site (i.e. how much variation in growth form is there).
12. Take a densitometer measurement
13. Conduct soil sampling. Label two whirlpacks with site label and xy coord of the Daubenmire plot and which is O and which is mineral soil.
    1. Clean corer with ethanol and flame sterilize. If ethanol runs out, then use water. Etoh shouldn't really break down DNA anyway.
    2. I will collect 2 soil cores per Daubenmire plot. The soil cores located randomly within each Daubenmire plot by dropping a pencil, and avoiding sampling right on top of a plant.
    3. Drill one core, and push the core up into the corer from the bottom.
    4. measure the depth of the O horizon and the mineral layer with a tape measure.
    5. Repeat last two steps for the second core.
    6. Pool the O horizon components from each core into one whirl pak and pool the mineral soil components into another whilr pack. If the O horizon is less than 1cm then discard it.
    7. If there is very little soil in the plot, then take 4 cores, or however much to get enough mass for measurements.
    8. Place the soil thermometer near the two cores at the approximate depth of the O horizon, and then push down further to measure the temp at the mineral horizon. Test the location first with a pin flag to make sure the thermometer won't break
    9. Do not freeze soil samples used for chemistry, but store in cooler.
    10. spray down corer with ethanol and set it on fire with the new torch!
14. Make more intensive veg. composition measurements in a 1m quadrat. Use slightly more resolved cover class categories (in online form) and assign every taxon within the quadrat to a cover class. This will give us abundance estimates for all taxa at a site. We can decompose the resulting matrix to get diversity indices for vegetation. We can also generate high quality structural diversity measurements too by noting the growth habit of each taxon. Data to be collected at this step are taxon, % cover, and growth habit (multi-stemmed forb, annual forb, graminoid, tree, shrub).
15. Generate random numbers for densitometer measurements and make those measurements. Densitometer measurements will be made with the handheld pvc style densitometer. Get the device level and look up. Count it as a + if vegetation is present in any quadrant of the view. Make 20 measurements throughout the site (80 total). As I walk around begin collecting veg. richness data.
16. Collect vegetation richness data. This involves walking around the site and listing all taxa that are present. If unsure what a taxon is (which will probably happen often), then count it by growth form (e.g. 8 unidentified forbs, or 3 unidentified grasses). This should generate a large species list that we can use to tally richness by growth habit. Note that growth habit can be augmented post hoc with more information (e.g. annual vs. perennial). NOTE: this observation step should help me choose what plants to sample.
17. Count number of downed trees within the plot that have a diameter of at least 8inches at the widest point and are at least 3m long. Again, this may not be needed, but shouldn't take long. If it seems to take too long, then will cut this measurement.
18. Begin sampling plants for DNA. Sample all trees and shrubs and at least 3 each of the most abundant forbs and graminoids, after that sample at least 4–5 rare things that occupy less than 1% of the cover in the plot and are just not that abundant in the area. Sample additional forbs and graminoids to capture differences in traits and phylogeny (e.g. dont just sample Fabaceous plants). Ideally, the plant taxa sampled will make up 80% or more of the total vegetative biomass at the location and will encompass at least 5 plant families (hopefully more).
    1. General notes for plant sampling: for any plant taxon that is distributed throughout the sampling location, generate xy coordinates and sample the individual nearest to those coordinates. For plants that only grow in patches, sample haphazardly. Sample only flowering/fruiting or post-fruiting plants, avoid vegetative plants (unless they don't reproduce every year) and obviously senescing plants. Try to sample phenotypic variation present (e.g. in leaf size, color, shape, floral color, overall size, etc.). Avoid obviously young or obviously old leaves. Try to pick "average" leaves that are not visibly discolored or severely damaged. Choose three leaves per individual sampled (if compound leaves, then 3 leaflets, each from a different leaf). Collect leaves from different portions of the plant (don't collect all leaves from same branch). The point is to get a representative sample of the foliar microbes on middle aged, average leaves for the plant. For taxa with basal rosettes and few leaves on stems, then sample the basal rosette. Do not mix radically different leaves (e.g. basal rosette and stem leaves on certain Asters) as these will undoubtedly differ in both traits and microbes and obscure the signal in the data. Try to choose leaves that are in the middle of the plant in terms of height above the ground. For trees, will just have to get whatever leaves/needles I can reach, or cut down with a rope saw.
    2. Make all trait measurements for one plant before moving to the next plant. Do all of one taxon before moving to the next taxon. Need to stay organized. Use the richness log that was taken on scratch paper, to keep track of which plant and which taxon I am on as I can't get counters into the android form.
    3. Label a whirlpack for the plant. Labeling of samples will be of the form: “Mountain\_site\_taxon\_indiv” where mountain will range from 1–6, site from 1–4, taxon from 1–25 followed by species name (e.g. 2AsLe, or 13PiCo), and indiv from 1–5. Label a larger bag for the plant taxon, in which all whirl packs for that plant will be stored. Label a larger bag yet for the whole plot.
    4. Measure phenology. Categorical: flowering, fruiting/flowering, fruiting, post-fruiting.
    5. If an epiphyte, hemiparasite,etc. make note of the likely associated host
    6. Pull leaves off of plant with forceps and scissorsand place in labeled whirlpack. Doublecheck the label!!!!For each plant do the following:
    7. Measure branching architecture: continuous. For branched plants, choose the three branches that leaves were sampled for sequencing, and measure the number of branching nodes before you get to a terminal branch that doesn’t have leaves. Measure the length of the branch to the tip of a leaf and then divide the number of branches by that length. This is called apical dominance index.
    8. Measure box measurements of plant.
       1. For trees, estimate height with a rangefinder if I can get one, or a Biltmore stick. Measure DBH
    9. Measure heights of each leaf sampled for DNA/Traits from ground. These values should be very nearly the same, so one value is to be recorded here
    10. Sterilize scissors/clippers and forceps. The forceps will be used to hold the leaf and the scissors to remove it from the plant. Sterilize by ethanol and napkin wipe to get most mass off, then flame sterilize.
    11. Choose three leaves to sample for DNA and use flagging, twisties to mark those branches if needed (e.g. a willow where it will be hard to remember where a leaf was removed, or a sage, where the leaves are small enough that you couldn't tell which branch was sampled) .Can use flagging and twisties to keep track of which leaves were removed that way analogous leaves can be measured with multispeq, just remove the flagging and twisties between plants.
    12. Choose one leaf that is analogous to those that were used for sequencing. Need to be right next to the leaf sampled for dna on the branch.
        1. Make microspeQ measurements of the leaf. If leaves are very tiny, then skip. As soon as measurement taken, then remove leaf and tape to paper that will go in a sleeve in binder
    13. Tape 2 more leaves to binder paper. Tape one leaf totally flat, and just tape by petiole for the two other leaves. Make sure the leaves in the binder are labeled with site, plant taxon, id, and which leaf was which (1, 2, or 3). Will make the following measurements at the campsite later:
        1. Perform penetrometer measurements on one of the petiole taped leaves. This involves sandwhiching it between a plexiglass deck and poking a hole through it with the penetrometer. Tape the leaf flat to the paper.
        2. Measure water droplet retention. This involves taping the leaf to the side of a binder that is resting on a flat surface. A level will be affixed to the binder. Tape the leaf so that most of the leaf is exposed (not covered by tape). Pipette a droplet of water onto this leaf from single use plastic pipette. Water will be out of water bottle. Gently raise the edge of the binder and note the angle at which the water droplet first visibly shifts. This is a goofy measurement but there is a lot of interspecific variation.
    14. Doublecheck all labels. Move to next plant and repeat.
19. Make sure I have voucher specimens for all forbs and graminoids sampled. Can skip vouchers for things that I have zero chance of misidentifying (e.g. trees).
20. Take samples back to car and place in freezer. Make sure all samples from one plot are in a big freezer bag to keep them organized. DO NOT put soil samples in freezer, but in the cooler.
21. Upon returning from the field:
    1. Leaves for DNA go in the freezer till they can be processed to remove epiphytes.
    2. Measure leaf area of removed leaves via camera. I dont think the hole from the penetrometer should be a problem here. It will be the same size in all plants, so if the camera is sophisticated enough to detect this hole it will be the same across all taxa. Keep track of which leaf is which.
    3. Weigh leaves for SLA (have UGs do this).
    4. After measuring SLA, grind and aliquot into tin cups for CN measurements. Store ground tissue in cheap plastic microcentrifuge tubes.
22. For leaves stored for DNA, separate epiphytes from endophytes via some sort of serial wash (likely have UGs do this too). The details need to be worked out here, but will likely involve agitation in a tween solution, followed by a serial rinse. The rinsate will be passed through a vacuum filter and the filter paper sequenced. Need to sort methods here exactly, but there is no rush as this wont happen till Fall, and in the worst case wont happen at all.
23. After washing off epiphyts perform DNA extraction. Ideally will standardize by mass, but not sure how practical this will be. If we have a lyophilizer would be easier. The rationale is that some species of plants will have more DNA per unit mass, so if we standardize dna conc through the use of a ISD we would be gaining insight into relative abundance of microbes to plant dna, but between plants this could correspond to different amounts of plant tissue. I am not sure if this really matters or not. Need to think through this more.

Post hoc data curation:

Assign plant taxa to a growth form (one of: woody stemmed shrub, tree, multi-stemmed forb, small annual forb, graminoid, pulvinate). This will allow us to see if certain growth habits have certain microbes, heightened richness, etc.