TODO:

Clean up the Daubenmire data. Wrangling this is a pain bc of the format output by the form

Get sample site info (various abiotic, land use, etc remote sensed data) from Shannon

REMEMBER:

Added too much standard to plates 19,14,9, 23.

Ha26\_ITSoC6 could have had two rounds of PCR1, unlikely but possible.

Remember that there are rewashes of about 100 ep samples that I didn’t remember rewashing. Check these and the non-rewashed samples to see what is going on.

Remember for Ha27 there are some triplicate PCRs, use those to show that triplicate was not needed, perhaps.

2\_1\_1\_2 has no EN, but it has 3 EPs...that are on EP plates. Check this out and see if one should actually be an EP

Check the EN dupes to make sure they aren’t EP dupes.

Check the EP dupes to see what they look like.

When starting fto process remember samples in asle and Harrison in Novaseq 2.

Oct 10: **FIXED** the mid key. I had copied from the wrong sheet of the mid spreadsheet so it was wrong. Figured this out because of the duplicates that showed up when I entered Yoon’s data. Good thing I figured this out!

**Oct 9: Edit: this is wrong I think. The midpair to midplate match seems wrong. This is likely because there have been various ‘master’ mid keys that have confusing field names and I probably merged things incorrectly bc of that.** made a mid key that follows the expected format. It is called “novaseq3\_demux” the older mid key that has more info and does not follow the expected format is called:

Sample\_mid\_key\_with\_notes\_not\_correctformat\_for\_parse\_count

I doublechecked the new one for duplicated sample names and mid locus pairings. We good. Used this:

rm(list=ls())

dat <- read.csv("~/Desktop/novaseq3\_demux.csv", header = T, stringsAsFactors = F)

head(dat)

table(dat$locus)

table(dat$project)

table(dat$wellposition)

table(dat$plate)

table(dat$client\_name)

table(dat$substrate)

table(dat$library)

table(dat$midplate)

dat <- dat[dat$library == "Novaseq3",]

table(duplicated(dat$samplename))

table(duplicated(paste(dat$forward\_barcode, dat$reverse\_barcode, dat$locus)))

write.csv(dat, file = "~/Desktop/novaseq3\_demux2.csv", row.names = F)

grep("\\-", dat$samplename)

grep("\\.", dat$samplename)

**Sept 7:c**leaned up mid key. No duplicated mid pairings. Some duplicated samples.

Sept 5 - finished making mid key. Called it reorderedMidkey for now (edit: this file is gone now). Since samples are on Novaseq2 and 3 there are a few instances where I used the same mid pairings for different samples and will thus need to demux separately for each library, then recombine after the headers have unique sample names.

Specifically I had these duplicates:

|  |  |  |
| --- | --- | --- |
| Novaseq2 | Novaseq3 | Mid plate |
| 15 | 18 | 16S0C3 |
| 15 | 18 | 16S0D3 |
| Combo | 26 | 16S0C6 |
| Combo | 26 | 16S0D6 |
| 8 | 29 | 16S0F3 |
| 8 | 29 | 16S0E3 |
| 21 | 30 | 16S0G3 |
| 21 | 30 | 16S0H3 |
| 31 | 23 | 16S0G5 |
| 31 | 23 | 16S0H5 |

Sepr 2 - lots more clean up. Mostly by hand though some with the nimbus using a program Gregg made today called axyprep clean up 2 josh (or something similar to that).

Sept 1 - Added ISD/COligo only blanks to Yoons Brazil plate yesterday so that we can use these as PCR blanks. I added five blanks. See the Yoon\_brazil\_bacterial folder for more. I also added some of Ha27 to fill up her plate. These will provide triplicate PCR for a few samples too, just to show that triplicate PCR doesnt matter. I quantified the plate today and saved the output. Doing normalization via “normalization\_tracking” Nimbus method. Used 1X Tris low EDTA as a diluent. Note that the EDTA was “low”, used the same diluent for all normalization, including for my phyllosphere samples. Normalized to 10ng/ul and 20ul for all samples: yoons or otherwise. Use 50ul tips

Sept 1 - finished all PCR2, save SUads. I didn’t nlabel three of the pcr 2 plates, but I have three matching plates (e.g. I have the 16s plate, but an unlabeled one that I assume is the ITS plate) so we should be good. THe ones I didnt label likely correspond to Ha 24 ITS, 20 16s, 14 16S

Aug 31 - finished getting template and mm into pcr2 plates. Added isd/coligos to suad’s plate and added some of Ha27 to fill up tphat plate. Made a sample to well key for suads stuff. Need to add mids to that key yet though.

Aug 27 - taq showed up yesterday. Decided to take sua’ds half plate and pass it through PCR1, which would make three full plates and one half plate (ITS and 16s with two dupes). I could fill up the remaining half plate with PCR reagent blanks, or check again with Gregg to see if has something else that needs sequenced. I could extract fungal cultures from Seif and put those in there too, but that is just a few samples. I dont need to sequence more technical replicates for my phyllosphere stuff. I want to save Su’ad’s leftover DNA in case of major fail, so decided to not fill up blanks with that.

Made up a batch of MM2 before class at 1:20. Will add to plates after class.

Aug 20 - more clean up for PCR1. Finished this step. No taq so can’t move on to pcr 2 until that shows up.

Aug 18-19, 2020: more clean up for PCR1.

Aug 17, 2020 more clean up: Mag bead clean up protocol:

Manually, it was done:

* Equilibrate Beads to room Temperature
* Add 24 ul of MagBeads to each well; DO this by aspirating 24 ul of mag beads, then 15ul amount of template from PCR dupe 1 and then dump this into pcr dupe 2. Pipette mix up and down 10 times. THis was done using a protocol that was loaded onto the integra pipettes
* Incubate at RT for 5 minutes
* Secure plate on magnet plate; incubate at RT for 5 minutes (until wells are clear)
* Remove 65 ul from each well; keep tips to left or right depending on the column to avoid bead pellet.
* Add 100 ul Fresh 80% EtOH to each well. Incubate 30 seconds. Remove 100 ul from each well
* Add 100 ul Fresh 80% EtOH to each well. Incubate 30 seconds. Remove 100 ul from each well
* Reaspirate from each well to assure maximum EtOH removal
* Allow plate to air dry for 7 minutes.
* Remove sample plate from magnet plate.
* Add 30 ul H2O; pipette mix 10+ times. Incubate 2 minutes at RT.
* Place sample plate back on magnet for 5 minutes or until all wells are cleared.
* Transfer 30 ul to labeled transparent plate (Plate1 PCR1 MIDPlate1 MIDPlate2)
* Transfer 10 ul from transparent plate to “PCR2” plate with Mastermix already added.
* Label Plate1 PCR2 MIDPlate1 MIDPlate2
* Seal “PCR2”s with bubble strips and run on thermocycler 35GSAF2 program

Aug 14, 2020 - started doing pcr clean ups. Did a couple plates. ALso checked that I have done pcr 1 for all the plates. I have.

Aug 3, 2020 - Ha 27 - 16sOe6, f6 and same for ITS. Ha28, 16soa7, b7 same for its. Ha29 - 16SOe3, f3 same for its.

Made a half batch of MM so that I would have enough for plate 30 and also for suads stuf

PCR 1 info: <https://microcollaborative.atlassian.net/wiki/spaces/MICLAB/pages/651558946/EPSCoR+NovaSeq+Run+3>

Aug 2, 2020 - DId PCR1 for Ha 24 (16sOB6A6 same for ITS), Ha 26 (16SOC6, D6 same), it is possible that I did ITSOC6’s pcr 1 twice. The thermocycler had the cancel screen on when I came in the morning to remove the plate, as if I had never started the run. ALl other thermocyclers looked good, so I figured I never started the run and so I had it do the run over.

Aug 1, 2020 ; DId PCR1 for Ha 18 (!6SoC3, d3, same for its) and Ha19 (16sOC5, D5, same for ITS). Ha 20 (16SOE5, F5, same for ITS), Ha 23 (16SOG5, H5, same for ITS)

July 24, 2020: Did PCR 1 for 16 (16SOc9, d9 and same for ITS). Then Normalized remaining plates (10,4,29,24,30)

PCR1 for 4 (16SOG2, H2,same for ITS)

PCR1 for 10 (16SOA3, B3 same for ITS)

PCR1 for 17 is 16SOE9, F9 same for ITS

July 23, 2020: Did PCR 1 for Harrison 11, possibly mixed up when adding template to columns 8 and 9, so beware. Had to do by hand bc the nimbus crapped out. (16SOG6, H6, ITSOG6,H6). Also did Harrison 12 with (16soc7,d7, same for ITS). Made more MM today and added to plates.

Did PCR1 for Ha9 (16SoE7, F7, same for its)

Forgot to spin down 11 and 12 before pcr 1. Did spin down the 9.

Did 14 (16SOG7, H7, same for ITS).

July 17, 2020: Did PCR 1 for Harrison 5 (16sog4 and h4, same for ITS). Harrison 6 (16SOA5,B5, same for ITS)

July 16, 2020: Normalized these plates: 3,17,16,6,27,5,20,18,23,19,14,26,12,9,28,11

Did PCR1 for Harrison 3 (16s oE4 and F4, ITSoE4, F4).**Still have 5sh plates to normalize**

Note on Novaseq 2 I included the following plates:

8, 15, 21, 22, 25, 31, asle 1, asle 2, corn1, partial 2 (combo 1),

Protocol:

1.Before starting, wipe down all surfaces with RNAse or bleach to get them clean (if bleach on metal, then clean with water or ethanol afterwards to avoid staining). Get plates out to thaw.

2. Plates should have had ISD and coligo added (following current guidelines, these may change, so look them up) and the nucleic acid concentration in each well quantified.

3. While thawing do the Nimbus daily QC. On the laptop hooked to the nimbus there is an icon on the desktop for calibration. Click on that, select daily, and go from there. Make sure to take the magnetic metal tip ejector strip off the trash first, or the qc will crash. Trash bin doesn’t need to be in the instrument for qc, should empty it when starting though. The machine will do some stuff with its testing tips and go over to the gripper station and do some conductance testing. Shouldn’t need plastic tips or water in any wells for the initial QC. Follow the prompts to finish QC, do daily. If in continuous use maybe could push to every other day? If the QC fails, then will just have to figure it out.

4. Get the dna concentration spreadsheets from the microplate reader onto the peta library so that the nimbus can read them. Obv. make sure these spreadsheets are labeled to match the plates and the information is backed up and accessible somewhere OTHER than the petalibrary. Follow layout from normalization worksheets used previously

5. Normalize dna. Use normalize nimbus program. “normalization \_tracking”

6. Add 50 ul tips. Follow instructions in program. See videos for walk thru. Put tips in the middle two tip slots, template in middle slot that is furthest from front of nimbus. Empty plate in middle slot closest to front. Put 1x low EDTA TE buffer in a new reservoir.

7. Make Master Mix. and load into EMPTY plates. Label the plates PCR1. REcall you need four plates per single template plate (PCR in duplicate for two loci). See <https://microcollaborative.atlassian.net/wiki/spaces/MICLAB/pages/651558946/EPSCoR+NovaSeq+Run+3>

7. Use the TwoLociDuplicatePCR\_Prep program to load template and primers into MM. Seal the primers and put in the fridge. Cap the plates for PCR with dome lids, vortex, and spin down. THen put into the appropriate PCR program on the thermocyclers.

6/22/2020 - date approximate. Finished prepping plates for Novaseq. All are labeled with name and number and are in the top freezer by the door.

Notes from Gregg regarding prepping plates for NovaSeq (here for posterity)

metal rack in the freezer and use the used barcodes (on top shelf of freezers)

use cheap transparent plates, sticker seals, for 96

5 ul 30 ng/ul SG stock plus 5 ul 10 ng/ul coligo stock plus 990 ul TE produces stock that is 150 pg/ul SG and 50 pg/ul coligo. The number of moles for each should be the same here because coligo is ~3 times shorter a molecule. 20 ul of this mixed stock plus 980 ul TE produces 3 pg/ul SG stock. 10 ul of this in 990 ul TE produces 0.03 pg/ul SG stock.

When 6 ul of this last stock is added to 30 ul of DNA extract, each SG concentration will be 0.005 pg/ul and each coligo concentration will be 0.0016 pg/ul. Maximum SG in PCR rxn will be 0.01 pg and maximum coligo will be 0.0033 pg.

control plate has SG and coligos, this would get added. Make note on plate if has been added. asterisk for addition. circle the asterisk after quantifying.

Quantify - use microplate reader. Where should I save output: petalibrary/microproject/microlab/dna quantification/newfolder for project

How should I format the file for using Nimbus to do normalization...or is this getting done by hand

Should be formatted as: samplename, conc as the two fields. conc is in units of ng/ul (for an example, look for files in the above referenced path that are called normalization worksheets).

Put finished plates in the fridge

6/11/20 - updated the spreadsheet for Novaseq 2 to avoid duplicates and include 31, Corn 1, and the partial plates, and the asle inoculum and fungul cultures from Seif

6/10/20 - Added 6 ul of 0.01 pg/ul of coligo and 0.03 pg/ul of the ISD to 30 ul of template to plates 31 (which has corn samples),29,16,30,10,28,5,corn 1,6,3,4,,26,18,12,17,27,20,11,23,24

Fucked up and added 20 ul to plate 23.

6/9/20 - Prepped 19,14,9 today. These three plates set in the fridge for a few weeks. I added 50 pg/ul of coligo and 150 pg/ul of ISD to these three plates. This was way more than what I was supposed to add, which was 0.01 pg/ul of coligo and 0.03 pg/ul of the ISD.

6/8/20 - have prepped plates 8, 15, 21,22,25 for sequencing by now. They are slated for Novaseq run 2. Samples were actually prepped several weeks ago. Added 6 ul of 0.01 pg/ul of coligo and 0.03 pg/ul of the ISD to 30 ul of template.

11/15/19 - read recent articles from the Weigel lab about metagenomics and amplicon sequencing and the ratio of host to microbial dna in plants.

I want to ask, “what is the ratio of host to microbial DNA (bacteria, fungi, archaea) in various plants and what are the correlates of variation among those plants in that ratio?”

Therefore, I need to get an accurate estimate of the ratio.

Could I use cpDNA to 16s for bacteria and archaea and the ratio of host ITS to fungal ITS for this?

To determine this I would need to show that cpDNA and ITS from the host behave quantitatively. This could be done with qPCR and sequencing. First with qPCR we show that when you add more cpDNA and more host ITS this behaves quantitatively. Second you sequence these same DNAs to show that read counts behave quantitatively. I think you would need to do this for a variety of plant taxa. IF everything behaves quantitatively, then off to the races.

Can we compare ratios across plants? This assumes equal extraction capability of DNA from plant cells from multiple species, I think. Say we get less plant dna from conifer needles, but the same amount of microbial dna. We would need to have some way to correct for differnces in extraction success among plants, I think. **I have weight of startiing plant material, maybe I would then do qPCR to find out how much plant dna I have and then divide that by mass to obtain an estiamte of dna per unit mass.** Of course, I would also get microbial dna per unit mass. For instance, if I went from 1ul/ng host : 5ng/ul bacteria in one sample to 10ul/ng host : 5ng/ul in another sample, then how wwouild I know that the ratio is not due to differences in extraction utility?

How to measure extraction efficiency?

Also, what about ploidy level?

When using metagenomics, it seems that bigger plant genomes would cause the ratio to shift too, which would preclude intertaxa comparisons.

If not, then consider doing metagenomics on a subset of the samples to obtain quantitative estimates of host dna to non-host dna. I don’t think this could be used to calculate a correction factor that would allow us to use amplicon data, but it might be a good doublecheck of the qPCR results and be useful in its own right. Would be a different paper though.

11/11/19 - checked through the data to see if I am missing anything. Only missing samples from 7-2-4 and a handful of others. I will look for 7-2-4. I do have about a hundred samples that I have two epiphyte samples for. I poked around for awhile and the best I can come up with is that we actually removed epiphytes twice for these samples for some reason. This is because they show up duplicated as soon as they are added to the spreadsheet, thus suggesting there was two of them in the bag of samples that we organized into plates. This means that we had two epiphyte tubes for that sample for some reason. Since I am not missing many samples I dont think these were mislabeled (and should have been somthing else). I dont recall accidentally washing samples twice for epiphytes, but I am at a loss what else could have happened. **I suggest looking at these carefully post bioinformatics and making sure they dont look strange. Also check that the endophyte samples from the potentially doublewashed leaves look ok. If everything checks out, then we simply have a lot of rewashed samples. If not, then remove them from the data. This is odd and bothersome, but I can’t think of anything else to do.**

11/8 - this week finished all plant and epiphyte extractions.

Note I dont think plate 13 exists anymore after combining plates differently.

11/5/19 - extracted 12 and 19. I made a blank yesterday for kit 3 that was mixed a bit with kit 7

11/4/19 - prepped plates 18 and 25. DIdnt do an extraction bc was tired and Abby was using machine

10/31/ extracted plates 9 and 14. 9 was not originally in the spreadsheet. Ankita also started combining the plates that were separated to remove the endophyte samples back into complete plates.

10/30 - extracted plates 16 and 10. Overfilled 10 at beginning so some contamination happened during vortexing. Jeff and will helped. Need to edit program to save tips on dispense

10/22 - extracted 29 and 31. Started prepping Johns samples. Finished kit

10/20 - undergrads continued to process corn and endophyte samples. The ground corn samples from friday sat in the refridgerator over the weekend instead of the freezer. Not ideal, but since samples appear to be randomized to some extent shouldnt be a big deal.

10/18 - processed corn plate 1 and the new plate 30, which is a composite between 12, 13, 18

Ground up the remaining corn samples. Started prepping for Monday.

10/17 - prepped corn plates for Gordon’s herbicide project. Combined EN samples from 12, 13, 18 to make a complimentary EN plate. Ankita combined these and made a new sample to well key called “New Plate EN Samples.xlsx”. I added this to the main sample well key on g drive, with the new plate being plate 30. Holding off on epiphyte extraction until we do some qpcr to see if we can get amplification. Also changed robot protocol slightly today so that I elute in 100 ul instead of 50, in case I am not saturating the membrane well enough.

10/16/19 - did plates 24 and 23, which were both epiphytes that I lyophilized. I dont think lyophiliziung made much of a difference. Spot checks showed quants of juyst a couple ng/ul. Row C of 24 probably got contaminated bc pipetter was drippy for some reason. 23 should be fine. Also I made one of the samples the blank for 4 and labeled it but the undergrads didnt update the spreadsheet so I dont know which it was. It was somewhere in teh middle and I think right. Like maybe e8 or 9 or so.

Oct 10 - 2019 - -prepped 6, 27. Extracted 26, 28. Changed protocol slightly today and spun a bit faster at the drying step. Trained Jeff and Ankita. They are pretty much good to go. Decided to try and lyophilize my epiphyte samples to concentrate them. I will dry them down, then resolubulize in powerbead (with vortexing). Maybe it will help.

Oct 9 - 2019 -

Extracted plates 20 and 17, shook, and spun

Oct 8 - 2019 -

Loaded plates 20 and 17 with PB/ranase, shook, and spun.

**PSS added**

Began training Will and Jeff. Showed them both how to use nanodrop. Quants varied tremendously among samples from 1 to 300. Most looked ok but seemed to have some absorption in the 230 area. I think my decision to not use PSS was a mistake. Am going to start using it now. So far plates 5, 11, 3 and 4 did not use PSS. IF PSS helps, then maybe go back through and clean up those plates somehow.

Chandler finished organizing sampmles into plates, including those few that hadnt been ground

Oct 7, 2019 - Loaded plates 5 and 11 into collection plates and didextraction. These finished up kit 2.

Sept 2, 2019 - Started extracting plates 5 and 11. Deciding to forgo the phenolic separation solution. We shall see about that decision. Using the Ingenta Assist Plus starting at step IR. Only got to addition of SL and havent loaded into plates. Took way too long

July 12, 2019 - Ankita finished organizing samples into plates. We have 1261 EP samples, which seems right, but only 780 endophyte samples. I am guessing we have a missing bag of endophyte samples somewhere, hopefully we can find it (edit: found it). We also have a lot of samples that dont seem to have a matching endophyte/epiphyte sample, and the numbers don’t match up with the missing endophyte samples. I am not sure what is going on there. It has been hard keeping everything organized.

\*We have a bag of endophyte samples that have been lyophilized but not ground. This will include many of the missing ones.

July 8, 2019 - Extracted plates 3 and 4. Took all day because I had to load plates. Will automate that part in hopes of speeding htings up. I think our centrifuge is waaay too slow. Need to ask around to find a faster one. Meanwhile I centrifuged for 2x the recommended time. Extracted DNA is being stored in the freezer at -80. Labeled with my name, the plate number, date, and epscor.

March 28 - finished lyophilizing. All look good, maybe slightly not dry enough, will have to keep an eye on them. All lyophilized samples stored in freezer in ziplocks to avoid rehydration. Added some spruce, pine and grass to lyophilizer so I can have some dummy samples to practice extraction on.

March 27 - I tried extracting 7 endophyte and 9 epiphyte samples today. Good news is the lyophilizing is sufficient to allow easy grinding. Bad news is cant grind in the collection plates and dont know what the bead homogenization plates are, will find out. See below. Foil sealing matts are not sufficient to prevent liquid contamination upon inversion so I tossed samples. Should have used toy samples from trees on campus instead of wasting my own samples, but I didnt think it would fail. Will use toy samples from now on. I used the following samples (made a note of this in the spreadsheet too):

5-1-1-9 en

2-3-6-1 ep

2-2-1-10 ep

2-2-3-1 ep

2-2-2-7 ep

2-3-1-1 ep

6-3-4-3 ep

7-1-4-10 ep

7-1-1-8 ep

4-1-2-2 ep

2-2-2-5 en

6-3-3-3 en

6-3-5-5 en

2-3-2-5 en

6-1-4-2 en

1-1-6-1 en

Called Qiagen and found out that the bead homogenizaiton plate is actually a bunch of microtubes that come racked and then fit into the tissue lyser: <https://www.qiagen.com/us/shop/lab-basics/plastics/collection-microtubes/#orderinginformation>

One would need to get the caps for them too if one wanted to actually use the tissue lyser.

March 26 - started lyophilizing samples that I poked holes in the lids of yesterday evening. Still doing -40c and 0 pressure for 24 hours. Have a few hundred left.

March 13 - over the past two days I have lyophilized half the samples. I am lyophilizing at -40C and a 0 vacuum (which I guess is as good as you can do) for 24 hours. I have lyophilized slightly more than 24 hours for some of the samples. They appear dry, but do not feel cold. NOTE: two samples 5-3-6-5, 3-2-4-2 fell out and got lost in my cooler. I didnt notice them for two days. They consequenly air dried. These should be checked to make sure they are not outliers after sequencing. I think since it is only two samples we might as well sequence them and retain any data we can.

March 4, 2019 - spent more time organizing project folder

March 4, 2019 - Sample preprocessing is complete except for lyophilizing (awaiting using pump in Linda’s lab). Ankita Sawant and William Herrick performed the majority of epiphyte separation using the protocol developed by Katherine Moccia. Samples were kept on ice during separation, but had to be thawed to parse. This is not ideal as the samples spend 20-30 minutes on the table during parsing, but is the best we could manage. We “squished” the wet leaves with a pipette tip to get as much liquid off as possible, thus we hope to have removed most of the epiphytes. Ankita also rewashed a handful of samples from grasses, forbs, and trees so we can see if their is much less DNA in the rewash. NOTE: I kept several blanks of the rinse solution to test for any inadvertent contamination. For most of the samples, I made a new solution the day of to avoid any contamination growing ( I cleaned bottle with water, then ethanol, then RNASE). The second round of samples should be doublechecked that they don’t have any odd contaminants because for that round I used the same solution that I had made up in the past, so it is possible that something could have grown in it, though unlikely. I took a blank of this solution. In the future solutions should be made the day of. To figure out which samples were the second batch see the dates on the freezer boxes. This will be added to the spreadsheet at a later time. Used autoclaved, pink tubes for epiphytes, except for very first batch when I didn’t have pink tubes. **Epiphyte tubes should be labeled “EP” and endophytes “EN”.** They should also be in different boxes, and, as mentioned, most of the EP samples are in pink tubes to make it easier to keep things organized.Samples are all stored at -80

SLA details: We scanned at 600 dpi and converted to 72 or 150. The problem was that I must have done something odd when I converted most of the files because the measurements were way too big. We assumed 1cm equals 56cm (which it does if you go from 600dpi to 150dpi). So if you are at 72 dpi then everything is half as many pixels, then if you had used 300 you would be halved again. So basically most of the data was 4.167 x too large (by my reckoning). I dont know how this happened, but think I must have messed something up when using Image Magick to convert the files. Next time I do this, make 1cm marks on most of the pages, at least all the ones scanned and converted in a given batch. That way calibration can be assured aftwerward. I just went through and divided all data by 4.167 except for data generated on the morning (<11:03 am on Oct 1) because that seemed correct (as determined via a separate batch of scans). When approximating the area of leaves via a ruler, the numbers we recorded with image j and transformed seem accurate.

FOR SAMPLING DATES see: “./data/siteData.csv” Final sampling date was 8/26. Samples were frozen at -18 in the field using the battery powered freezer. For longer sampling bouts, I went down to -12 or so to save battery power. I got all samples frozen by the evening that I collected them, except for the two sites that required backpacking. I kept samples on ice in a softsided cooler in the field, however really didnt work well for the backpacking sites because I had to use a smaller freezer. Check those sites to make sure they are not outliers (these are Big Sandy and Fremont).

All sample data was collected in an android form and in the field trait measurements made in a write in the rain notebook. These latter data have been digitized. The android data are in the data folder. One can access the form here: “You (Erin B. Vivaswat S, Alex B, John C) should all be able to log into the URL shown below using your uwyo email and the password "guest".

<https://buerklelab-202118.appspot.com/>

Trait measurements: leaf toughness was done in the field on fresh leaves, typically at the end of the day. Used a penetrometer. Aimed for center of leaves and avoided midrib or other prominant veins. Used a plexiglass deck to smash the leaf and hold it flat. Hole in deck for the penetrometer. In very few instances, I did the toughness measurements the next day, but tried to avoid this to avoid bias.

Leaves were taped to paper in the field. One leaf per plant. Originally tried to do replicates per plant but it was way to much work. Leaves chosen were as similar to the ones sampled for microbes as possible. Same part of plant, size, shape, etc. These taped leaves were used for water drop test and SLA

A single drop of water was dripped onto each leaf using a 1ml disposable plastic pipette. This was about 4-5ul, then the leaf was tilted until the water droplet slid. The angle at which this happened was recorded. I facilitated doing all this by taping the leaf to paper and putting that paper on a binder, which I lifted. Angle was measured with a protractor. These same leaves were used for SLA. When possible I used the same leaves for SLA, etc. that I used for the multispeQ. This happened almost all the time, except when many needles were needed to get the multispeq to register.

Multispeq recordings were made on leaves as similar to those chosen for sequencing as possible. Same amount of light, general location, angle of repose etc. Normally I choose a leaf right next to the ones I measured. In cases where the leaves were over my head and standing there with the multispeq would have been very inconvenient, I placed the multispeq with a leaf on the ground or in a bush that was receiving about the same amount of light. I went through and cleaned up these data and decided not to use several metrics (see readme, copied here)

“See this url for info on all measurements: <https://photosynq.org/help/instruments_What_does_the_MultispeQ_measure>

Leaf angle: Do not use. Often I laid the multispeq near the plant while taking measurements, so the angle won’t be correct.

For the same reasons do not use these variables:

Compass

Compass direction

Pitch

Roll

Thickness is suspect for many conifers and small plants for which I measured multiple leaves at once (so that the sensor could be covered). For these samples, I added a N under “thicknessReliable”. Just use values associated with “Y”.”

June 18–19 \_ Sampled site #1 at Libby Flats. Decided to take one multispeQ reading due to time. If a good reading didn’t come through, then tried again. If still nothing good, then moved to next plant. I think thickness readings will be useless for things like Polemonium, where a bunch of leaflets get squashed into the device. Will have to check these readings to see if they are good. Same for Astragalus as the leaves were very small and I may have gotten the midrib often. Needles don’t work well in multispeq. Had to pull off a bunch and put into device. Leaf angle and direction will not be useful metrics. Daubenmire coordinates for this site were: 1: 47,12; 2: 8,36; 3:21,30;4:18,11.

**LABELING CONVENTION USED: e.g. 1\_2\_3\_4** where the first digit is the region (1), the second digit is the site (2), the third digit is the plant taxon (3), the last digit is the individual (4). Also there is an EP or an EN added to denote if the sample is endophyte or epiphyte (EP). EP samples are almost all in pink tubes, but there are a few in clear tubes bc I ran out of pink ones. In general, I sampled 20 sites, 3 sites each at 7 locations, 7 plant taxa at each site, and 10 individuals per taxon.

I choose plants so that they composed 80% or more of the biomass present as determined occularly. This means I sampled trees present, dominant shrubs, forbs, and grasses. I tried to get unique plants, uncommon plants, and very abundant plants. The latter to ensure I had continuity across sites and I didn’t end up with different plants at every site. ID was done by myself or Ernie Nelson

Permits were gathered for all collecting. This process has been documented elsewhere. Copies of permits are stored here however.