NutNet Soil Sampling Protocol – 2010/2011

Goals and Rationale

Field Sampling and Logistics

Sampling will occur in all plots of sites that have been applying the treatment for at least 2 years (sites started in 2007, 2008, and 2009). Sites sampled in 2009 may want to wait for a year to sample at their discretion. Sampling will occur in the 10 x 200 cm biomass clip strip after biomass and litter are removed. The sampling kit mailed to each site will contain 30 bags for soil samples, 30 bags for root cores, and 30 15 cm by 2.5 cm PVC tubes for the soil cores. Soil and root cores will be 2.5 cm in diameter and 10 cm deep. 4 cores will be taken and emptied into the soil bag (yielding about 200 cc and between 200 and 300 g of soil per plot). The last core will be left in the PVC tube and placed in the root core bag.

All 30 soil and 30 root cores (6-8 kg total) will be mailed directly to Nicole DeCrappeo at USGS (Corvallis, OR) where samples will be sieved, homogenized, and distributed to the following labs:

1. Nicole DeCrappeo (100 g fresh for PLFAs and nematodes)
2. Elsa Cleland at UCSD (intact root core for rockin' roots research, additional optional deep cores for soil profiles),
3. Noah Fierer at CU (5 g frozen for magical, microbial, miracles)
4. Kirsten Hofmockel ISU (10 g frozen for extraordinary, extracellular, enzymatic experiences)
5. Eric Seabloom (remainder, 100-150 g) air dried for c/N, pH, etc. and archiving)

Analyses and Measurements

Root Biomass (Cleland): We will estimate live root biomass using a modification of the standard LTER methods for measuring standing fine root biomass (Bledsoe et al. 1999). Briefly, each soil core will be placed in 2 L of deionized water and gently stirred to separate roots from the soil matrix. Live roots tend to float and are lighter in color than dead roots or soil organic matter. The live roots will be hand picked from the solution using tweezers and placed in a 0.5 mm mesh tea strainer. The sample will be allowed to sit so that the soil particles sink to the bottom, and the remaining clear water will be passed through a second sieve to capture any remaining roots. The collected roots will be gently rinsed in a stream of deionized water to further remove soil particles. The extracted, clean root biomass will be placed in weighing tin and dried at 40 C for 48 hours in a forced-air drying oven before being weighed and archived for future tissue nutrient analysis. Root biomass will be expressed as grams biomass per core volume. The remaining soil particles at the bottom of the sample will be passed through a 2 mm sieve and all rocks will be collected, dried and weighed (along with soil moisture data from other sampling efforts this will facilitate calculations of process rates per gram of dried, rock-free soil). All data will be made available to researchers from each site.

Bledsoe, C. S., T. J. Fahey, F P. Day, and R. W. Ruess. 1999. Measurement of static root parameters: biomass, length, and distribution in the soil profile. Pages 413-436 in G. P.

Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins, editors. Soils methods for long-term ecological research. Oxford University Press, New York, New York, USA

Root profiles (Cleland): The depth distributions of roots vary widely, even among grassland ecosystems, making it challenging to accurately estimate below-ground production across the range of NutNet sites. We propose to estimate the proportion of below-ground biomass captured in our 10 cm deep cores for root biomass (see above) with additional measurements of root biomass at multiple depths, following the methods of Schenk & Jackson (2002). These soil cores will be taken to a depth of 90 cm in 15 cm increments at 3 locations (one location for each block) adjacent to the NutNet plots to generally estimate root profiles at each site. Briefly, a 1 inch diameter slotted soil corer will be driven into the soil to 15 cm and then extracted, being careful not to lose soil during extraction. This soil will be placed in a marked plastic bag, the soil corer will be re-insterted into the same hole and then pushed to 30 cm depth to extract the next 15 cm sample, etc, to a total depth of 90 cm, or until an impermeable layer is encountered. Root biomass will be extracted as described above and all data shared with participating sites. Realistically if the soil is rocky, this could be extremely challenging (if not impossible), which may limit the range of sites for which we can calculate root depth profiles.

Schenk, H.J. & Jackson R. B. (2002) The global biogeography of roots. *Ecological Monographs*, 72: 311–328.

Microbial Diversity (Fierer): We will use a newly-developed pyrosequencing method to simultaneously survey communities of bacteria, archaea, fungi, and other micro-eukaryotes (including protozoa, nematodes, etc.) in each of the collected samples. The method is similar to one we have previously published (see Lauber et al. 2009. Appl. Environ. Micro., Fierer et al. 2008 & 2010. PNAS, Rousk et al. 2010. ISME J) the only difference being that we will capture both 16S and 18S rRNA genes with the same primer set. Each sample will be surveyed to a depth of approximately 2,000 sequences per sample and from these data we will be able to determine: alpha diversity levels (using rarified taxonomic and phylogenetic indices), community composition (relative abundances of taxa), and the extent of taxonomic/phylogenetic similarity between the communities found in all possible pairs of soil samples to be analyzed. All data from a given site will be made freely available to the researchers from that site.

Extracellular Enzymes (Hofmockel): We will measure microbial activity using potential extracellular enzyme assays (e.g. Sinsabaugh et al. 2003, Hofmockel et al. 2007). Each sample will be assayed for a suite of enzymes involved in decomposition of organic matter and nitrogen and phosphorous mineralization. These enzymes can be grouped into several broad categories based on the type of substrate that they can degrade, including cellulose and hemicellulose (cellobiohydrolase), polysaccharides (glucosidase), chitin (N-acetyl-glucosaminidase), protein (leucine aminopeptidase) and phosphorous (phosphotase). Briefly, we will make a soil slurry of ~1 g of soil and 100 mL of 50 mM NaHCO3 buffer. The suspension will then be aliquoted into 96-well microplates in 16 replicate 200-µL aliquots for each enzyme assay. All enzyme assays,

will be analyzed fluorometrically using 4-methylumbelliferyl- and 7-amido-4-methylcoumarin-labeled substrates. Fluorescence will be measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters.

Phospholipid Fatty Acid Analysis (DeCrappeo): We will estimate microbial biomass, relative taxa abundances, and fungal to bacterial ratios using phospholipd fatty acid (PLFA) analysis (Bailey et al. 2002). Fatty acids are found in cell membranes of microbes, and certain groups have signature lipids that serve as identifiers. These signature lipids are used to create a taxonomic fingerprint of the microbial community, which includes gram-positive and gram-negative bacteria, actinobacteria, fungi, and protozoa.

Nematodes (DeCrappeo and Biederman): Soil nematodes will be extracted using the sugar centrifugation method (Kaya and Stock 1997), which separates live and dead adults, juveniles, and eggs from the soil matrix using a series of sieves in conjunction with density-dependent flotation. Nematode counts and genus level identifications will be performed on a Leica inverted interference contrast compound microscope. Nematodes will be categorized into one of the following six trophic groups based on their feeding structures: bacterial feeders, fungal feeders, root associates, plant parasites, omnivores, and predators (Porazinska et al. 2003).

Nutrient Pools (Seabloom/Borer):
We had a long discussion about how to allocate our scarce to nonexistent resources to get the most information from pre- and post-treatment soil analyses. Sarah, Noah, Eric, and Elizabeth can all kick in some funds to do this work. Here are the costs of analyses roughly:

Lab	Cost Per Sample	Analysis
Jean Knops	$3.50	C:N
A&L Labs	$10.00	Texture
A&L Labs	$8.00	pH, Buffer pH, Ca, Mg, K, P and OM, CEC, Base Sat.%
UMN Labs	$4.00	pH

We came up with the following prioritized list of analyses and have all agreed that we can cover the costs up to level 3 for now which will give us all analyses (texture, pH, macro, and micronutrients) in the three control plots at all sites from the pre-treatment soils. In addition we will have pH and C/N in all pre- and post-treatment plots. I have attached a spreadsheet with the details but it would be great to get your feedback on this.

Prioritized soil analyses tasks	Cumulative Cost	Cost per PI (4)
1. All analyses at pretreatment control plots	$3,225	$806
2. C/N and pH in all plots -- pre treatment	$13,350	$3,338
3. C/N and pH in all plots -- post-treatment	$22,350	$5,588
4. P, K, and cations in all plots -- pre-treatment	$33,150	$8,288
5. P, K, and cations in all plots -- pre-treatment	$42,750	$10,688
6. Texture in all plots -- pre treatment	$57,750	$14,438