**Soil Microbial Abundance and Diversity**

Determining the soil microbial abundance/diversity within cropland and rangeland soils is a vital component to understanding biogeochemical transformations (Atlas and Bartha 1986) and ultimately, long term productivity associated with management practices which are applied to a site within the landscape (Dick 1992). Changes in microbial abundance/diversity can be affected by abiotic and biotic factors such as, temperature, soil moisture, disturbance (mechanical, burning, grazing, etc.), chemical addition, community structure, soil depth and plant community (Doran 1980; Fierer et al. 2003; Frostegård et al. 1993; Fuentes et al. 2006; Gupta and Germida 1988; Söderström et al. 1983; Zogg et al. 1997). Through regular measurements of soil microbial abundance/diversity during a typical growing season in cropped and grassland/rangeland soils we can better understand which microbes are present in certain types of soils, how those communities are affected by given abiotic and biotic factors and microbe’s roles in nutrient cycling. Microbial abundance/diversity data from across the United States will provide larger datasets and a more robust understanding of these communities for the long term agroecological research (LTAR) network.

**Objective:**

Each site should measure soil microbial abundance/diversity within the fetch of the eddy covariance towers in both business as usual (BAU) and aspirational (ASP) treatments. This will provide inter-annual variability in microbial abundance/diversity, along with information on the effect of management on microbial populations across the US landscape.

The procedure for soil microbial abundance/diversity is universal in terms of Phospholipid fatty acid analysis (PLFA), the chosen method for abundance/diversity measurements. This procedure was chosen due to cost, multiple setups within the LTAR project allowing for QA/QC procedures and the USDA-ARS Grazinglands Research Laboratory (GRL), El Reno, Oklahoma has volunteered to run all samples from the LTAR sites. The Grassland Soil and Water Laboratory in Temple, TX, (Dr. Hal Collins), is the secondary site for QA/QC and to prevent any backlog of sample analysis. **The main objective is to quantify microbial abundance/diversity, inter-annually (three times a year) and annually and determine the effect of BAU or ASP management at each LTAR site on the measured parameters. S**ampling events are outlined in this protocol to ensure that changes in cropped soil and rangeland soil microbial abundance/diversity are captured, as these two ecosystems can have very different growth patterns in terms of plant community.

**Protocol:**

Plant communities are dominated by perennial species that have either a warm-season (C4) or cool-season (C3) growth pattern. Understanding these growth patterns will determine when to sample soils to understand dynamics of microbial abundance/diversity. Cool-season growth pattern species typically grow in spring-early summer and again in the fall (Figure 1). Warm-season growth pattern species generally start to grow as the soil warms and air temperatures increase (Figure 1) and peak in early to mid- summer.

Figure . Example growth rate of cool-season and warm-season plants over one year.

Sites that have predominantly cool-season species should be monitored based on plant growth patterns for example in the Southern Plains sampling might occur in February, late May-April and July (Table 1). These dates are flexible and should correspond to regional growth and precipitation patterns. Perennial soils that are predominantly cool-season species should follow the same sampling as cropped soils, with the addition of late growth and once after the first frost. Cropped soils, under warm-season species should be sampled three times a season in association with start of growth, peak growth and senescence of the aboveground biomass. Rangeland soils that are dominated by warm-season species should be sampled during the same times as cropped warm-season soils. It should be noted that these are suggested sampling times. Inter-annual variation will alter the exact time of sampling. In general sampling should coincide with start of growth, peak growth and senescence of the aboveground biomass.

Table 1. Sampling date suggestions for cool-season and warm-season cropped and

rangeland soils for polylipid fatty acid analysis.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Sampling Date | Procedure |
| Cool-Season | Cropped Soil | February, Late-May or April, July | Gravimetric Soil Water Content, Poly Lipid Fatty Acid analysis, Soil to archive |
| Rangeland Soils | February, Late-May or April, July, September, After Frost | Gravimetric Soil Water Content, Poly Lipid Fatty Acid analysis, Soil to archive |
| Warm-Season | Cropped Soil | April, July and September | Gravimetric Soil Water Content, Poly Lipid Fatty Acid analysis, Soil to archive |
| Rangeland Soils | April, July and September | Gravimetric Soil Water Content, Poly Lipid Fatty Acid analysis, Soil to archive |

Samples should be taken within the fetch of an eddy-covariance tower or within the plot of greenhouse gas monitoring structures. If sampling within the fetch of an eddy-covariance tower, create at least 5 replicated plots within the fetch, spaced 25 meters apart. Soil samples, 0-15cm, should be taken from each plot in triplicate, homogenized and put in an airtight bag and placed on ice. Upon transport to the local facility, remove a subsample for gravimetric soil water content analysis and place 10 grams of soil into an airtight bag, label and place in the freezer. If sample areas are around stationary GHG chamber plots, follow the same protocol, taking three samples from each plot and homogenizing them. This is the preferred method as there will be concurrent soil data to support biogeochemical nutrient cycling. Please make sure that each sample is labeled with:

Date sampled

 Name of LTAR Site

 Name of Plot

 Replicate Number

At the final sampling of the year prepare stored soil sampling for the season for processing. Weigh out approximately 2 grams of soil into 13X100 mm kimex tube with a Teflon coated lid. Place tubes in a desiccator until dry. Then place lid on each tube and ship overnight to the address below. Please indicate whether the samples are from cropped soil or rangeland soil and if they are predominately warm or cool-season species. Then ship, overnight to:

USDA-ARS, Grazinglands Research Laboratory

C/O Brekke Peterson Munks or Lauren Hanna

7207 West Cheyenne Street

El Reno, OK 73036

Phone: 405.262.5291

Samples will be analyzed using the 96-well plate PLFA method (Buyer and Sasser 2012). It is important to note if the system is rangeland or cropped soil to determine if we should do a secondary procedure to assess arbuscular mycorrhizal presence. After the fatty acids from the microbes have been extracted from the soil, soil extracts will be analyzed using GC-FID technology with MiDi Sherlock software to determine abundance and community diversity of soil microbes.

**References:**

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