LTAR Leaf Sampling Protocol Draft v1.0, 7 January 2016.

The overarching objective of plant tissue sampling in LTAR common experiments or observatory measurements is to quantify the chemical composition of crop and rangeland plant species, with particular regard to mineral elements that may limit agroecosystem function and goods/services provision. Plant tissue composition data have broad applicability. They can be used to understand spatial and temporal variation in plant nutrient content and supply, identify the extent to which one or more mineral elements may limit crop and rangeland plant growth, and determine the influence of management, disturbance regimes, climate, or edaphic factors on crop and rangeland productivity, ecosystem services, commodity quality and nutritional value. Plant tissue nutrients are also important for understanding ecosystem nutrient use efficiency and are a complement to soil nutrient analyses. The protocol aims to provide sampling guidance that is

1. Applicable to the major research questions likely to be addressed across the LTAR network.
2. Insures collection of adequate quantities of plant tissue of sufficient quality for most plant macro- and micro-nutrient analysis,
3. Adequately represents variability within the experimental units at each site.
4. Maximizes integration with other LTAR data streams, such ANPP/yield, soils, community composition sampling,
5. Insures comparability across LTAR sites.

Tier 1 Measurements (All sites): Tissue C, N, and P at leaf or whole plant or ANPP level

Tier 2 Measurements (as needed): Ca, K, Mg, micronutrients

Biotic Scales of sampling

1. Rangelands:
   1. Individual plants
      1. Leaf-level sampling. Used to indicate forage quality, an index of plant photosynthetic capacity.
      2. Whole plant sampling. a measure of whole plant nutrient uptake, an index of soil nutrient availability.
   2. Community sampling. Two approaches
      1. Sampling multiple species in a community. Used to identify species differences in nutrient uptake/access to soil nutrients. May distinguish between natives and invasive/weedy species, or other agriculturally or biotically important functional groups. If all species sampled, can be combined with a measure of species abundance to estimate total aboveground nutrient pools.
      2. Sampling aggregate samples of the community- homogenized ANPP samples. Provides a measure of total nutrient pool in aboveground plant biomass.
2. Crops
   1. Individual, whole plant, or aggregate community sampling similar as that for rangelands.
   2. Could reference standardized sampling procedures for specific crops [such as in Jones (1998) Chapter 2 in Kalra (ed) Handbook of Reference Methods for Plant Analysis. CRC Press, Boca Raton, FL].
   3. Possibly add sampling of harvestable commodity (grain, fruit, etc.) as indices of commodity quality/end-use properties/nutritional quality – allows potential interface of LTAR with post-harvest and human nutrition groups in ARS.

Spatial Scales of Sampling

The spatial scales of sampling will vary depending on the research objective, and to an extent vary between crop and rangeland systems. The spatial scale of interest is field scale (i.e. fetch of the eddy-covariance towers) to allow data to be used in combination with most LTAR measurements. The number and array of subsamples necessary to accomplish this will depend on the degree of spatial heterogeneity, and variation.

Temporal Scales of Sampling

This protocol assumes a once-per-year sampling at a time corresponding to peak growth for most species. Some research questions may warrant more frequent sampling, as determined by local investigators.

Sampling Procedure

1. Leaves
   1. Sample mature leaves in full sun just below the growing tip on main branches or stems. Sample just prior or at the beginning of reproductive growth.
   2. Sample enough leaves to yield 10 g of dried tissue (see post-harvest handling). A suggested minimum is to sample leaves from a minimum of 5 plants, but more may be required for small-leaved plants or in location with high heterogeneity. Local investigator judgement prevails in defining adequate sampling intensity.
   3. Avoid senesced leaves and those with signs of herbivory, disease, mechanical damage, or contamination by soil, dust or chemical residues.
2. Plants
   1. To sample whole plants, select plants representative of the population, and cut as close as practical to the soil level. Clip above the lowest portions of the plant stem that may be contaminated by soil if required.
   2. Collect enough plants to adequately represent each experimental unit. A suggested minimum is 5 plants, however for small plants or in heterogeneous locations a great number may be needed, according to local investigator judgement.
   3. As with leaf sampling, avoid plants with substantial herbivory, disease, mechanical damage, or contamination.
3. Communities.
   1. Collect leaves or whole plants, as appropriate, from each species in the community, or from the dominant or representative species from the functional groups of interest.
   2. Alternatively, collect a minimum of 10 g of ground tissue from homogenized ANPP samples (see post-harvest handling).

Post-harvest handling

The purpose of post-harvest handling is to preserve samples with minimal loss of dry weight or changes in chemical composition from the living state. Tissue composition can change post-harvest when tissues become hot, desiccated, or begin to decompose. To avoid exposing collected samples to these conditions:

1. Immediately place collected samples on ice until return to the laboratory. Keep samples in zip-lock bags if needed to minimize desiccation; however this increases the importance of keeping the samples cool and stabilizing the samples promptly once back at the lab.
2. If sampling of contaminated plants cannot be avoided, follow decontamination procedures in Campbell and Plank (1998, Chapter 3 in Kalra (ed) Handbook of Reference Methods for Plant Analysis. CRC Press, Boca Raton, FL).
3. In the lab, immediately dry the samples at moderate temperatures not to exceed 80 °C to constant weight. The time required will vary with the size and nature of the sample, but generally 24 to 48 hours.

Sample Storage and Archiving