

Appendix 1. Protocols for monitoring of carbon sequestration in forests and in soils.

## **A. FOREST MENSURATION**

Forest biometricians have developed measurement and statistical methods to determine the total quantity of biomass in various forest ecosystem components, such as standing timber, woody debris, and the shrub, forb, and grass layers. Additional relationships exist between aboveground biomass and belowground (root) biomass that allow for reasonably accurate estimation of root mass. These are (mainly) non-destructive techniques that can generally detect changes in forest biomass (and biomass C) within a 5 or so year time increment.

### **Materials to be Measured**

With respect to forest biomass accretion, three vegetation strata will be measured; trees (including saplings), tall shrubs, and low shrubs and herbs (including ferns, grasses, and forbs). In addition, down woody materials, including coarse and fine woody debris, will be sampled.

### **Background**

A detailed discussion of sampling procedures to be used to assess forest carbon and its change with time should be prefaced with a short discussion of the relative proportion of carbon in various components, and their potential change with time. In forests, vascular aboveground biomass (about half of that biomass is carbon) is dominated by trees. A series of examples from three sets of data from forests in Minnesota make that very clear: (1) conifer plantations of varying ages sampled in northern Minnesota (Ohmann 1984); (2) forested wetlands sampled across central and northern Minnesota (Swanson and Grigal 1991); and (3) upland forest stands sampled within the Boundary Waters Canoe Area (Ohmann and Grigal 1985). All these data show similar distributions of biomass among vegetation strata (Figure Forest-1).

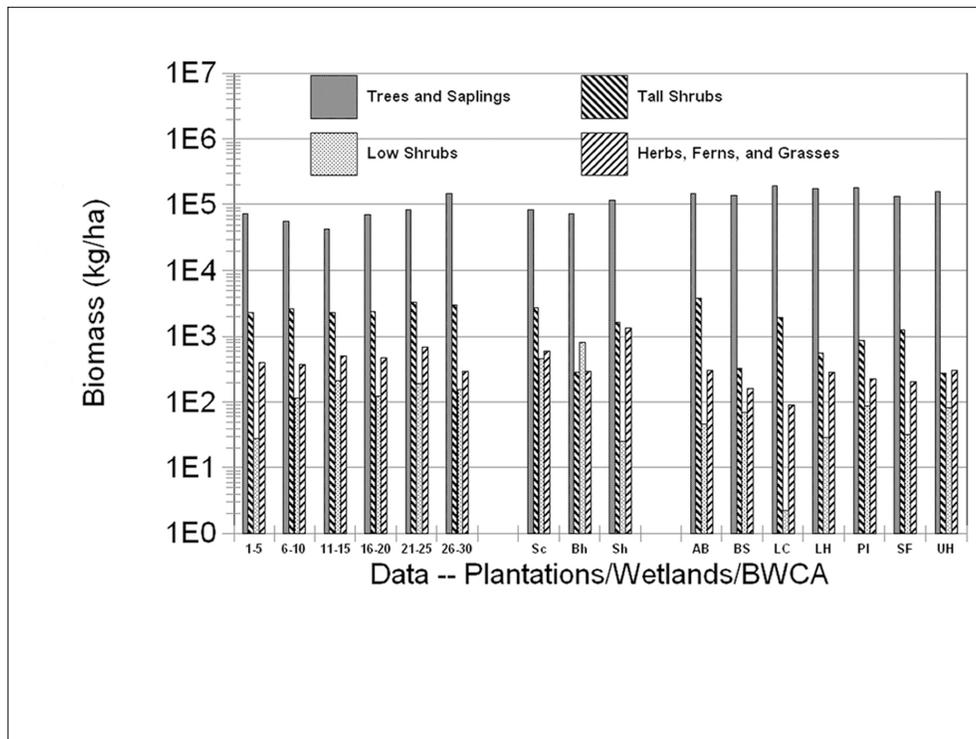


Figure Forest-1. Vascular aboveground biomass distribution among vegetation strata of forest stands from Minnesota. Bars to left represent 53 conifer plantations from northeastern Minnesota, classified into six groups based on time (years) since establishment (Ohmann 1984). Central bars represent 70 forested wetlands sampled across central and northern Minnesota, classified as Sc = conifer swamp, Bh = high-density treed bog, and Sh = hardwood swamp (Swanson and Grigal 1991). Bars to right represent 194 upland forest stands sampled within the Boundary Waters Canoe Area, re-classified as AB = aspen-birch, BS = black spruce, LC = lowland conifers (predominantly northern white cedar and balsam fir), LH = lowland hardwoods (predominantly ash and elm), PI = pine, SF = spruce-fir, and UH = upland hardwoods (maple, basswood) (Ohmann and Grigal 1985).

In all cases, biomass of trees and saplings is one to two orders of magnitude higher than that of any other strata, at about  $100 \text{ t ha}^{-1}$  (Figure Forest-1). The tall shrub stratum ranges around  $2 \text{ t ha}^{-1}$ , with generally greater mass in the plantations (Figure Forest-1). Low shrubs contribute about  $0.1 \text{ t ha}^{-1}$ , and are most important in the wetland forests (Figure Forest-1). Finally, non-woody forbs, ferns, and grasses contribute about  $0.5 \text{ t ha}^{-1}$ , with variable distribution among the forest types but greater mass in the plantations and wetlands (Figure Forest-1).

This distribution of biomass among vegetation strata, with well over 95 percent in the trees and sapling, illustrates the importance of accurately sampling tree biomass, and conversely, the lesser importance of the other strata in terms of a carbon inventory. Uncertainty of  $\approx 10$  percent in tree biomass is greater than the sum of the biomass of all the other vascular strata. That knowledge can be used to allocate resources in sampling forest biomass.

Estimated biomass of woody debris ranges from less than  $10$  to  $40 \text{ t ha}^{-1}$  for forests in eastern and

continental areas of North America (Duvall and Grigal 1999; McCarthy and Bailey 1994; Muller and Liu 1991; Sturtevant et al. 1997; Tyrell and Crow 1994). An inventory of woody debris in 563 forest stands in northcentral Minnesota found an average of about 23 t ha<sup>-1</sup>, evenly divided between snags (standing dead trees) and logs (dead wood on the forest floor) (unpublished data). The range of debris biomass among forest types was between about 10 and 30 t ha<sup>-1</sup> (Figure Forest-2). Because about 10 percent of the aboveground biomass in forest stands in Minnesota is in woody debris as logs (Figure Forest-1, Figure Forest-2), any reasonable carbon inventory requires accurate estimates of biomass in woody debris. That estimate is more important than that in any vegetation strata except trees.

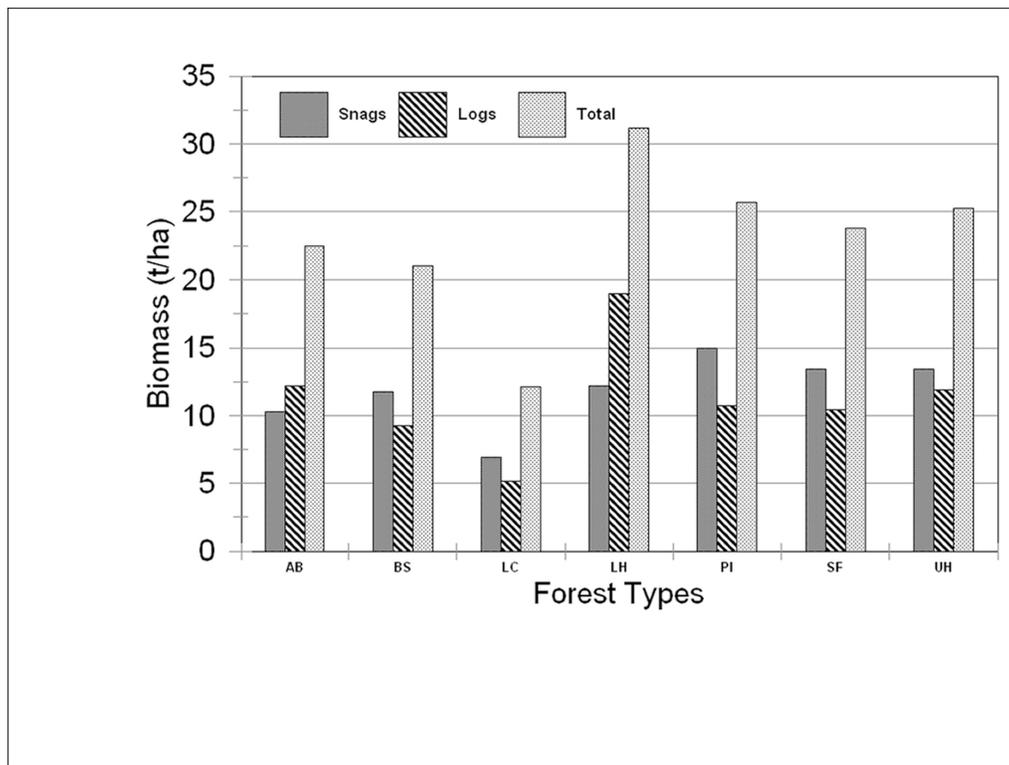


Figure Forest-2. Biomass distribution in woody debris among 563 forest stands from north-central Minnesota. AB = aspen-birch, BS = black spruce, LC = lowland conifers (predominantly northern white cedar and balsam fir), LH = lowland hardwoods (predominantly ash and elm), PI = pine, SF = spruce-fir, and UH = upland hardwoods (maple, basswood) (unpublished data).

## Field Methods

### *Basis of Protocols*

The relative size of the carbon pools of the various components of the forest ecosystem, in conjunction with two criteria that: (1) where feasible, standard methods should be used to allow for greater comparability of the results of these monitoring studies with those obtained from other studies of C sequestration; and (2) sampling methods should be sufficiently documented so

that they may be replicated in the future by individuals not involved in the initial study, leads to a proposal to base the forest biomass (C) sampling on the USDA Forest Service's Forest Inventory and Analysis (FIA) program. This program has been in continuous operation since 1930 (<http://fia.fs.fed.us/library/fact-sheets/default.asp>, assessed 26 October 2008). The FIA program has a very broad mission, and collects, analyzes, and reports information on the status and trends of forests in the U.S.: how much exists, where it exists, who owns it, and how it is changing, as well as how the trees and other forest vegetation are growing and how much has died or been removed in recent years. These activities have led to a well-documented set of protocols, including methods to analyze uncertainty (<http://www.fia.fs.fed.us/library/field-guides-methods-proc/>, assessed 28 October 2008).

Because the primary goal of the current project, monitoring changes in forest biomass over time at a few selected sites, differs somewhat from the goals of FIA, their protocols will require minor modifications for this application.

### *Sample Design and Plot Layout*

#### Sample Design

The current FIA program consists of three phases. Phase 1 uses remotely sensed data such as aerial photographs and satellite imagery for initial plot measurement and stratification. Phase 2 consists of field sampling at an intensity of about one site for every 2500 ha. The major data that are collected at that intensity relate to the tree strata. Phase 3 consists of a subset of Phase 2 plots (approximately one for every 16 plots) that are measured for a broader suite of attributes, including tree crown conditions, lichen community composition, understory vegetation, down woody debris, and soil properties (Sampling and Plot Design.pdf, <http://fia.fs.fed.us/library/fact-sheets/default.asp>, assessed 26 October 2008).

Sampling changes in forest biomass over time at a few selected sites, as in the current work, will require modifications of methods used in Phase 3, but with a much higher intensity of sampling per unit area. Sampling will be directed at trees, tall shrubs, low shrubs, herbs (including ferns, grasses, and forbs), and woody debris.

At each forest site/treatment, at least three plots will be established. This is a much higher intensity than that used by FIA, but is necessary to provide some measure of uncertainty of the ultimate estimates. Depending on budgets, more plots per site/treatment could be established.

Plots will be located by a restricted randomization scheme. At each site/treatment, a rectangular grid will be established, with each grid point a possible plot center. Portions of the grid that represent desired conditions will be selected *a priori*. In other words, unrepresentative areas (because of differing soil, vegetation, topographic position, etc.) will be excluded from potential sampling. Three (or more) plot centers will then be randomly located among the acceptable grid points. They will be located by GPS, and plot and subplot centers (see below for layout) will be permanently marked by metal re-bar.

## Plot Layout

Each plot in the FIA sampling scheme consists of four subplots (Figure Forest-3). This layout will also be used to assess changes in forest biomass over time, but some of the details of the layout will not be used. For example, because of the strong interest in C change in soil, sampling for that component will be carried out using a different, more intensive, scheme. Similarly, the “Lichens plot” will not be used; lichens and mosses will be sampled as part of the forest floor sampling. Those samplings are discussed in other portions of this document.

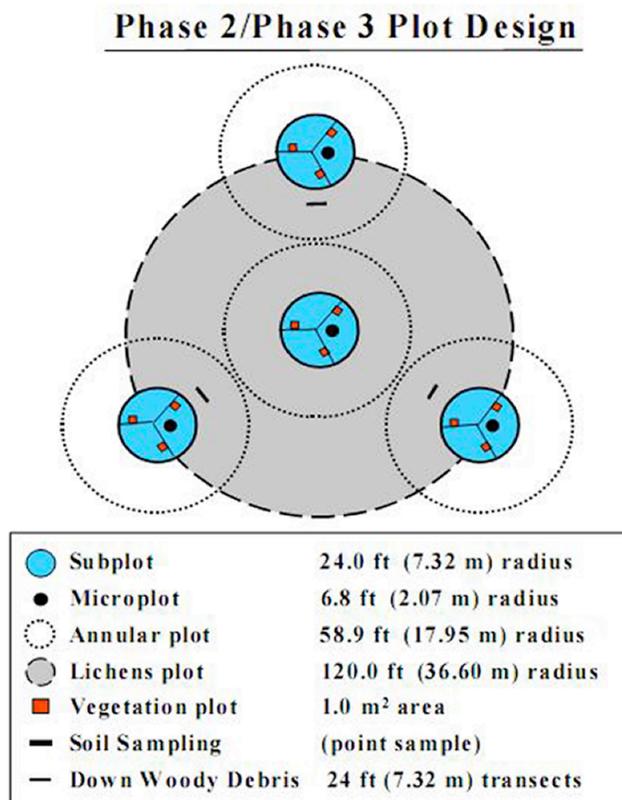


Figure Forest-3. Schematic layout of FIA plot layout used for Phase 2 and Phase 3 sampling. Subplots are oriented around the central subplot (subplot 1) at 360° (subplot 2), 120° (subplot 3), and 240° (subplot 4), at 36.6 m from center to center (from Sampling and Plot Design.pdf, <http://fia.fs.fed.us/library/fact-sheets/default.asp>, assessed 26 October 2008).

## *Tree Sampling*

Trees are sampled on each subplot (7.32 m radius). FIA was originally established to provide inventories of forest resources for industrial use, and that remains one of its foci. As a result, the size criterion for trees versus saplings, and the resulting difference in their sampling, retain some

of that orientation. However, for compatibility and comparability of the results of our monitoring studies with those obtained from other studies of C sequestration, that criterion will be retained.

Detailed procedures for tree sampling can be found in the National Core Field Guide, Version 4.0 (<http://www.fia.fs.fed.us/library/field-guides-methods-proc/>, assessed 28 October 2008). Briefly, trees at least 5.0 inches (12.7 cm) in diameter at breast height are measured within the subplot. These include all live and standing dead trees. Trees with a diameter at least 1.0 inch (2.54 cm) but less than 5.0 inches are termed saplings, and are sampled within the microplot (2.07 m radius). The center of the circular microplot is 90° and 3.7 m offset from point center of each subplot. All live saplings are measured. At successive samplings over time, all saplings that grow into each microplot thereafter are included until they grow to 5.0 inches or larger, at which time they are measured within the subplot and provided with a positional reference.

Tree measurements include species, diameter, height, and location (azimuth and distance from subplot center). Additional protocols, such as the definition of standing dead, can be found in the field guide (National Core Field Guide, Version 4.0).

### *Tall Shrub Sampling*

Although the FIA estimates percent cover of vegetation by both height stratum and by species, they do not attempt to estimate biomass of non-tree vascular vegetation (Phase 3 Field Guide - Vegetation Diversity and Structure, Version 4.0, October, 2007, <http://www.fia.fs.fed.us/library/field-guides-methods-proc/>, assessed 28 October 2008). The FIA sampling scheme will therefore be modified to allow estimation of biomass of tall shrubs (defined by FIA as woody plants with height > 0.5 m at maturity – Phase 3 Field Guide).

Three vegetation plots – also termed quadrats in some of the FIA documentation – are located on each subplot (Figure Forest-3). Plots are 1 m<sup>2</sup> (3.28 x 3.28 ft). They are located on the right sides of lines at azimuths of 30°, 150°, and 270° from the subplot centers. Two corners of each quadrat are permanently marked at 15 and 18.3 feet (4.57 and 5.57 m), horizontal distance, from the subplot center.

Detailed tall shrub data will be collected on one quadrat per subplot, that at 30°, and more extensive tall shrub data on the other two quadrats. The detailed data will be a tally of all tall shrub stems by diameter class, and the extensive data will be simply height and cover. This sampling is based on the lesser importance of tall shrubs to total aboveground vascular biomass.

### Stem Tally

On the selected quadrat (that at 30°), the diameter at 15 cm aboveground of all woody stems with a diameter at breast height of < 2.5 cm will be measured as a semi-continuous variable in 2.5 mm classes with a template and tallied by size and species (Figure Forest-4).

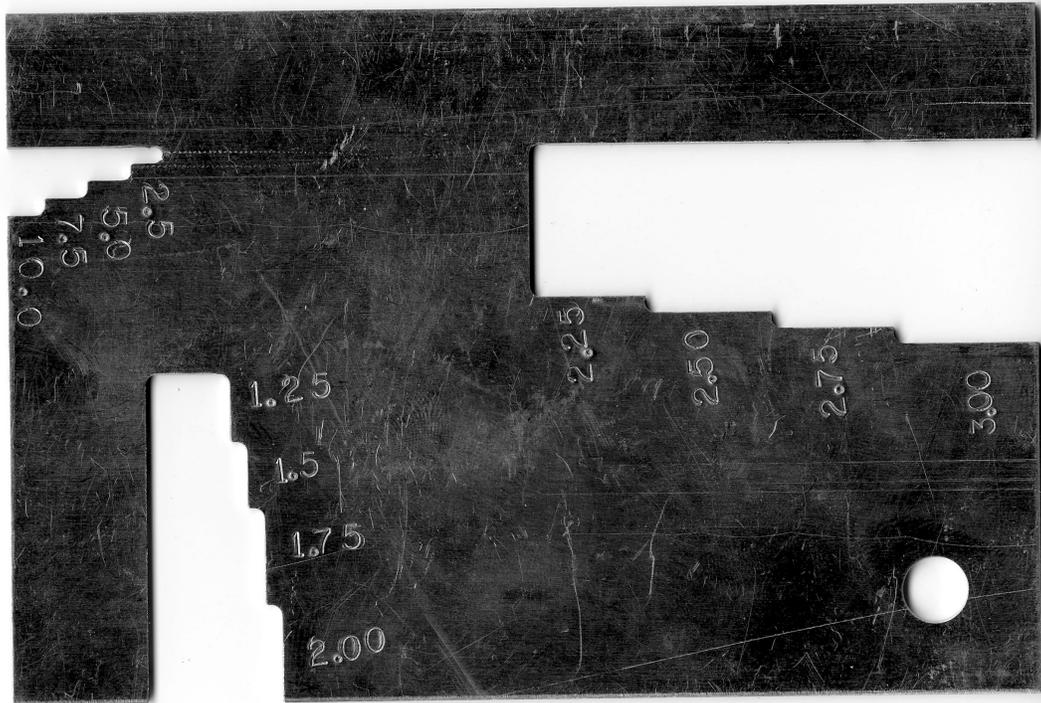


Figure Forest-4. Template used to measure shrub diameter at 15 cm aboveground as a semi-continuous variable in 2.5 mm classes.

### Extensive Data

On all three quadrats, extensive data for tall shrubs will be collected. These data will be canopy cover of tall shrubs by height classes, and will generally follow FIA protocols. Canopy cover is based on a vertically-projected polygon described by the outline of the foliage, ignoring any normal spaces occurring between the leaves of plants (Phase 3 Field Guide). A rapid canopy cover estimate is made, ignoring overlap among species, and consists of the total canopy cover of the foliage of all tall shrubs by layer above the ground surface. Cover will be estimated in the following classes: 1-5%, 6-10%, 11-20%, 21-40%, 41-60%, 61-80%, and 81-100%. Two heights classes will be used; > 0.5 to 2 m and > 2 to 5 m. These approximately correspond to the FIA layers of > 2 to 6 ft and > 6 to 16 ft.

### *Low Shrub and Herb Sampling*

Low shrubs (defined by FIA as woody plants with height < 0.5 m at maturity – Phase 3 Field Guide) and herbs, including ferns, grasses, and forbs, will be sampled similarly. As with tall shrubs, FIA does not attempt to estimate biomass of non-tree vascular vegetation (Phase 3 Field Guide - Vegetation Diversity and Structure, Version 4.0, October, 2007, <http://www.fia.fs.fed.us/library/field-guides-methods-proc/>, assessed 28 October 2008).

As with this modification of the FIA sampling scheme, biomass estimates for low shrubs and herbs will be focused on the three vegetation plots (quadrats) on each subplot (Figure Forest-3). In this case, the same sampling scheme will be used for all quadrats. As with tall shrubs, the intensity of sampling is based on the lesser importance of low shrubs and herbs to total aboveground vascular biomass.

### Cover Estimates

Specifically, canopy cover will be estimated for low shrubs, ferns, forbs, and grasses (separately) within each of three height classes as subdivisions (approximate halving) of FIA vegetation layers. Height classes will include 0 - 0.25 m and > 0.25 - 0.5 m (the sum roughly corresponding to the 0 - 2 ft layer), and > 0.5 - 1.0 m (half of the > 2 to 6 ft layer). Total canopy cover and canopy cover within each height class will be estimated, but the majority of life forms will probably have canopy cover in only one layer, so that the total and layer canopy covers will be identical. Canopy cover is based on a vertically-projected polygon described by the outline of the foliage, ignoring any normal spaces occurring between the leaves of plants. The following canopy cover classes will be used: 0, <1, 1-5%, 6-10%, 11-20%, 21-40%, 41-60%, 61-80%, and 81-100%. The 0 class will be used for plants rooted in the quadrat but with no foliage in the height class. Cover for any height class cannot be greater than the total cover for that life form.

### Clipping

Cover data will be converted to biomass estimates using locally-developed relationships (see **Numerical Methods**). To obtain the data for these relationships, biomass will be determined and cover estimated for each life-form on one auxiliary quadrat per subplot. These auxiliary quadrat (clip plots) will be identical in size (1 m<sup>2</sup>) to the quadrats used for sampling low shrubs and herbs, and will be located on the right sides of a line at an azimuth of 90° from the subplot centers. Two corners of each auxiliary quadrat will be temporarily marked at 15 and 18.3 feet (4.57 and 5.57 m), horizontal distance, from the subplot center. In subsequent samplings over time, the azimuth will shift to 210° and 330° from the subplot centers. If additional sampling is carried out, azimuths of 60°, 180°, and 300° from the subplot centers will be used.

Biomass will be determined on the clip-plots by clipping each life-form at ground level and returning the material to the laboratory for oven-drying and determining mass. Canopy cover will be estimated as on the permanent sample plots.

### *Sampling Down Woody Materials*

Down woody materials can be an important pool of carbon in forest ecosystems. Down woody material is dead material on the ground in various stages of decay, and for this inventory it includes coarse woody debris (CWD) and fine woody debris (FWD). In the case of sampling these materials, as with trees, the FIA protocols will nearly wholly be followed (Phase 3 Field Guide - Down Woody Materials, Version 4.0, October, 2007,

<http://www.fia.fs.fed.us/library/field-guides-methods-proc/>, assessed 28 October 2008).

Briefly, the basis of the sampling of down woody materials are linear transects wherein material that intersects the transect line is inventoried. That procedure will be briefly described below, but details will be found in the field guide (Phase 3 Field Guide - Down Woody Materials). In the sampling, CWD includes downed, dead tree and shrub boles, large limbs, and other woody pieces that are severed from their original source of growth and on the ground. CWD also includes dead trees (either self-supported by roots, severed from roots, or uprooted) that are leaning > 45 degrees from vertical. As the name implies, CWD is generally larger material (pieces > 3.0 inches (7.5 cm) in diameter at the point of intersection with the transect). Material smaller than CWD is considered FWD, and includes downed, dead branches, twigs, and small tree or shrub boles that are not attached to a living or standing dead source. It can be connected to a larger branch, as long as this branch is on the ground and not connected to a standing dead or live tree. Only the woody branches, twigs, and fragments that intersect the transect are counted. More detail on the definitions and criteria for each class can be found in the field guide (Phase 3 Field Guide - Down Woody Materials).

Sampling for CWD is along three transects that originate at the subplot center and extend 24.0 ft horizontal distance (the radius of the subplot) at azimuths of 30°, 150°, and 270° (Figure Forest-3). In the case of FWD, only one transect is established on each subplot, along the 150° azimuth. Because FWD is generally present in higher densities than CWD, a shorter transect is used. The transect begins at 14 ft (slope distance) from the subplot center and extends out either 6 ft (for small – 0 to 6 mm diameter – and medium FWD – > 6 mm to 24 mm) or 10 ft (for large FWD – 25 mm to 75 mm).

Individual pieces of CWD intersected by a transect are tallied by measuring the diameters at the point of intersection, and at the small end and the large end (depending on decay class). Total length between those latter two diameters is also recorded. The decay class of the CWD (1 = sound, freshly fallen, intact logs; 2 = sound, mostly intact; 3 = heartwood sound; 4 = heartwood rotten; 5 = no structural integrity) is noted (details in Phase 3 Field Guide - Down Woody Materials). In the case of FWD, individual diameters are not recorded but simply the counts in each of the three size classes are recorded.

## **Numerical Methods**

The data that are collected will be summarized and used to estimate biomass and related carbon mass.

### *Trees*

#### Aboveground

Aboveground tree biomass is usually estimated through the use an allometric equation

$$M = a \times D^b \quad (1)$$

where  $M$  is aboveground tree biomass and  $D$  is tree diameter, usually measured at 1.3 m aboveground (Kittredge 1944, Ter-Mikaelian and Korzukhin 1997). The standard method to obtain estimates for  $a$  and  $b$  in Eqn. (1) is by least-square regression of data of  $M$  and  $D$  pairs measured from destructively sampled trees that represent the diameter range of the stands under investigation. This is a laborious and time-consuming process. As a result, applicability of equations beyond the specific population of trees that were sampled has been explored and tested. For example, theoretical models have been developed to describe the M-D allometry. One model assumes the presence of an M-D scaling relationship irrespective of species, site and genetic factors, wherein  $b$  is  $8/3$  (2.67) and  $a = 0.10$  (West et al. 1999). A recent analysis of 279 studies indicated an average empirical value of  $b = 2.368$  and  $a = 0.14$  (Zianis 2005). These discussions are cited to indicate a source of uncertainty in tree biomass estimates.

Although ideally both  $a$  and  $b$  should be developed locally for any stand-level biomass estimates, the cost-benefit of such an approach must be considered. If one accepts the premise that variability in  $a$  and  $b$  are very important, then a “new” relationship should be developed at each site and time. There is, for example, a suggestion in the literature that different  $b$  values are necessary for different growth stages of trees (juvenile, adult and mature) (Pilli et al. 2006).

An alternate approach is to use the series of biomass estimation equations from Alemdag (1983, 1984). Those equations, based on trees sampled in Ontario, encompass all the species likely to be found in Minnesota. The equations are similar to Eqn. (1), but use both tree dbh and total height as independent variables. Although tree height may not be measured as accurately as diameter in standing trees, it may help distinguish biomass differences in different growth stages. There is generally a strong height-diameter relationship among forest trees, and this may introduce issues such as some questions in propagation of uncertainty (Zianis 2008). In summary, however, inclusion of tree height (an FIA variable) in estimating tree biomass probably contributes to accuracy and precision. Because our primary interest is in longitudinal studies, the same suite of equations is likely sufficient to detect differences in biomass over time.

### Belowground

Biomass estimation equations for tree roots are relatively uncommon in the literature, and there is no comprehensive set of equations for Minnesota species. An alternative is to base belowground biomass estimates on data from Santantonio et al. (1977), who tabulated root biomass estimation equations from a large number of studies and also provided a figure with individual data points showing the relationship between tree dbh and root mass, on an estimation equation for root mass from New Hampshire that was based on aboveground mass (Whittaker and Marks 1975), and from an estimation equation from Minnesota based on diameter (but with only 17 observations) (Perala and Alban 1994). An expression of root mass as a function of tree diameter, computed as the average of the literature sources, yields an expression similar to Eqn. (1) but where  $M$  is belowground tree biomass and  $D$  is tree diameter, and where  $a = 0.031$  and  $b$

= 2.39. Comparison with the average empirical values from Zianis (2005), cited above, indicates that although tree root biomass varies with diameter, it is approximately 22 percent of aboveground biomass or about 18 percent of total tree biomass.

### *Tall Shrubs*

There are extensive data from northern forests that relate tall shrub stem diameter to biomass (Telfer 1969, Grigal and Ohmann 1977, Ohmann et al. 1976, Brown 1976, Roussopoulos and Loomis 1979, Ohmann et al. 1981, Connolly and Grigal, 1983). Most of the equations describing these relationships have been summarized by Smith and Brand (1983). Many of the equations are based on shrub diameter at 15 cm aboveground, and in many cases the diameter is “measured” as a semi-continuous variable in 2.5 mm classes (using a template – Figure Forest-4). The allometric relationship is

$$M = a \times D^b \quad (2)$$

as in Eqn. (1), except in this case where M = tall shrub biomass and D is shrub diameter class in mm or cm.

When using a template for measuring the shrub diameter and developing the allometric relationship, as described in the field methods, D in Eqn. (1) is not the shrub diameter but the maximum size of that diameter class (e.g., diameters in the class 10.0 to 12.5 mm are all less than 12.5 mm). Some data have indicated that this approach tends to inflate the estimated total biomass if there are many stems in the smallest size classes (Balogh 1983). An approach that has been used is to fit the diameter-density distributions for individual shrub species to a linearized power function

$$\ln(N) = c + d \ln(Dm) \quad (3)$$

where  $\ln(N)$  is the natural log of the estimated number of stems of that diameter class and  $\ln(Dm)$  is the natural log of stem diameter class in mm or cm. The slope of this equation ( $d$ ) for each species is the change in number of individuals with diameter. Using this approach, the expected number of individuals by diameter class was then estimated by Eqn. (3) and biomass was computed using Eqn. (2) (Ohmann and Grigal 1985).

Using the procedures described above, and the relevant species-specific biomass estimation equations from the literature (Smith and Brand 1983), biomass by species will be estimated for tall shrubs on each of the intensively-sampled quadrats. Tall shrub biomass has also been estimated by cover and height (e.g., Peek 1970), albeit for individual clumps of single species. Relationships will be developed (linear, allometric, simply broad classes?) between total tall shrub biomass and canopy cover/height from the intensively-sampled quadrats, and those relationships will be applied to the extensively-sampled quadrats within each site/location at each sampling time. The relationships will be developed with  $n = 12$  (3 plots x 4 subplots/plot x 1 intensive quadrat/subplot).

### *Low Shrubs and Herbs*

Biomass estimation equations, using canopy cover as the independent variable, have been developed for herbs, ferns, and low shrubs (Ohmann et al. 1981) and applied for stand-level estimates. Undergrowth biomass can be reasonably estimated by the relationship

$$\text{Mass} = e \times C^f \quad (4)$$

where Mass = biomass, C is canopy cover in percent, and  $e$  and  $f$  are constants of the relationship. These equations are generally species-specific, but in the case of this inventory similar mathematical expressions will be used for each life-form. Locally-developed relationships (Eqn. (4)) will be developed for each site/location at each sampling time.

These relationships will be based on the data from the auxiliary clip-plots by clipping each life-form at ground level and returning the material to the laboratory for oven-drying and determining mass. Canopy cover will be estimated as on the permanent sample plots. For each life form, the relationships will be based on  $n = 12$  (3 plots x 4 subplots/plot x 1 clip plot/subplot).

### *Understory Belowground Biomass*

Although the magnitude of root biomass of understory vegetation is small compared to that of trees, some general estimates can be made using data from the literature. Reasonable ratios between biomass of roots and shoots are about 1.5 for tall shrubs and about 2 for herbaceous vegetation.

### *Down Woody Materials*

Biomass and C content of woody debris is usually based on the calculation of volumes using techniques described by Van Wagner (1968). The biomass and C content is then estimated by combining the volume with estimates of density and C concentration of woody debris (e.g., as reported by Duvall 1997). Both density and C vary with decay class, and so the computation is carried out by decay class. For this study, FIA protocols and algorithms will be used.

## **Limitations of the method**

### *Potential magnitude and sources of error*

Unfortunately, there are numerous sources of error (i.e., uncertainty) in estimating forest biomass (C) change over time. These techniques require many field measurements, and measurement errors are a potential source of error. Translation of those measurements to biomass via various estimation equations also introduces error. Uncertainty in the equations may be related to their functional form, the precision of the estimation, and their applicability to a specific site(s).

### *Potential strengths and weaknesses*

These methods are relatively inexpensive to implement and require no significant maintenance. This would not be the case if unique tree biomass estimation equations were developed for each site/time of sampling. A major weakness of biomass estimation is the estimation of belowground C.

### *Applicable timeframe of measurement*

Biomass estimation techniques should be able to detect incremental changes in aboveground biomass, primarily of trees, occurring over about a five- to ten-year period. Shorter periods of observation require proportionally higher intensity of sampling and measurement.

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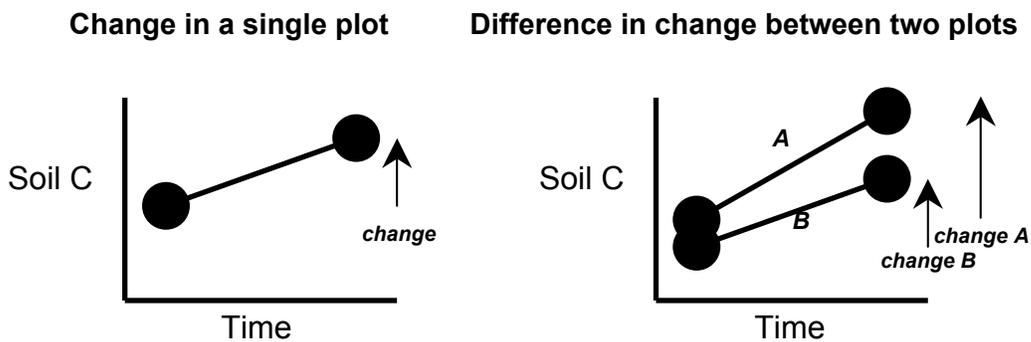
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## B. SOIL SAMPLING

### *Preliminary considerations*

This project addresses questions about changes in a single plot and differences in change between two plots, where those two plots may have different treatments (Fig. Soil-1).



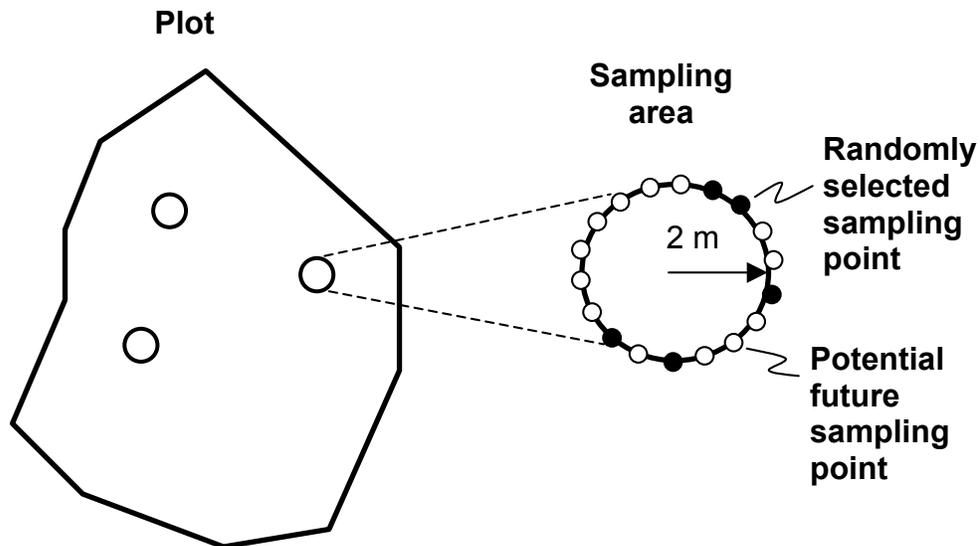
**Figure Soil-1. Changes in a single plot vs. comparing changes between plots**

The statistical observational unit is the plot. Soil sampling areas are subsamples within the plots. Comparison of single plots having different treatments does not allow conclusions about the treatments, only that the plots are different or not. Statistically based conclusions are valid only for the plots, although managers might choose to extrapolate (i.e., nonstatistically) to other locations. To address the question “Do different treatments differ in their magnitude of change?” would require multiple plots of each treatment with the treatments randomly assigned to the plots; that is beyond the scope of this study.

The plot may be adjacent to land-uses similar to the plot, or surrounded by other land uses. If surrounded by other land uses, there needs to be a decision about whether or not the plot should be divided a central measurement area and a buffer area.

### *Where to sample within the plot*

The general sampling layout is to have multiple sampling areas within each plot and one or more sampling points within each sampling area (Fig. Soil-2). Future resampling may be conducted within the same set of sampling areas, which will yield higher statistical power if variability within a sampling area is much less than variability across the entire plot. However, if this is not the case, or if sampling areas cannot be exactly relocated, or if sampling areas have been disrupted by initial sampling, future resampling should be conducted on a different set of sampling areas.



**Figure Soil-2. General soil sampling scheme, with multiple sampling areas within a plot, and one or more sampling points per sampling area.**

Classical statistical analysis is based on randomly located sampling areas. Conversely, systematic grid-based locations may be more conducive to laying out the plot and geostatistical analysis. The concern with systematic sampling is that there may be an unknown pattern in the plot, e.g. from a previous land use or a future land use, that may bias results should the grid coincide with the pattern. Using randomly located sampling areas is a safer approach.

For a given number of sampling areas per plot ( $n$ ), the highest statistical power occurs if

there are multiple sampling points per sampling area ( $p$ ). For a given number of total sampling points per plot ( $p_{\text{total plot}}$ ), the highest statistical power occurs if there is only one sampling point per sampling area. For a situation where both  $p_{\text{total plot}}$  and  $n$  are adjustable, rather than given, and the project is constrained by limit funds, the highest statistical power can be attained by

$$p = (\sigma^2_{\text{points}}/\sigma^2_{\text{areas}})^{0.5}(\text{cost}_{\text{area}}/\text{cost}_{\text{point}})^{0.5}$$

where  $p$  is the number of sampling points per sampling area,  $\sigma^2_{\text{points}}$  is the variance among sampling points within an area,  $\sigma^2_{\text{areas}}$  is the variance among areas, and  $\text{cost}_{\text{area}}$  &  $\text{cost}_{\text{point}}$  are the costs per area and per point, respectively. Good estimates of the variances and costs require *substantial* preliminary sampling, which usually is not feasible. From a practical standpoint, if sampling is conducted with machinery that is expensive (time and resources) to move from one sampling area to another within a plot, but is efficient in taking samples at multiple points once it is set up, then multiple sampling points per sampling area are worthwhile. In contrast, if samples are collected by hand, then a single point per sampling area will likely be most cost-effective to produce a high statistical power.

#### *Desired sensitivity and uncertainty*

This project address questions about changes in a single plot and differences in change between two plots, where those two plots may have different treatments. The numbers of soil sampling points needed to address these questions depend on the desired levels of sensitivity and uncertainty, and on the variability of soil C among sampling areas within a plot. The variability of soil C within a plot will vary with the cross-sectional area of the sample. Typically, the larger the cross-sectional area, the smaller the variability among sampling points and among sampling areas, because small-scale variability is captured within the sample. However, the quantitative relationship for a given plot can be determined only with intensive sampling of that plot.

The analysis presented here assumed one sampling point per sampling area and resampling of different sets of sampling areas. Sampling more than one point per sampling area and

resampling the same sampling areas may or may not decrease the number of sampling areas required, depending on the structure of the spatial variability (e.g. Homann et al. 2008). Further, transforming the variable, soil C, may lead to statistical distributions that are more normal, thereby more closely meeting the assumption of normality and leading to more justifiable results. This analysis used the following desired levels of sensitivity and uncertainty.

**Change in soil C in a single plot**

sensitivity: detect a real change of 20% of existing soil C

uncertainty: real change = measured change  $\pm$  10% of existing soil C

**Difference in change in soil C between two plots**

sensitivity: detect a real difference in change of 20% of existing soil C

uncertainty: real difference in change

= measured difference in change  $\pm$  10% of existing soil C

*Number of sampling areas (n) for a single plot*

The minimum detectable change for a single plot ( $MDC_{plot}$ ) is based on a two-sample t-test with equal sample sizes (Zar, 1999), where the two samples represent two sampling times whose sampling points are independent from each other. Alpha = 0.05. Power = 0.8.  $df = 2n-2$

$$MDC_{plot} = s_{plot}(2)^{0.5}(t_{0.05(2),df} + t_{0.2(1),df})$$

where  $s_{plot} = s (1/n)^{0.5}$  and s is the within-plot sample standard deviation, which is taken as the best estimate of the within-plot population standard deviation, which is assumed to be constant over time; n is number of sampling points at each sampling time;  $df = 2n-2$ . The equation can be solved for n, given any desired  $MDC_{plot}$  and s. Table Soil-1 shows the n required to conclude there is a change, if a real change of 20% occurred, based on s from several studies.

Merely concluding that there is a real change does not indicate the magnitude of that change. The magnitude and its uncertainty are indicated by (although technically not equivalent to)

the 95% CI of the real change.

$$95\% \text{ CI of real change} = \text{measured change} \pm s_{\text{plot}}(t_{0.05(2), df=2n-2})$$

The desired 95% CI may be expressed in terms of existing soil C:

$$95\% \text{ CI of real change} = \text{measured change} \pm \text{fraction of existing soil C}$$

For example, for a measured change of 20%, and a fraction of existing soil C of 10%,

$$\begin{aligned} 95\% \text{ CI of real change} &= 20\% \text{ measured change} \pm 10\% \text{ of existing soil C} \\ &= 10 \text{ to } 30\% \text{ of existing soil C} \end{aligned}$$

The n required to limit the uncertainty of real change to this level is presented in Table Soil-1.

Table Soil-1. Sample numbers required to detect a minimum change of 20% and 10% of the existing soil carbon content in a single plot at a 95% confidence interval for selected soils in Minnesota. Sample numbers are those required at each sampling interval.

System	Soil depth	Soil C (kgC/m <sup>2</sup> )	s (% of existing soil C)	sample numbers required to detect 20% change	sample numbers required to detect 10% change	
Cedar Creek Abandoned fields <sup>1</sup>	0-10 cm	1.5	18	18	26	
	10-30cm	1.6	12	9	13	
	30-50cm	1.3	15	13	19	
Cedar Creek Forest <sup>1</sup>	O horizon	0.6	31	50	75	
	0-10 cm	2.1	18	18	26	
	10-30cm	1.7	17	16	24	
	30-50cm	1.3	18	18	26	
Cedar Creek field 76 <sup>2</sup>	0-10	1.88	36.12	67	93	
Western MN <sup>2</sup>	lowest	0-15	2.0	9.15	6	8
	highest	0-15	1.7	42.34	93	150
	all groups	0-15	2.1	27.39	42	65
Waseca, MN <sup>3</sup>	Clarion	0-15	3.1	6.45	4	5
	Nicollet	0-15	3.3	3.64	3	3
	Webster	0-15	4.0	6.75	4	5
Nemadji State Forest <sup>2</sup>	0-10	4.3	34.33	60	84	
	10-25	1.7	26.74	37	52	
UMORE Park, Rosemount, MN <sup>2</sup>	0-15	4.17	10.69	7	11	

<sup>1</sup>Data from Homann and Grigal, 1966.

<sup>2</sup>Unpublished data, Nater and Brozowski.

<sup>3</sup>Data from Adams, 1984

*Number of sampling areas (n) for difference in change between two plots*

The minimum detectable difference in change between two plots ( $MDD_{\text{change}}$ ) is based on a two-sample t-test of change. Alpha = 0.05. Power = 0.8.

$$MDD_{\text{change}} = S_{\text{change}} (2)^{0.5} (t_{0.05(2),df} + t_{0.2(1),df})$$

where  $S_{\text{change}} = S_{\text{plot}}(2)^{0.5}$ ; n is number of sampling points on each plot at each sampling time;  $df = 4n-4$ . The equation can be solved for n, given any desired  $MDD_{\text{change}}$  and s. Table Soil-2 shows the n required to conclude there is difference in a change, if a real difference in change of 20% occurred; for example if one plot changed 10% and the other 30%.

Merely concluding that there is a real difference in change between two plots does not indicate the magnitude of that difference. The magnitude and its uncertainty are indicated by (although technically not equivalent to) the 95% CI of the real difference in change.

95% CI of real difference in change

$$= \text{measured difference in change} \pm S_{\text{change}}(t_{0.05(2),df=4n-4})$$

The desired 95% CI may be expressed in terms of existing soil C:

95% CI of real change

$$= \text{measured difference in change} \pm \text{fraction of existing soil C}$$

For example, for a measured difference in change of 20%, and a fraction of existing soil C of 10%,

95% CI of real difference in change

$$= 20\% \text{ measured change} \pm 10\% \text{ of existing soil C}$$

$$= 10 \text{ to } 30\% \text{ of existing soil C}$$

The n required to limit the uncertainty difference in change to this level is presented in Table Soil-2.

Table Soil-2. Sample numbers required to detect a minimum change of 20% and 10% of the existing soil carbon content between two plots at a 95% confidence interval for selected soils in Minnesota. Sample numbers are those required at each sampling interval.

System	Soil depth	Soil C (kgC/m <sup>2</sup> )	s (% of existing soil C)	sample numbers required to detect 20% change	sample numbers required to detect 10% change	
Cedar Creek Abandoned fields <sup>1</sup>	0-10 cm	1.5	18	33	50	
	10-30cm	1.6	12	16	22	
	30-50cm	1.3	15	24	35	
Cedar Creek Forest <sup>1</sup>	O horizon	0.6	31	100	148	
	0-10 cm	2.1	18	33	50	
	10-30cm	1.7	17	31	45	
	30-50cm	1.3	18	33	50	
Cedar Creek field 76 <sup>2</sup>	0-10	1.88	36.12	131	183	
Western MN <sup>2</sup>	lowest	0-15	2.0	9.15	10	14
	highest	0-15	1.7	42.34	180	270
	all groups	0-15	2.1	27.39	80	120
Waseca, MN <sup>3</sup>	Clarion	0-15	3.1	6.45	5	7
	Nicollet	0-15	3.3	3.64	3	3
	Webster	0-15	4.0	6.75	6	8
Nemadji State Forest <sup>2</sup>	0-10	4.3	34.33	119	165	
	10-25	1.7	26.74	73	101	
UMORE Park, Rosemount, MN <sup>2</sup>	0-15	4.17	10.69	12	17	

<sup>1</sup>Data from Homann and Grigal, 1966.

<sup>2</sup>Unpublished data, Nater and Brozowski.

<sup>3</sup>Data from Adams, 1984

### *Materials*

Soils should be sampled to at least a depth of 50 cm, and preferably to the current rooting depth or the anticipated rooting depth under a different management practice. Soils should be sampled by layers designated by depth from the surface of mineral soil. Layers should be at least 10 cm thick, because thinner layers have high uncertainty with respect actual thickness that is sampled.

Soils should be sampled by corers with minimal core compaction or by quantitative soil pits. A large cross-sectional area of the sample is beneficial, because typically, the larger the cross-sectional area, the smaller the variability among sampling points, as the small-scale variability is captured within the sample. However, the quantitative relationship for a given plot can be determined only with intensive sampling of that plot.

Soil organic C (kg/m<sup>2</sup>), hereafter call soil C, should be calculated for each individual sampling point (or for each sampling area if multiple sampling points per sampling area are composited prior to chemical analysis):

$$\text{soil C (kg/m}^2 \text{ per layer)} = \text{C concentration (\% of oven-dried mass)} / 100\% \\ \times \text{soil mass (kg oven-dried / sample)} \\ \div \text{cross-sectional area of sampler (m}^2 \text{ / sample)}$$

where each variable is measured for each sampling point, and soil is defined as the material within the sample volume that contains C.

An *equivalent* expression is

$$\text{soil C (kg/m}^2 \text{ per layer)} = \text{C concentration (\% of oven-dried mass)} / 100\% \\ \times \text{soil bulk density (g/cm}^3\text{)} \\ \times \text{layer depth (cm)} \\ \times (1 - \% \text{ rock volume}/100\%) \\ \times 0.001 \text{ kg / g} \times 10,000 \text{ cm}^2 / \text{m}^2$$

where

$$\begin{aligned} \text{soil bulk density (g/cm}^3\text{)} &= \text{soil mass (kg oven-dried / sample)} \\ &\div \text{cross-sectional area of sampler (m}^2\text{ / sample)} \\ &\div \text{layer depth (cm)} \\ &\div (1 - \% \text{ rock volume}/100\%) \\ &\div 10,000 \text{ cm}^2/\text{ m}^2 \end{aligned}$$

where each variable is measured for each sampling point, and rock is defined as material within the sample volume that is not included in the estimate of soil C. In most cases, this would be what is normally referred to as stones that do not contain C. In some cases, large woody roots would also be excluded and their C (kg/m<sup>2</sup>) would be estimated by some other technique.

If soils have significant calcium carbonate concentrations, they will need to be treated with acid prior to analysis of C, otherwise C derived from carbonates could be mistakenly measured as organic C.

For examining change, initial samples should be saved so they can be chemically analyzed along with future samples. If not, actual differences between two samplings may be confounded by differences in analytical techniques and instruments. But the initial samplings must be stored under conditions such that their C concentrations will not change. We recommend air (or mild-oven) drying, followed by freezing (storage at ~ -20° C).

### *Limitations of the method*

Large numbers of sampling areas per plot are required to yield adequate levels of sensitivity and uncertainty (Tables Soil-1 and Soil-2). Resampling the same areas might reduce the number of required sampling areas, but this depends on plot-specific spatial variability (Homann et al. 2008). Relocating sampling areas may be improved by documenting high-precision, high-accuracy GPS coordinates, and by placing a metal marker, e.g. rebar, at depth in the soil.

If the within-plot standard deviation, *s*, is to be used to determine required numbers of sampling areas (Tables Soil-1 and Soil-2), it needs to be established to relatively high certainty. Unfortunately, this can only be accomplished with a preliminary study that

measures a large number of sampling areas on each specific plot.

Soil sampling will disrupt portions of the plot. The magnitude of the disruption will depend on the type of sampling. Exact locations of sampling and spatial extent of disruption should be carefully documented so subsequent sampling does not occur in the disrupted areas.

Longterm storage of samples may be challenging.

Soil sampling and processing may have to be adapted to each plot, because of high coarse fragments that make sampling difficult; coarse woody debris and trees in forests, which prevent sampling soil beneath them; changes in coarse woody debris and trees in forests, which change which areas can and cannot be sampled; C-containing soil aggregates that do not disperse under conventional soil processing procedures (Homann et al. 2004).

#### *Potential sources of error*

There may be seasonal cycles in soil C due to root death and decomposition. In forests, there may be seasonal changes in O layer C due to autumnal litterfall and its subsequent decomposition. Sampling at the same time of year would alleviate this potential problem.

Estimates of C concentration, coarse fragments, and bulk density are sometimes taken from different data sets, hence different sampling points, and used to estimate soil C ( $\text{kg/m}^2$ ). This has two potential consequences:

- 1) biasing soil C values for individual samples, and biasing the average soil C value of several samples at a single point in time – although if the bias is similar at two points in time, the change between the two points in time may be relatively unbiased;
- 2) creating unknown uncertainty in the soil variability estimates if covariances between the variables are not taken into account.

The initially specified depth defines the lower boundary of the system whose changes we

wish to quantify. Unfortunately, the same lower boundary may be difficult to identify in subsequent sampling if there is (i) erosion from the site, (ii) sediment deposition to the site, (iii) movement of soil within the site, and (iv) compaction or expansion of soil due to changes in organic matter or other processes. Under these circumstances, applying the initially specified depth from soil surface to the subsequent sampling depth will define a lower boundary that is different from the initial one, which will influence the evaluation of change in soil C within the system. Similarly, evaluations of change in the individual layers may be influenced. There are three approaches to contending with these processes:

1) For processes (i), (ii), (iii), and (iv), ignore the processes and define the lower boundary at subsequent sampling to be at the initially specified depth from the soil surface. This can create substantial bias if only a surface soil layer (e.g. 0-10 cm) is analyzed, because the 0-10 cm at subsequent sampling does not represent the initial 0-10 cm depth. It creates much less bias if the full profile is analyzed as a single layer, e.g. 0- 100 cm depth. However, analysis of the full profile may be insensitive to significant soil C changes at the soil surface because the uncertainty in full-profile soil C would overshadow those changes.

2) For processes (i), (ii), (iii), and (iv), place an identifiable marker at the lower boundary during the initial sampling. In subsequent samplings, sample to the depth of the marker. Clearly, disturbance and its effect on C is an issue with this approach.

3) For process (iv) only, define the lower boundary by mass of inorganic matter ( $\text{kg m}^{-2}$ ) rather than by depth. This approach assumes that the amount of *in*organic matter in the system is constant, while the organic matter and its C constituent can change. The system may be defined, for example, as the surface 200 kg of inorganic matter  $\text{m}^{-2}$ . In practice, the soil must be sampled in relatively thin layers (e.g. 5 to 10 cm thick) of known cross-sectional area, mass of inorganic matter is computed – which can be done by subtracting soil organic C and associated organic matter from soil mass – and successively deeper layers or portions of layers are summed until the specified mass of inorganic matter is reached. The amount of C associated with those layers or portions of layers is then summed. The technique has been used for equivalent soil depths of up to 50 cm (Homann and Grigal 1996, Homann et al. 2001, and a slightly less rigorous approach by Ellert et al. 2002). Compared with typical

fixed-depth analysis, the technique requires more layers to be sampled and analyzed because they are thin, and the technique requires more computation. The technique is substantially more challenging in rocky soils, both for sampling and computation.

#### *Potential strengths and weaknesses*

The greatest strength is the direct measurement of soil C mass, in contrast to measurement of gas fluxes and their associated uncertainties. There is a physical sample in hand that can be analyzed, and reanalyzed if required. The weakness is the need to preserve samples in an unchanged state so they can be measured concomitantly with samples taken decades in the future.

Large numbers of sampling areas are required. If relatively few sampling areas are measured, there will be little chance of observing change and accurately estimating the magnitude of change.

Procedures of soil sampling can be sufficiently documented so as to be largely repeatable decades later. However, any changes in soil sampling procedures – whether intended or not—may create unknown bias.

#### *Applicable timeframe of measurement*

Typically soil sampling is relatively ineffective measuring changes in soil C due to sequestration in timeframes shorter than a decade even for sequestration processes with relatively high rates of C accrual. It is much better suited to measuring differences occurring over decades to centuries.

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