**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 of a research site and the management practices which are applied to the landscape (Dick 1992). Changes in microbial abundance/diversity can be affected by abiotic and biotic factors such as, temperature, soil moisture, disturbance (mechanical, cattle, 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 rangeland soils we can better understand which microbes are present in certain types of soils, how those communities are affected given abiotic and biotic factors and better understand microbe’s roles in nutrient cycling. Microbial abundance/diversity data from across the United States will provide larger datasets 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 United States Department of Agriculture-Agricultural Research Service Office at the 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, ran by Dr. Hal Collins, is the secondary site for QA/QC and to prevent any backlog of sample analysis. **The main objective of each LTAR site is to report microbial abundance/diversity, inter-annually (three times a year) and annually and the effect of BAU or ASP management on such parameters.** Two different sampling 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:**

Rangeland plant communities are dominated by perennial species that have either a warm-season (C4) or cool-season (C3) growth pattern. Understanding these parameters will help to know when to sample soils to capture microbial abundance/diversity. Cool-season growth pattern species typically grow in spring-early summer and once 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).

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

Cropped soils that are predominantly cool-season species should be monitored in February, late May-April and July (Table 1). Rangeland soils that are predominantly cool-season species should follow the same sampling as cropped soils, with the addition of September or October and once after the first frost. Cropped soils, under warm-season species should be sampled in April, July and September or close to senescence. 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 stationary greenhouse gas structures. If sampling within the fetch of an eddy-covariance tower, try to 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 Facility

Name of Plot

Replicate Number

At the final sampling of the year prepare a box with ice packs and insulation for shipping samples to GRL. Place all samples in the box. 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:

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C/O Brekke Peterson Munks or Lauren Hanna

7207 West Cheyenne Street

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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 presents. 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.

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