**NutNet Add-on Projects**

**Soil Sampling & Shipping Protocols**

**Descriptions of add-on experiments:**

**Jialiang Kuang (Dr. Jizhong Zhou’s Lab) – PFLA, GeoChip, BioLog description**

Microorganisms play indispensable roles in regulating global cycling of C, N, P. One of the main goals of this project is to understand the long-term responses of microbial functional diversity and the community assembly mechanisms to nutrient addition across space and varied environmental conditions.

We will conduct high-throughput amplicon sequencing to examine the taxonomic and phylogenetic composition of microbial communities. For functional structure, we will assess the functional potentials using the functional gene-array-based high-throughput technology (GeoChip 5.0), which has been widely used for profiling the functional structure, diversity, metabolic potential/activity and dynamics of microbial communities in terrestrial ecosystems. In this study, we are particularly interested in the microbial functional traits related to the nutrition and focused on the genes involved in C, N, and P cycling. We will use BioLog (EcoPlates) to measure the metabolism of 31 carbon sources among communities to evaluate the differentiation in C degradation. We will also perform phospholipid fatty acid (PLFA) analysis to quantify the microbial biomass of soils.

**Anita Risch – Soil organic matter priming and microbial substrate use**

The input of labile C to soil, e.g., by root exudates, can induce a loss of soil organic matter (SOM) by accelerating microbial activity. This process is called ‘priming’. Although priming may have a strong overall effect on the soil C balance, the magnitude and controlling factors are uncertain. Fertilization may reduce priming as plants have to acquire less nutrients and exudate less substrate, which may preserve SOM. NutNet represents the ideal set-up for study priming effects by providing a global gradient of environmental conditions combined with fertilization. We will use the Control, N, P, and NP plots only.

We will study priming by adding 13C-labelled glucose or glycine, a N-free and a N-containing substrate as model compounds for labile roots exudates. We will measure the release of CO2 from the substrate and SOM using an isotopic CO2 spectrometer (Picarro). This will allow us to (1) track the use of labile substrate by microbes and (2) the vulnerability of old SOM through priming. For the latter, we will measure radiocarbon from selected samples to estimate the fertilization effect on new and old C.

**Jeremiah Henning – roots endophyte colonization**

Plant associations with root endophytes are critical to the maintenance of plant diversity and plant productivity. While several studies have documented the disassociation of plants with arbuscular mycorrhizal fungi following nutrient enrichment, these results are often tied to the background nutrient content of soils. However, much less is known about the response of other root endophyte associates, like dark septate endophytes and pathogenic oomycotan fungi, to nutrient enrichment. Additionally, with our dried roots, it would be super easy to measure root traits, like specific root length, if anyone was interested in those data. We will use ~2g of dried root material to measure for the presence of arbuscular mycorrhizal, dark septate endophyte, and pathogenic oomycotan colonization of plant roots. Roots will be cleared, stained using trypan blue, and will be mounted on microscope slides, for any subsequent analyses folks may be interested in. We are interested in understanding 1) macroecological patterns of root endophytic fungi colonization and 2) how nutrient amendment impacts fungal colonization patterns at local sites.

**Anita Krause – CN and micronutrient description**

Carbon, nitrogen, and micronutrient data are part of the core measurements taken at all Nutrient Network sites and provide part of the basic information needed to assess the effects of multiple resource limitation and top-down control on community structure and productivity.

**Brief descriptions of sampling and shipping:**

**1. Field sampling** (see Appendix I for details)

* Complete the NutNet sampling data sheet (<https://nutnet.umn.edu/datasheet>) for your annual project sampling of NutNet. Data will become available to network members once compiled by the data manager at the University of Minnesota.
* Complete our add-on sampling data sheet to provide basic information for your site, such as location and climate condition (see Appendix II).
* Take soil cores for different add-on experimental analyses.

**2. Sample preparation and storage** (see Appendix I for details)

* + Keep soil samples **on ice** and transport to lab within 24 hours.
  + Preserve the soil samples at 4~8°C **without frozen** (we need living microbial cells).

**3. Send us soil samples** (See Appendix III for international shipping notes)

* Ask Jialiang Kuang ([kjialiang@ou.edu](mailto:kjialiang@ou.edu)) or Daliang Ning ([ningdaliang@ou.edu](mailto:ningdaliang@ou.edu)) for special import permit and detailed notes.
* Please contact Jialiang before you ship the soil samples.
* Send the **unfrozen** soil samples with **ice packs** to Jizhong Zhou’s lab by ‘**FedEx** **International Priority**’ shipping.
* We prefer **FedEx**. Please contact your local FedEx for more specific information in shipping the soil samples to US. If you have to choose other carriers, please contact Jialiang and Daliang for more detailed information.
* Please provide enough ice packs for a longer shipping period in case that the samples get hung up in customs.
* Please note that the recipient have to be **Daliang Ning**.
* Please avoid arrival during the weekends and holidays.

**Appendix I. Detailed Protocol for Soil Sampling and Shipping**

In short, please (1) contact Jialiang Kuang ([kjialiang@ou.edu](mailto:kjialiang@ou.edu)) and Daliang Ning ([ningdaliang@ou.edu](mailto:ningdaliang@ou.edu)) 1~2 weeks before sampling, (2) complete our sampling data sheet (Appendix II), (3) take soil cores following the procedure below, (4) keep samples on ice and transport them to lab as soon as possible.

**Detail procedures**

Our soil sampling procedure follows all the experimental designs of NutNet (<https://nutnet.umn.edu/exp_protocol>, <https://nutnet.umn.edu/nutrients>) and is modified based on the original soil sampling protocol for nutrient analyses.

1. Sampling time:

Soil cores will be collected at the peak of the growing season from all of the plots.

1. Preparation for sampling:

* soil corer - 2.5-cm (1-inch) diameter
* cooler (or foam box) with enough ice (or ice packs)
* plastic soil sample bags (can use commercial slider bags or zip bags for food)
* 75% ethanol spray and paper towel (or use commercial alcohol wipes) for sterilizing and cleaning the soil corer and spatula
* spatula for soil collection
* gloves
* pen and marker pen
* portable handy scale (or a representative bag of 325g of soil for comparison)

1. Sampling:

For a field site with a regular 10-treatment design (Figure 1), a total of 30 soil samples will be taken (i.e., 3 blocks \* 10 treatments in Figure 1A). For each core sampling plot shown in Figure 1B (i.e., white 2.5\*2.5 m plot), soil cores are collected from within this year’s biomass clip strips after removal of the live vegetation and litter.

We aim to collect ~325g of soils for each core sampling plot. Roughly, collect six soil cores (soil corer - 2.5 cm x 10 cm) from within this year’s biomass sampling strips. Because soil composition can vary greatly between sites/plots and change weight per volume, please use the portable handy scale or the representative bag to adjust the number of soil cores and make sure enough soils are sampled.

Litter and vegetation should be removed from the soil surface before collecting each sample. Composite and homogenize the sub-samples into a single sample for each core sampling plot.

Label each bag (with permanent marker, Sharpie preferred) with the following information: date of collection, name of collector, name of sampling site, and block/plot/treatment identification.

Store soil samples in a cooler with ice / ice packs (but DO NOT use dry ice to freeze the samples).

Use 75% ethanol and paper towel (or alcohol wipes) to sterilize and clean the soil corer and spatula between samples that are collected from different plots.

Diagram

Description automatically generated

1. Keep the soil samples in cooler with ice / ice packs and transport to lab within 24 hours.
2. Packing and shipping
3. Prepare tools

Carton box, foam box as cooler, enough frozen ice packs, wadded paper, other files required by courier.

1. Consult a courier for notes, proper documentation and labeling requirements.
2. Pack samples into a foam box, surround the samples by enough ice packs. Fill any empty space with wadded paper.
3. Band the foam box and outer packaging (cardboard) tightly by tape.
4. Send as soon as possible after packing. Please let Jialiang and Daliang know once you send.
5. Please note that the recipient have to be **Daliang Ning**.

**See Appendix III for international shipping notes before shipping samples to USA.**

**Appendix II. Sampling Data Sheet**

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| --- | --- | --- | --- |
| **1- Site name and contact information** | | | |
| Name of Site |  | | |
| Office Phone |  | Fax |  |
| Technician name |  | Email |  |
| Address |  |  |  |
| **2- Basic information of location and climate** | | | |
| Name of variable | Values/Info. | Variable type | Description |
| PI |  | unique | Principal investigator names separated by semicolons |
| Habitat |  | categories | e.g., old field; forest meadow; savanah; tallgrass prairie; tundra; shrubland |
| Country |  | unique | Country names |
| State |  | unique | State or Provence |
| County |  | unique | Local scale political divisions such as counties, municipalities, etc. |
| Latitud |  | continuous | Latitude from -90 (S) to +90 (N) in decimal degrees |
| Longitud |  | continuous | Longitude from -180 (W) to +180 (E) in decimal degrees |
| Elev |  | continuous | Elevation (m) |
| Slope |  | continuous | Degrees from 0 to 90 (or more realistically 30 or your will be falling off) |
| Aspect |  | continuous | Degrees from 0 to 359 |
| Precip |  | continuous | Mean annual precipitation in mm |
| Precip\_Jan |  | continuous | Mean January precipitation in mm |
| Precip\_Jul |  | continuous | Mean July precipitation in mm |
| Jan\_high |  | continuous | Mean monthly high in January in degrees C |
| Jan\_low |  | continuous | Mean monthly low in January in degrees C |
| Jul\_high |  | continuous | Mean monthly high in July in degrees C |
| Jul\_low |  | continuous | Mean monthly low in July in degrees C |
| **3- Sampling Information** | | | |
| Sample Collection Date & Time (MM-DD-YY) | | |  |
| Sample Label | Block | Plot | Treatment |
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This is an example. Please compile the sampling data sheet in our provided excel file.

**Appendix III. Detailed Shipping Notes for International Soil Samples**

Here are some common notes. It is highly recommended to consult the shipping company and relevant departments in your school/country for formal regulations and requirements. A colleague who has sent fresh soil samples to USA is also helpful.

Ask Jialiang Kuang ([kjialiang@ou.edu)](mailto:kjialiang@ou.edu)) or Daliang Ning ([ningdaliang@ou.edu](mailto:ningdaliang@ou.edu)) for highlighted files in following text.

* **Export license and some certification**
* Many countries take items including viruses, bacterium, toxins etc. very seriously. Please check whether you need to apply for an export permit before preparing to ship samples.
* Some countries or regions may ask for certification or agreement to meet the law of toxic or hazardous substance control. You may check it with relevant departments.
* **Import Permit for soil samples**
* The USDA has strict regulations regarding transporting soils into the US from foreign countries. Please contact Jialiang Kuang ([kjialiang@ou.edu](mailto:kjialiang@ou.edu)) or Daliang ([ningdaliang@ou.edu](mailto:ningdaliang@ou.edu)) for Import Permit files.
* Both of these (“Permit to receive soil” and “Soil sample label”) should be attached to the outside of the shipping container.
* Please note that the “Soil sample label” (i.e., PPQ Form 550 label) becomes barcoded label, which is not allowed to reuse or duplicate. In addition, each unique barcode is associated to one package. Thus, please contact us to generate your unique barcode before you plan to ship the package, and we can generate multiple labels if you have more than one package.
* **Commercial Invoice**
* In most cases, you must state declared value for customs with the appropriate country currency, even if your shipment contains only samples or research materials and it is not for resale.
* Ask an experienced people or the shipping company for the source of applicable commercial invoice. We provide two examples of “commercial invoice”.
* You are usually required to submit three signed commercial invoices.
* **FedEx Air Waybill (reference provided)**
* Please fill the FedEx Air Waybill form following our provided example of “Air Waybill”.
* Choose FedEx International Priority.
* **Pasting on the outside of package**
* Please check following files/labels are pasted on the outside of package:

Export license and/or certification in necessary,

Import permit for soils,

Commercial invoice,

Air Waybill.

*If you use a shipping company to ship the soils, please make sure they know the permits need to be on the outside of the package. We have had packages returned to the sender because the permit was on the inside of the box instead of the outside.*

It is very important to follow these instructions and regulations in your country. If your shipment is stopped at customs, the USDA can deny entry to any packages and the samples will be returned to you.

The package should be shipped directly to Daliang Ning at this address:  
Daliang Ning  
Institute for Environmental Genomics, University of Oklahoma  
101 David L Boren Blvd, SRTC 2030  
Norman, OK 73019

(405) 651-5322

Because of the relatively limited budget for the international shipping, we greatly appreciate if you could cover the shipping cost. If this is not possible for your group, please contact Jialiang and Daliang for more detailed information.