Chapter 2. Plant Sampling Guidelines[3]

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This document provides guidelines for sampling plant shoots and roots with additional guidelines and references for determining plant quality. Plant biomass including rhizodeposition provides the majority of net primary production that fuels above and belowground ecosystems. Thus high caliber plant data is invaluable to preparing carbon budgets, improving C and N processes model, is valuable for understanding and predicting processes such as decomposition and N-cycling.

The ratio of C:N is an easy parameter to measure; however, it has been shown that C:N is not sufficient for predicting decomposition (Franck et al., 1997; Gorissen et al., 1995; Palm and Rowland, 1997). Palm and Rowland (1997) recommended that lignin, soluble C (soluble sugars, (if %N> 1.8%)) soluble phenolics, total N, total P, total C, and ash-free dry weight be included in a minimum data set of parameters used to characterize plant input quality for decomposition and soil organic matter studies.

The impact of plant residues on CO_2, N_2O emission is dependent upon the quality of the residue (e.g., C:N ratio, N concentration) and the size of residue. The amount of N_2O evolved varies among type of residue incorporated and the particle size of the residue (Ambus et al., 2001; Shelp et al., 2000). The incorporation of crop residues can provide a source of readily available C and N. Greater emission of N_2O follow incorporation of residues with low C:N ratios, such as legumes of horticultural crops as compared with cereal straw incorporation (Baggs et al., 2002). Smaller crop residue particles, allow for increased microbial attack, and thus greater production of N_2O (Ambus et al., 2001). Such residues can enhance metabolic activity and form local anaerobic zones, giving favorable sites for denitrification and contribute to "hot spots" of N_2O emission (Ball et al., 1999). Homogenous mixing of residue into soil increased the amount of N_2O released compared to applying a layer of residue in soil cylinders (Ambus et al., 2001). The quality of plant residue can alter the balance of N immobilization and mineralization, thus indirectly impacting substrate availability for N_2O formation.

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General plant sampling guidelines

At a minimum the plant information should give an indication of the biomass input into the system. For row crops and some horticulture, the species (crop), cropping history, planting date, row width, crop rotation, phenological stage or age at time of sampling are important metadata to be recorded. Harvestable yield andabove plant biomass should be determined per unit area. Root biomass is desirable. Plant biomass should be determined by individual researchers based on the vegetation/crop sampled. Timing and frequency is system dependent, although the age and physiological stage should be recorded.

Plant aboveground sampling crops:

Harvestable yield can be determined by hand, or with a plot scale or full-scale combine as appropriate for size of plot used. It is important to include if the yield is reported as dry weight or standard moisture, which varies by crop. The standard moisture used needs to be explicitly stated. An estimate of maximum non-grain biomass produced should be determined at physiological maturity (black layer for corn) (Wilhelm et al., 2010) and R6 prior to leaf drop for soybean. Narrow row or perennial species can be harvested within a known area, such as a 1-m^2 frame or hoop.

Plant aboveground sampling for quantifying weeds

In general, most plant sampling techniques that are used for crops apply equally well to invasive species. However, the patchiness of weed infestations may sometimes require slightly different approaches to select representative samples. The sampling methodology and accounting for the mass of weeds (weight/unit area) will depend upon the nature of the experimental plots and the field environment. In small plot studies, sampling could be based on the overall sampling scheme for the crop species, and then separate of the weeds from the crop. Individual weeds types should be considered, including height and perhaps distance from the row. If in a more extensive area, then a transect method might be quite satisfactory. If in a pasture or other large field, then it might be reasonable to use a random method (systematic random points). This extensive sampling could be combined with more intensive random sampling in particular areas of interest. In any case it would be necessary to know the area sampled so that scaling-up and calculation of the mass of weeds per unit area can be calculated.

Unique techniques might be required for special situations. One of these might be if the desired sampling area uniformly had either C3 or C4 plants present in it and the species that is considered the weed were the opposite, then bulk samples could be obtained and the delta ^{13}C determined and a ratio of C3 to C4 plants calculated based upon stable C isotope analyses.

If the question were relative leaf area of weeds or ratio of weed to crop leaf area or if some other physiological metric were desired, then of course entirely different approaches would be required.

Plant aboveground sampling for grazing lands

Pasturelands and, to a greater extent, rangelands are complex associations of multiple species with varying spatial heterogeneity, so quantifying aboveground biomass and its distribution is less direct than for croplands. All of the principles discussed above in the weeds section, transect and random methods, surrogate methods (C3 vs. C4), and combinations thereof, could apply for grazing lands with the exact approach depending on the complexity and size of the plant community. For diverse rangeland communities with numerous species, sampling just the dominant species, their representation and distribution, and binning the remaining minor species, perhaps in functional plant groups as appropriate, helps streamline the characterization of plant biomass inputs. The particular approach will depend on project objectives, and will likely vary among sites. Refer to Stohlgren et al. (1998) and Sorrells and Glen (1991) for a more in-depth discussion on vegetation-sampling techniques in grasslands. Since grazing lands are typically multi-species plant communities, a descriptor of those communities (perennial cool-season grass, clover; northern mixed-grass prairie) is important.

Plant handling

Plant material should be analyzed fresh or freeze-dried, especially if soluble compounds are to be assayed (Allen, 1989). However, an acceptable compromise is to dry the material at or below 45^{o}C, with adequate ventilation to minimize microbial or enzymatic breakdown (Allen, 1989; NREL, 1996). After drying, the material should be ground to pass through a 1-mm mesh.

Determination of equivalent dry-weight at 65^{o}C permits results to be expressed on dry-weight basis (Palm and Rowland, 1997). Since biochemical composition varies among species and physiological stage, (Constantinides and Fownes, 1994; Heal et al., 1997) it is important to include the age or physiological stage of the material, and identify organs included.

Root sampling guidelines

Root plasticity and variability (spatial and temporal) together with sampling challenges make it very difficult to accurately measure root biomass. As noted by Taylor (1986, all root biomass sampling techniques (e.g., soil cores, monoliths, minirhizotron, etc.) are hampered by high variability, loss of fine root biomass, and high labor requirements. In a cropping system, the aboveground vegetative biomass and the root system represent the available organic source C inputs in the soil, unless manure or other organic amendment was applied, which adds additional inputs. Understanding the role of C translocated belowground is critical to understanding the soil C cycle. Therefore, attempting to quantify belowground biomass is desired.

Depth

Rooting depths of annual crops range from about 0.5 m to around 3.0 m (Borg and Grimes, 1986; Dardanelli et al., 1997; Merrill et al., 2002; Stone et al., 2002) in contrast to perennial root crops

such as alfalfa (*Medicago sativa* L), which can reach depths of 6 m after several growing seasons (Borg and Grimes, 1986). However, most crops have the majority of the root biomass within the surface 60 cm. Therefore, if resources are limited, roots cores should focus on the surface 60 cm (Allmaras and Nelson, 1971; Allmaras et al., 1975; Mitchell and Russell, 1971; Weaver, 1926).

Unlike most annual cropping systems, rangelands are characterized by heterogeneity in plant community composition. Within rangeland habitats, the plant community includes three rooting types based on depth: widely spreading, *superficially rooted* (0 to 10 cm) species such as cacti; *shallowly rooted* species such as grasses, which have the majority of their dense fibrous root systems in the upper 40 cm of the soil, although some roots usually penetrate much deeper; and *deeply rooted* species, which include shrubs, half-shrubs, and forbs with primary taproot systems often penetrating to depths >1 m but with lateral roots in the upper soil layers (Lauenroth and Milchunas, 1992). In rangelands dominated by grasses, about 75 to 80% of total root biomass is in the top 30 cm of the soil, and about 44 to 57% is in the top 10 cm (Sims et al., 1978; Jackson et al., 1996; Reeder et al., 2001).

Number of cores

Due to the heterogeneous nature of soil and the non-random and non-uniform distribution of roots, variability among samples will be high, not to mention the issue of variability among techniques. Taylor (1986) in his review of root sampling techniques estimated that to have 90% confidence 40 samples with a sample volume of cm^3 would be needed and that was in relatively uniform loess soil. Rarely is it feasible to take that number of samples; therefore, researchers need to contend with high variability.

Plant patchiness causes wide variation in root mass and distribution that occur in rangeland ecosystems (Milchunas and Lauenroth, 1989), as do differences in plant community composition associated with topography and soil type (Lauenroth and Milchunas, 1992). A stratified sampling protocol across the factors (topography and plant species) controlling spatial patterns is required (Burke et al., 1999; Reeder, 2003).

When to sample

As with all plant parameters, it is important to record at least the age and preferably the physiological stage at the time of sampling. Ideally, it would be best to sample at peak root biomass. However, this is not necessarily well defined for all crops, but in general the transition in annual species from vegetative to reproductive growth represents peak root:shoot ratio (Russell, 1977; Klepper, 1991). Based on minirhizotron data Liedgens et al. (2000) reported that maximum root density occurred about 10 d after pollen shed at most positions to the plant row for corn. Wheat maximum root biomass is at anthesis (Siddique et al., 1990). Root growth of soybeans also appears to reach a maximum about seed set and begin declining after seed development starts (Mitchell and Russell, 1971). Measuring at physiological maturity would likely mean some of the belowground biomass is already been lost to decomposition. Siddique et

al. (1990) reported that root-to-shoot ratio declined from 0.55 at anthesis to 0.4 at maturity, thus root measurements at maturity will underestimate total root biomass. Alfalfa is a perennial species, so root development would be expected to be considerably different than annual species; both the biomass and the chemical composition will change depending on how many years since planting, and from that stand point alfalfa may be more similar to perennial than to annual species.

Wide yearly variation in root biomass is common in rangeland systems and result primarily from annual variability in climatic factors (precipitation, temperature, evapotranspiration and solar radiation) which affect net primary production and plant species composition (Reeder et al., 2001). Wide intra-seasonal fluctuations in root biomass also occur. In rangelands dominated by cool season grasses, maximum root biomass usually occurs in late spring or early summer (Coupland, 1992), whereas in habitats with a large warm season grass component, maximum root mass usually occurs toward the end of the growing season. However, fluctuations in root mass relate to temperature and precipitation (Lauenroth and Whitman, 1977; Milchunas and Lauenroth, 2000), so erratic temperature and precipitation patterns can suppress or accelerate plant production and alter the time at which maximum root biomass occurs (Reeder et al., 2001).

Sampling depth and horizontal

Root sampling to 60 cm, does not capture all roots, but it is the zone of maximum root density for most species. If resources allow, sampling throughout the root-depth would be ideal. It is relatively easy to use a hand probe for sampling the surface 60 cm. Sampling likely would require the use of a hydraulic probe. Hand probes come with wet and dry tips; it is advisable to purchase some of each. Increment the sample as resources permit.

Horizontal root distribution is neither uniform nor random (Allmaras and Nelson, 1971); therefore, it is advisable to collect samples at several horizontal positions relative to the plant between two rows. For example in corn or soybean with 76 cm row spacing, taking a probe near a plant 1-3, 13, 25 and 38 cm from row will capture some of the horizontal distribution. Three or more subplot locations within a plot are recommended. If resources are limited the root material can be pooled. In narrow row crops like wheat or drilled soybeans, the four horizontal positions would be next to a plant, center of inter-row, next to the next plant and the next inter-row. This strategy also can work in alfalfa, especially if it was planted with a nurse crop like wheat or oat.

Root density (g cm^{-3}) should be reported. Therefore, the volume of soil sampled must be recorded. The volume of soil is calculated as follows, $volume = h\pi r^2$ where h=height, r=radius.

Root processing

Store the soil cores with roots in plastic bags or plastic pails, at (4^oC) until they can be washed, preferably within one week. Roots can be washed from the soil with hydropneumatic elutriation

as described by Smucker et al. (1982). Commercial elutriators are available from Gillison's[4] fabrication (http://www.gillisons.com/products.htm).

Alternatively a low budget, simple method for root-washing can be used. This method requires the following items: 2 mm sieve, 0.6 mm sieve (or something similar), spray nozzle on hose, sink with soil trap, plastic buckets or tubs, small containers (about 250 mL capacity), tray for final cleaning, forceps, and sample bag for roots. Preliminary separation of roots from soil may be done in a plastic bucket. First crumble or break into smaller pieces as water is added using spray nozzle. Soak sample in water for about 30 minutes. Hand-mix the sample, decant liquid off through bigger sieve repeat as needed. The sieve will trap the roots; this method loses some of the fine roots. Dump the entire sample into sieve and wash with nozzle. Place well cleaned big clumps of roots into small labeled containers. Wash out as much soil as possible from the bigger sieve then return soil to bucket, repeat hand-mixing and sieving until roots are no longer visible. Soil is retained in the bucket. Roots are collected into a smaller container in water. Root samples can be stored at 4^{o}C until final cleaning.

Roots need to be separated from debris, a shallow dish with plenty of water is needed, which allows roots to be seen and picked out from debris. Roots should be blotted dry, wet weight measured samples can be stored frozen at this point if needed. Otherwise, freeze dry and determine dry weight. If a freeze-drier is not available, dry at 45^{o}C. The low temperature assumes there will be analysis beyond dry weight. If there is enough root biomass, determine ash-free biomass of a small sample. Determine dry weight by drying a subsample at 60^{o}C. If no chemical analysis is to be completed, the entire sample can be dried at 65^{o}C. Determine ash-free weight at 550 to 600^{o}C Details for determining ash-free biomass can be found at http://www.nrel.gov/biomass/analytical_procedures.html.

Optional plant quality assessment

Total C and N in the biomass are desirable and may be determined by combustion methods. It is recommended that ash-free biomass be determined (NREL, 2005). Additional optional quality assessment and or nutrient content may be desirable. For example, chloride can be determined by extracted with shaking in 0.01 M $CaSO_4$ and determining Cl concentration with a mercury (II) thiocyanate colorimetric method (480 nm) (Gavlak et al., 1994) measured on a Technicon AutoAnalyzer II (ALPKEM, Clackamas, OR) (Technicon, 1973) or comparable instrument. All other mineral can be determined by ICP-OES such as on a Varian Vista-Pro CCD Simultaneous ICP-OES (Varian Incorp., Palo Alto, CA) following a concentrated HNO_3 acid microwave digestion procedure based on USEPA 3051 and USEPA 3051A methods (US-EPA, 2007) using

[4] The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

a Mars Xpress Microwave Digester (CEM Corp., Mathews, NC) or similar instrument, and following manufacturers recommendations.

Standard biomass analytical procedures

Standard procedures are available at http://www.nrel.gov/biomass/analytical_procedures.html complete with background references, step-by-step protocols and sample calculation. Currently, the methods can be downloaded free of charge. In addition this web site has a biomass feedstock composition and property database, which has information on agricultural residues, wood, herbaceous energy crops and other potential biofuel sources. Alternatively there are several approaches for characterizing plant quality. One is to use a sequential extraction scheme. Sequential extraction allows isolation of more specific components with a limited amount of plant material; however, it is time consuming, expensive, and has more potential for experimental error (Palm and Rowland, 1997). A second method is to do separate extractions of a limited number of components. For example, lignin could be extracted without first extracting starch. Separate extraction tends to reduce the experimental error (Palm and Rowland, 1997). Neutral detergent fiber and acid detergent fiber (Van Soest and Wine, 1968; Van Soest et al., 1991) are common methods for determining digestibility of forage crops.

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